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ALTERED REGULATION OF IL-6 SYNTHESIS IN PSORIATIC FIBROBLASTS.
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IL-6 is a cytokine with the capacity to link cutaneous inflammation with keratinocyte hyperproliferation, making it a potential candidate in the initiation and/or maintenance of psoriatic lesions. Elevated IL-6 production in lesional psoriatic skin, probably derived from dermal fibroblasts, has been described by our group. In this study the pattern of IL-6 synthesis of fibroblasts from lesional and uninvolved psoriatic skin was compared with fibroblasts from normal healthy skin. The B9 bio-assay and northern blotting were used to monitor the IL-6 synthesis under basal culture conditions with or without added growth factors or serum. At least three times elevated IL-6 activities were found under basal conditions in the supernatants of lesional psoriatic fibroblasts as compared to normal fibroblasts. Serum or growth factors restored this difference in IL-6 production. In addition only in normal fibroblasts serum induced an increase in IL-6 production on a per cell basis. The synthesis of IL-6 in lesional psoriatic fibroblasts thus appeared considerably less dependent on external stimuli. However, serum unexpectedly reduced almost completely the expression of IL-6 mRNA in lesional psoriatic fibroblasts in contrast to normal cells. This inhibitory effect of serum was fully restored by cycloheximide. These results imply an altered pattern of IL-6 synthesis in lesional fibroblasts, which could play a pathogenic role in psoriasis.

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IMMUNOSUPPRESSIVE EFFECTS OF 1,25-DIHYDROXYVITAMIN D3 ON EPIDERMAL CELLS.
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Calcitriol or 1,25-dihydroxyvitamin D3 (1,25(OH)2-D3) is the biologically active form of vitamin D. We have previously shown that this hormone has potent immunosuppressive effects on the human allogeneic mixed epidermal cell lymphocyte reaction (MECLR), and can act in synergism with cyclosporin A.

In the present work, we further evaluated the effects of 1,25(OH)2-D3 on the cell populations involved in MECLR by incubating stimulator epidermal cells (EC) and effector lymphocytes separately during 2 h with efficient concentrations of calcitriol, or of the vehicle. Cells were then washed five times and mixed in the cultures. Pretreatment of lymphoid cells with 1,25(OH)2-D3 induced a 47 % decrease of the proliferation, but calcitriol-pulsed EC resulted in a more pronounced, 85 % inhibition versus the control level. The EC-targets of 1,25(OH)2-D3 during MECLR were further analyzed after separating Langerhans cells (LC) and LC-depleted keratinocytes, using an immunomagnetic particle technique. Pretreatment of LC induced a 30 % decrease of the proliferation, compared to vehicle-treated-LC. These calcitriol-pulsed LC did not decrease the proliferation induced by unmodified autologous EC. As expected, LC-depleted keratinocytes failed to stimulate allogeneic lymphocytes. When added to autologous unmodified EC however, calcitriol-pulsed keratinocytes induced a 85 % decrease of the proliferation, compared to vehicle-treated keratinocytes. The phenotypic expression of HLA DR, -DQ, and -DP antigens on EC, assessed by immunoalkaline phosphatase staining, was not modified after a 2h or 18h-pulse with 1,25(OH)2-D3. The inhibitory effect of calcitriol on EC was only partially reversed by the addition of several dilutions of neutralizing antibodies directed against TGF-ß.

In conclusion, calcitriol may limit the immune response in human skin through decreased antigen presentation mediated both directly on LC as well as indirectly through the modulation of the production of cytokines by keratinocytes.

3

INTERLEUKIN-3 IS PHYSIOLOGICALLY SECRETED BY NORMAL KERATINOCYTES IN NEONATAL MICE.
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IL-3 is a multifunctional cytokine, produced principally by T cells, that has been shown to induce de novo expression of the Thy-1 antigen on Thy-1 depleted murine haematopoietic cells from bone marrow. It is thought to represent one of the earliest factors responsible for T lymphopoiesis. Production of IL-3 by murine keratinocytes is still controversial; most studies have employed transformed keratinocyte cell lines and IL-3 responsive indicator cells that respond to other haematopoetic cytokines. We cultured normal neonatal murine keratinocytes and demonstrated that supernatants could support the growth of FL5.12, a pre-B cell line from fetal liver that responds to IL-3 and no other known keratinocyte cytokine; this activity could be neutralized using antibodies to rIL-3. IL-3 transcripts were detected using reverse PCR of IL-3 mRNA purified from similar cultures of neonatal keratinocytes. We next analyzed mRNA derived from intact neonatal tissues by reverse PCR. Transcripts for IL-3 were detected in epidermis, thymus, and spleen but not in dermis. Identity of PCR amplified cDNA products was confirmed by Southern blotting with ^{32}P-labelled IL-3 cDNA. The source of IL-3 mRNA in epidermis is likely to be the keratinocyte, since few dendritic epidermal T cells (DETC) are present between days 0-2. We hypothesize that authentic IL-3 produced by neonatal epidermal keratinocytes induces Thy-1 on precursors of DETC in neonatal epidermis and acts as an epidermal T lymphopoietic growth factor in the mouse.

4

FIBROBLAST-INDUCED MODULATION OF THE UROKINASE-PLASMINOGEN ACTIVATOR-RECEPTOR (U-PA-R) IN SQUAMOUS CARCINOMA CELLS (SCC-4) AND NORMAL HUMAN KERATINOCYTES.
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For the functioning of plasminogen-plasmin proteolytic cascade in the skin, the presence of active enzymes is necessary. In this cascade, the binding of uPA to its receptor determines its proteolytic activity in the extracellular micro-environment in processes like tumor invasion and skin repair. During these processes epithelial and mesenchymal cells are not longer separated by a basement membrane and can come in direct contact with each other, possibly leading to a modulation of uPA activity. To study the modulation of the levels of uPA and its receptor in vitro, use has been made of a human squamous carcinoma cell line SCC-4 and normal human keratinocytes (NHK) cultured for 6 days either alone or together with lethally irradiated mouse fibroblasts (3T3 cells). Human uPA and human plasminogen activator inhibitor-1 (PAI-1) in conditioned media (CM) were determined using ELISAs. The amount of functional uPA-R protein in isolated cell membranes was analysed using ^{125}I-DFP-uPA crosslinking. The uPA, uPA-R and PAI-1 mRNA expression in parallel cultures were assayed using Northern Blotting with specific cDNA probes.

The uPA concentration in CM derived from NHK and from SCC-4 cells was approximately 10 ng uPA/µg DNA and increased about two to three times in the presence of 3T3 fibroblasts. The functional uPA-R was present in isolated plasma membranes and its amount was reduced when SCC-4 cells were co-cultured with 3T3 fibroblasts, whereas the opposite was seen in NHK. At the mRNA level no significant changes were found. In viable 3T3 fibroblasts uPA-R was not detected at both protein and mRNA levels. While the content of PAI-1 protein was increased (from 5 to 30 ng/µg DNA) when NHK were co-cultured with 3T3 cells, the PAI-1 content remained unchanged in SCC-4 cells (± 10 ng/µg DNA). When co-cultured with 3T3 cells the uPA and PAI-1 mRNA expression were increased in SCC-4 cells and decreased in NHK. Although fibroblasts induced an increase in the total amount of uPA protein in both normal and malignant keratinocytes, the biological activity of uPA which is regulated through binding to its receptor and the amount of PAI-1 is clearly different between normal and malignant keratinocytes. This may be relevant in the physiological process of wound healing and the pathological process of tumor invasion.

5

A TRIMETHYLXANTHINE SPECIES IS A MAJOR LYMPHOCYTE ATTRACTANT IN NORMAL HUMAN SKIN AND PLASMA.
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We have previously demonstrated low molecular weight peripheral blood lymphocyte (PBL) attractant activity in chamber fluid and stratum corneum samples from abraded normal human skin. We have shown that this material is similar or identical to a component in normal human plasma, as determined by reversed phase (RP)-HPLC, UV spectrophotometry and PBL migration bioassay (Bacon et al, Eur J Immunol 20:565-571,1990). We have now purified the material in plasma to homogeneity by successive ultrafiltration and RP-HPLC and have subjected the underivatized biologically active factor to electron impact mass spectrometry. Major ions at m/z 194 (molecular ion), 165, 137, 109, 82, 67, 55 and 42 indicated the presence of trimethylxanthine (TMX), but the positions of the methyl groups could not be assigned by this method. Standard 1,3,7-TMX (caffeine, Sigma) and 1,3,9-TMX (isocaffeine, Sigma) induced dilution-related responses in the PBL migration assay. The plasma-derived TMX had a UV absorbance spectrum similar to that of caffeine (UV maximum 273 nm), but could be distinguished from isocaffeine (UV maxima at 239 and 269 nm). The third positional isomer, 1,3,8-TMX, has not been obtainable for study. Normal human plasma therefore contains PBL attractant material which is either 1,3,7-TMX (caffeine) or 1,3,8-TMX. A similar or identical compound is present in normal human skin. Its source is uncertain, but is likely to be dietary if its structure is 1,3,7-TMX. This material may play a role in lymphocyte adhesion and trafficking.

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IMMUNOGLOBULIN GENE-REARRANGEMENT ANALYSIS IN THE DIFFERENTIAL DIAGNOSIS BETWEEN CUTANEOUS PSEUDO-B-CELL LYMPHOMAS AND B-CELL LYMPHOMAS.
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The differentiation between pseudo-B cell lymphomas and cutaneous B cell lymphomas (CBCL) using histologic and clinical criteria may be very difficult. Therefore the differentiation is based on immunohistochemical analysis (IHA) showing polyclonality of cytoplasmatic and surface immunoglobulin (Ig) in pseudolymphomas and monotypic light chain expression or lack of detectable Ig in CBCL. Immunoglobulin gene rearrangement analysis is a more sensitive method than IHA to demonstrate clonal B cell populations. The value of this method in the differential diagnosis between pseudo-B cell lymphomas and primary CBCL has not been established. In the present study the presence of clonal rearrangements of Ig genes was investigated in 7 pseudo-B cell lymphomas, 8 primary CBCL and 10 cases CBCL using a Jh, Cκ, Cλ and κde probe. In all cases the diagnosis was based on the results of IHA. Clonal rearrangements of one or more Ig genes were demonstrated in 4 of 7 cases of pseudo-B cell lymphomas, 6 of 8 cases of primary CBCL and in all secondary CBCL. The observation that cutaneous pseudo B cell lymphomas as defined by immunohistochemical criteria often contain occult monoclonal B cell populations implicates that differentiating between both conditions is not always possible by means of gene rearrangement analysis. These findings support the concept that cutaneous pseudo-B cell lymphomas and primary CBCL are part of a continuous spectrum of B-cell lymphoproliferative skin disorders.

7

IL-8- AND GRO-LIKE NEUTROPHIL ATTRACTANTS IN PSORIASIS.
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In previous studies we could show, that psoriatic scales contain at least nine biochemically distinct neutrophil activating proteins (ANAPs). Three of these ANAP-components we structurally identified as the 69- and 72-residue forms of IL-8 as well as melanoma growth stimulatory activity, MGSA or "gro". The aim of this study was a further definition of the other ANAP components. We separated extracts obtained from pooled psoriatic scales by CM-cation-exchange HPLC and could identify two distinct peaks (α-ANAP, ß-ANAP). α-ANAPs were separated by different reversed phase- and size exclusion-HPLC-methods into five different components, whereas ß-ANAP by similar methods could be separated into four components. Apart from the three major ANAPs we could isolate two peptides of low potency neutrophil chemotactic activity (ED_{50}= 100 ng/ml). Aminoterminal amino acid sequencing revealed identical sequences as found for the 69- and 72-residue forms of IL-8. Since upon SDS-PAGE analyses these ANAP-factors were of 1-2kDa lower M, than that found for IL-8 we conclude that these components represent C-terminally truncated IL-8 forms. Two of the α-ANAPs we isolated in addition to MGSA/gro showed upon SDS-PAGE analysis a similar M, and upon PMN-chemotaxis a dose response profile characteristic for MGSA/gro. Since it is now known that three genes for gro do exist it is suggested that the major form isolated from psoriatic scales represents gro α, whereas the two minor forms we now isolated could represent gro ß and gro γ.

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TGF-ALPHA & BETA EXPRESSION IN PSORIATIC PLAQUES DURING THERAPY.
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We have previously reported that psoriatic plaques show increased expression of TGF-alpha RNA in suprabasal keratinocytes (J. Invest. Derm. 1990; 95: 229-232). Subsequently, we have carried out sequential biopsies in patients with psoriasis undergoing topical tar therapy. Prior to treatment, patients have one biopsy taken from a psoriatic plaque and one from adjacent clinically normal skin. The next biopsy is taken after 14 days therapy from a clearing lesion, and the last biopsy from the site of a former psoriatic plaque 4 weeks after commencement of treatment. Levels of TGF-alpha & beta mRNA have been assessed using in situ hybridisation and levels of TGF-alpha assessed using monoclonal antibody (Oncogene Science) immunohistochemically.

In 6 patients studied to date, all have shown clear elevation of TGF mRNA levels in the upper cellular layers in untreated psoriatic epidermis. During therapy this elevated TGF mRNA falls, and after 4 weeks of treatment at clinical clearance the level detected is similar to that seen in the biopsy taken from clinically unaffected skin of that same individual. Antibody studies show high levels of TGF-alpha in both clinically normal skin and in untreated psoriatic plaques. During therapy the level of TGF-alpha falls below that seen in clinically normal skin. In contrast, mRNA for TGF-beta is reduced in untreated psoriatic plaques by comparison with clinically uninvolved skin, and appears to rise during therapy. These observations strongly suggest that an imbalance of TGF-alpha and TGF-beta is associated with expression of psoriatic lesions.

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PUVA TREATMENT DOWNREGULATES THE RELEASE OF TNF ALPHA, IL-1 BETA AND IL-8 BY PERIPHERAL BLOOD MONONUCLEAR CELLS.
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Recently, we could demonstrate that PUVA treatment of peripheral blood mononuclear cells (PBMC) derived from psoriatics downregulates IL-6 production. In order to study whether this is specific for IL-6, the effect of PUVA treatment on the release of IL-1 beta, IL-8 and TNF alpha was studied. PBMC were treated with 8-methoxypsoralen (8-MOP) followed by a single UVA exposure (20kJ/m2). Culture supernatants were harvested after 12 hours and tested for IL-1 beta (IRMA), IL-8 (ELISA) or TNF alpha (L929 bioassay). In comparison to untreated cells, PUVA-treated PBMC produced significantly decreased amounts of IL-1 beta, IL-8 and TNF alpha. These data were also confirmed by Northern blot analysis showing reduced mRNA expression for IL-1 beta, IL-8 and TNF alpha. In neither of both test systems was reduction by 8-MOP or UVA alone observed. In addition, PBMC were isolated from 3 psoriatics, who underwent PUVA therapy. In comparison to PBMC obtained before beginning PUVA, production of IL-1 beta, IL-8 and TNF alpha was significantly downregulated in PBMC isolated after completion of PUVA therapy. The present data demonstrate that PUVA treatment both in vivo and in vitro causes significant suppression of the proinflammatory cytokines IL-1 beta, IL-8 and TNF alpha at the protein and the mRNA levels. Therefore one may conclude that this effect contributes to the antiinflammatory effect of PUVA.

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IS ELEVATED SERUM BETA-ENDORPHIN IN PSORIASIS RELATED TO INTENSITY OF SKIN INFLAMMATION.
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Activated T lymphocytes migrate to psoriatic epidermis. Since lymphocytes as well as monocytes are able to produce opioids, the density of infiltrate in psoriatic lesions may affect serum beta-endorphin(b-End) levels.On the other hand, stress, which is known to provoke psoriasis flare, might increase opiod release. The study of endogenous b-End in psoriasis might help to elucidate the role of stress in the pathogenesis of the disease. Serum beta-endorphin was quantitated in 103 patients with psoriasis vulgaris by radioimmunoassay according to Carr.

Serum b-End was found to be markedly ($p<0.001$) increased in patients with psoriasis (14.4) compared to normals (6.0). The highest value was found in patients with active plaque lesions (17.2), whereas symptom-free patients showed reduction in b-End (10.3). The serum beta-End in patients with skin lesions that lasted longer than 3 months (15.3) was greater than shortly following flare (12.5). Its level correlated neither with stress nor with patient age at onset of psoriasis. However, inverse relationship between serum b-End concentration and itching was noted.

Data indicated that b-End is produced by inflammatory cells which migrate to psoriatic lesions (lymphocytes, macrophages, etc.) rather than it is released from nervous system in response to chronic stress.

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EFFECTS OF SPHINGOSINE ON THE HUMAN STRIPPING MODEL AND THE PSORIATIC LESION.
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Ornithine decarboxylase (ODC) is a useful marker for epidermal proliferation and its induction is mediated by activation of the enzyme protein kinase C (PK-C). Inhibitors of PK-C, such as sphingosine, therefore block the ODC induction. It has been hypothesised that PK-C inhibitors might be of therapeutic value in psoriasis. Here we describe the effects of sphingosine on human skin following sellotape stripping (an in vivo model for induction of epidermal proliferation), and report a preliminary trial in chronic plaque psoriasis.

Following tape stripping, sphingosine (0.1 and 0.2 M) was shown to reduce the induction of ODC by 52% and 66% respectively, a finding in line with previous experiments using mouse skin. Contrary to previous reports, however, a marked cytotoxic effect was observed; eight hours after application edema and erythema were seen, and there was histological evidence of a toxic dermatitis.

In contrast, no significant improvement in a modified psoriasis area severity index score was found following 12 days of 2-daily application of 0.1 M sphingosine in ethanol to two psoriatic lesions. Although we can not exclude with certainty that bioavailability during treatment might have been suboptimal, the striking inhibition of ODC induction following tape stripping suggests sufficient penetration of sphingosine to inhibit epidermal growth grossly.

The present study does not lend support for the earlier optimism that protein kinase C inhibitors might be of relevance in the treatment of psoriasis.

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PROTEIN KINASE C ISOENZYMES IN HUMAN KERATINOCYTES.
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The family of protein kinase C (PKC) consists of at least seven subtypes with different biochemical characteristics. The enzyme activity of the "classical" subtypes (α, βI, βII, γ) is regulated by phospholipids and calcium, whereas the novel subtypes (δ, ε, ζ) all lack the calcium binding domain. Furthermore, the different subtypes have been suggested to play discrete roles in the regulation of cellular responses. Previously, we have shown that PKC plays a crucial role in the regulation of keratinocyte proliferation. In the present study, we investigated the expression of PKC subtypes in human epidermis and keratinocytes by the use of monospecific antibodies. PKC α and β were found to be highly expressed in proliferating keratinocytes. During keratinocyte differentiation, the levels of PKC α and β were found to be markedly reduced. In contrast, the expression of the novel of PKC subtypes did not significantly change during cell proliferation and differentiation, with PKC δ being the most abundant subtype. In transformed keratinocytes, PKC expression was markedly down-regulated with a preferential loss of the "classical" subtypes. In conclusion, the present data provide first evidence for discrete roles of PKC subtypes in keratinocyte biology.

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HLA-DR, -DQ AND -DP GENES IN NICKEL, CHROMIUM AND/OR COBALT SENSITIVE INDIVIDUALS: GENOMIC ANALYSIS BASED ON RESTRICTION FRAGMENT LENGTH POLYMORPHISM. Lennart Emtestam, Henrik Zetterquist, Ulla Wedén, and Olle Olerup. Departments of Dermatology, Immunology and Center for Biotechnology, Karolinska Institute, University Hospital, Huddinge, Sweden.

We recently found an HLA-DQA locus association in nickel sensitive subjects. The purpose of the present study was to investigate if our previous finding could be confirmed in an independent study, and also to include studies on chromium and cobalt sensitive subjects.

Using TaqI or MspI digested DNA and probes for DQA, DQB, DRB, DPA and DPB alleles were defined on the basis of Restriction Fragment Length Polymorphism (RFLP). The HLA class II allele frequencies of nickel (n=72), chromium (n=28), and/or cobalt (n=42) sensitive subjects were compared to the frequencies of 250 healthy controls.

No significant changes in the frequencies of DR, DQ or DP alleles were found in the nickel, chromiun and/or cobalt sensitive subjects.

The increased concordance rate of nickel sensitivity in monozygotic compared to dizygotic twins indicates a genetic etiologic component. Also, the specific proliferation of metal specific T lymphocytes, in vitro, is HLA class II restricted. However, in the present study we could not confirm our previous finding of an HLA class II association in nickel sensitive individuals. Thus, it is unlikely that genes located in the HLA class II region are associated with the tendency to develop metal sensitivity.

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PEPTIDE BINDING STUDIES DEMONSTRATE PROMISCUOUS RATHER THAN SELECTIVE CHARACTER OF MHC RESTRICTION, Wolf-H. Boehncke, Toshi Takeshita*, Jay A. Berzofsky*, Ronald N. Germain*, Department of Dermatology, Univ. of Ulm, Germany; *National Institutes of Health, Bethesda, USA

MHC molecules sample and present antigens which then become available for antigen-specific T cell activation.

The binding characteristics of antigen to several mutant versions of an MHC molecule were determined introducing single amino acid substitutions into the antigen. Binding to MHC molecules and recognition by T cells were tested by in vitro competition and in vivo stimulation assays.

Most amino acid substitutions did not affect antigen presentation or recognition. Residues relevant for binding/recognition form a motif similar to a non-related peptide. Substitution of a glutamine by alanine yielded a heteroclytic peptide with a much higher potency to bind to MHC molecules as demonstrated by in vitro competition. This substitution of the bulky glutamine side chain by the CH_3-group of alanine most likely represents the removal of a binding obstacle rather than the introduction of a structure supporting peptide binding.

Thus, MHC restriction is permissive rather than selective resulting in presentation of all peptides that can be accomodated in a given binding grove. We provide evidence for the existence of binding motifs for class II-associated peptides as is the case for classI-associated antigens.

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Functional and morphological characterization of a spleen accessory dendritic cells by monoclonol antibody 4F7

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Recently we have reported on the production of the monoclonal antibody 4F7, which recognizes an epitope being upregulated on dermal and epidermal dendritic cells. This antibody detects an epitope on spleen and lymph node dendritic cells. The $4F7^+$ spleen dendritic cell was further characterized. In situ this mo ab recognizes very few labeled cells in the white pulp of the spleen and approximately < 1% of spleen single cell suspensions as evidenced by FACS analysis in untreated animals. The $4F7^+$ spleen dendritic cells were highly enriched (98%) using 4F7 mo ab labeled immunomagenetic particles. Electron microscopy of such preparations showed typical characteristics of accessory dendritic cells. All of these cells expressed plasma membrane associated MHC class II antigen. Immunohistological characterization of the cells with mo abs revealed beside the expression of Ia molecules positive staining for $Fc_\gamma R$ II and C3bi/R molecules. There was no expression of MACII, MACIII, L3T4, Lyt 2, Thy.1.2, T3, IL-2 receptor, LFA-1α, NLDC 145, MIDC 8 and F4/80 on freshly prepared $4F7^+$ spleen cells. After enrichment and cultivation for about 3 days, these cells showed no adherent properties and a decrease of $FC_\gamma R$ II and C3bi/R expression. The capacity of $4F7^+$ dendritic cells to activate allogeneic T-cells in primary MLR was similar to freshly isolated Ia^+ Langerhans cells. The 4F7+ epitope may represent a functional marker exclusively expressed on dendritic cells in epidermis, dermis, lymph node and spleen with common phenotypical and functional characteristics.

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UVB INDUCED SUPPRESSION OF THE EARLY INITIATING PHASE OF CONTACT HYPERSENSITIVITY TO PICRYLCHLORIDE CAN BE TRANSFERRED BY IMMUNE LYMPHOCYTES. Y. Sontag*, J. Garssen#, F.R. de Gruijl*, J.C. van der Leun*, W.A. van Vloten* and H. van Loveren#, Dept. of Dermatology, Utrecht University,Utrecht(*) and RIVM, Bilthoven(#), The Netherlands.

Murine type IV reactions depend upon the sequential activities of 2 different antigen(Ag)-specific T cells (H. van Loveren et al., Lymphokines 14:405-429, 1987). The early acting T cell produces an Ag-specific, non-MHC restricted factor that mediates Ag-dependent local serotonin release from mast cells. The resulting increased vascular permeability -measurable as a swelling 2 hrs after challenge- enables the influx of late acting Ag-specific, MHC-restricted T cells. These "classical" T_{dh} cells produce lymphokines that attract the 24-hr perivascular infiltrate of non-specific inflammatory cells.

In the present study the influence of UVB radiation on the early and late phases of contact hypersensitivity (CHS) to Picrylchloride (PCl) was studied. DBA/2 mice were irradiated with suberythemal doses of UVB (220 mJ/cm^2) on the shaved backs for 4 consecutive days. Four days later mice were sensitized with 5% PCl on the shaved abdomens. Four days after sensitization mice were challenged on the ears with 0.8% PCL and ear swelling was measured 2, 4, 24 and 48 hrs after challenge. There is a biphasic response, with maximum swellings at 2 and 24 hr. The 2-hr swelling was almost completely suppressed and the 24-hr swelling by about 50% in UVB-treated mice. Because both swellings were suppressed by UVB, we wondered if this suppression could be transferred by immune lymphocytes. Similar results were obtained when immune lymphocytes from UVB-treated donors were transferred to naive recipients ($5*10^7$ cells/animal) which were then challenged. These results suggest that the known suppression of CHS by UVB may in part be due to suppression of the early initiating phase of CHS and that the state of suppression can be transferred with lymphocytes from UVB treated donors.

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PREDOMINANCE OF LESIONAL CD8+ T LYMPHOCYTES IN BULLOUS DRUG REACTIONS. Michael Hertl, Heribert Bohlen, Andreas Kuhn, Jürgen Geisel, Claudia Boecker, Hans F. Merk, Department of Dermatology, University of Köln, Germany.

Bullous drug reactions are severe hypersensitivity reactions whose pathogenesis is hypothesized to be T cell dependent. The goal of this study was to isolate and characterize T lymphocytes found in cutaneous lesions in these drug reactions. Eight epidermal and dermal T cell lines were generated from two patients with penicillin-induced bullous exanthemas and from positive patch test reactions to this drug. The majority (>90%) of the cultured T cells was CD3+CD4-CD8+ and HLA-DR+ which correlated with a marked dermal and sparse epidermal infiltrate of CD8+ cells in situ. Functional studies included Con A mitogenesis assays to examine suppressor activity. Cytotoxicity was tested against B cell lines after preincubation with penicillins or stimulation with a bifunctional (αCD3αCD19) antibody. Drug dependent T cell proliferation was investigated using x-irradiated autologous and allogeneic B cell lines as presenting cells. Four epidermal and dermal T cell lines from penicillin-induced patch test reactions did not exhibit any suppressive activity but showed marked cytotoxicity. Furthermore, a distinct proliferative response to penicillin could be observed. Lesional epidermal T cells from a second patient with ampicillin-induced bullous exanthema were found to show cytotoxicity, but no suppressor activity for Con A mitogenesis. Additionally, these T cell lines showed a marked proliferative response in cultures with autologous B cells which were preincubated with penicillin. In the identical patient, T cells from patch test reactions to penicillin were mainly CD8+ and exerted cytotoxic rather than suppressor activity. Our data indicate that the majority of infiltrating T lymphocytes in bullous drug lesions are CD8+ cytotoxic cells which may contribute to blister formation in that they lead to degeneration and necrosis of keratinocytes.

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TRADITIONAL CHINESE HERBAL THERAPY IS AN EFFICACIOUS TREATMENT OF RECALCITRANT ATOPIC DERMATITIS. M.P. Sheehan, M.H.A. Rustin, D.J. Atherton, C. Buckley, D.J. Harris, J. Brostoff, L. Ostlere, Departments of Dermatology, The Royal Free Hospital and School of Medicine and The Hospital for Sick Children and Department of Immunology, University College and Middlesex School of Medicine, London.

There has been considerable interest in the possibility that Traditional Chinese Herbal Therapy (TCHT) is a new treatment for atopic dermatitis (AD).

A randomized double-blind placebo-controlled trial of TCHT was therefore undertaken in adult patients whose AD was refractory to standard therapy. Patients received 2 months' treatment of either the active formulation of herbs (TCHT) or placebo herbs followed by a crossover to the other treatment after a four week washout period. The median percentage change in erythema after eight weeks for all patients on active treatment was 91.40 (95.2% confidence interval 56.66 to 97.78) compared to -10.6 (95.2% CI -33.33 to 0) with placebo. Similarly, the median percentage change in surface damage after eight weeks for all patients on active treatment was 85.7 (95.2% CI 56.66 to 96.30) compared to -17.3 (95.2% CI -50.00 to 0) with placebo. The 95.2% CI for the difference between these medians was 75.4 to 125 for erythema ($p < 0.0001$) and 80.79 to 125 for surface damage ($p < 0.0002$). Of the 31 patients who completed the study and expressed a preference, 20 preferred that phase of the trial in which they received TCHT compared to 4 patients who preferred placebo (95.2% CI for this proportion was 0.45 to 0.81, $p < 0.02$). There was a significant subjective improvement in itching and sleep during the TCHT treatment phases. No side effects were reported by the patients although unpalatability of the decoction was frequently experienced.

Although formal long-term studies are not yet available, Traditional Chinese Herbal Therapy appears to have great potential benefit for patients with atopic dermatitis.

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IS THERE A REGULATORY ACTION OF INTERLEUKIN-6 IN SYSTEMIC SCLERODERMA?

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As increased serum levels of the cytokine IL-6 are demonstrable in several autoimmune diseases and IL-6 was found to elevate collagen and glycosaminoglycan synthesis of fibroblasts, our interest focused on the action of IL-6 in Systemic Scleroderma (SS). We could demonstrate increased serum levels of IL-6 in patients in an inflammatory phase of SS (n=34), whereas SS patients in stationary phases (n=31) did not show any significant differences in serum levels as compared to healthy controls (n=40). To give an answer to the question whether increased serum levels are due to an unspecific inflammatory response or to increased amounts of IL-6 produced by fibroblasts, we studied the concentration of IL-6 in human primary skin cultures, in human fibroblast monolayer cultures of controls and SS as well as the IL-6 mRNA steady state levels of SS fibroblasts by northern blot analyses. We found, that in primary dermal cultures IL-6 is produced by the dermis with the maximum at the days 2-4 after explantation. That could point to an increased formation of IL-6 when the cells proliferate intensively. In monolayer cultures IL-6 caused a time and concentration dependent stimulation of fibroblast proliferation. This effect was inhibited by IL-6 antibodies. In SS monolayer fibroblasts only some of the examined cultures showed increased IL-6 concentrations in the medium and again some exhibited increased mRNA steady state levels as compared to controls. A high positive correlation of 85,5% between serum levels of IL-6 and procollagen-III-propeptide in SS patients could be found. The above raised question whether IL-6 has some regulatory function in the SS fibrosis remains open.

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Interleukin-4 (IL-4) stimulates collagen gene expression in monolayer fibroblast cultures. P. Gillery[1], C. Fertin[1], A. Bonnet[1], J.F. Nicolas[3] J. Banchereau[4], B. Kalis[2], F.X. Maquart[1].Laboratoire de Biochimie, CNRS URA 610 (1), Laboratoire de Recherche en Dermatologie (2), 51095 Reims Cedex ; INSERM U 209(3), 69437 Lyon Cedex 03 ; Shering-Plough, Laboratoire de Recherches Immunologiques(4) 69570 Dardilly ; France.

The role of mast cell and T-lymphocyte infiltration in the physiopathology of fibrosis, especially scleroderma, has been emphasized by several groups. Among the products of these inflammatory cells, IL-4 is one of the candidates for the stimulation of fibroblasts to produce increased amounts of collagen and other extracellular matrix macromolecules. We studied the effects of IL-4 on collagen gene expression in monolayer culture of dermal fibroblasts. Fibroblasts were grown by routine techniques. When confluent, they were incubated in quadruplicate in DMEM + 1% fetal bovine serum supplemented with various concentrations of IL-4. Total protein and collagen syntheses were measured by ^{14}C-proline incorporation and fluorometric measurement of hydroxyproline. Collagen gene expression was measured by Northern blot and dot blot analysis of pro α 1(I) mRNA, (generous gift of Dr Vuorio, Turku, Finland), using a 36B4 cDNA probe (generous gift of LGME-U184, Strasbourg, France) as reference. IL-4, 10 to 100 U/ml stimulated collagen synthesis in a dose-dependent manner. It was at least as active as TGF-β. A correlated increase of the pro 1(I) collagen mRNA was observed. IL-4 might be implicated in the physiopathology of scleroderma lesions. (Work supported by grants from INSERM (CRE 900703 and 900704), CNRS and the University of Reims).

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TRANSFORMING GROWTH FACTOR-ß STIMULATES EXPRESSION OF COLLAGEN VII IN NORMAL CUTANEOUS CELLS BUT NOT IN CELLS DERIVED FROM MUTILATING DYSTROPHIC EPIDERMOLYSIS BULLOSA SKIN. Adrian König, Jorma Lauharanta and Leena Bruckner-Tuderman, Departments of Dermatology, University Hospitals of Helsinki, Finland and Zürich, Switzerland

Transforming growth factor-ß (TGF-ß) stimulates synthesis of collagen VII, the major anchoring fibril protein, in vitro. Treatment of cutaneous cells with 5 ng/ml of TGF-ß significantly increased the expression of collagen VII by cultured keratinocytes or fibroblast-keratinocyte co-cultures as shown by immunofluorescence staining and immunoblotting of keratinocyte extracts. This inductive capacity of TGF-ß was utilized to assess collagen VII production in cells from a patient with dystrophic mutilating epidermolysis bullosa (EB), a blistering disorder in which the skin is devoid of collagen VII. The patient's keratinocytes and fibroblasts did not synthesize detectable amounts of collagen VII, but expressed another basement membrane protein, laminin, in a normal manner. In contrast to normal cells, no stimulation of collagen VII production was achieved with co-culturing of the EB cells, or with addition of TGF-ß to the cultures. Mixed co-cultures of normal fibroblasts and EB keratinocytes showed strong expression of collagen VII in the normal cells but no synthesis in the EB counterparts. These results suggest that an inability of the EB cells to synthesize collagen VII protein, rather than excessive degradation, contributes to the lack of anchoring fibrils and skin fragility in this patient with severe mutilating dystrophic EB.

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FIBROBLAST INDUCED CONTRACTION OF DERMAL EQUIVALENTS IS INHIBITED BY FRAGMENTS OF TYPE I COLLAGEN. Phil Stephens, Edward J. Wood and Michael J. Raxworthy*, *Department of Biochemistry and Molecular Biology, University of Leeds, LS2 9JT, UK and *3M Health Care Ltd, 3M House, Loughborough, LE11 1EP, UK.

Fibroblasts seeded into a hydrated collagen lattice give rise to a tissue like structure known as a Dermal Equivalent (DE). DEs fabricated in bacteriological grade dishes contract over a period of days producing floating lattices. Such a model has the potential for allowing *in vitro* investigations into some of the remodelling processes that occur during wound healing. In previous studies we observed that freshly isolated human dermal fibroblasts were unable to cause contraction of collagen lattices. This was investigated by comparing the contraction profiles of DEs fabricated using freshly isolated fibroblasts, normal cultured fibroblasts and fibroblasts exposed to fragments of type I collagen. Collagen was digested using *C. histolyticum* collagenase which was subsequently inactivated by the addition of 1,10-phenanthroline hydrochloride. Lattice contraction was calculated by measuring three lattice diameters. Freshly isolated fibroblasts from a number of human tissue types of varying ages caused no contraction of DEs over a thirty day period. However, the same cells maintained in culture through a number of passages caused DE contraction over the same period of time. Cultured cells (passage number 12) incubated with fragments of type I collagen for 5 hours and seeded into DEs caused a contraction of 32.1% (SD±0.006) after 23 days and 65.8% (SD±0.006) after 30 days, whereas control cells caused contractions of 90.1% (SD±0.015) and 90.5% (SD±0.011). This suggests there is blockage of the fibroblast cell surface receptors and that they play an important role in the interaction of the cells with the lattice and its subsequent contraction. Freshly isolated fibroblasts may be unable to contract collagen lattices due to loss of receptors through protease cleavage or blockage of them by fragments of extracellular matrix proteins such as collagen or fibronectin.

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LAK-CELL TRAFFIC IN METASTATIC MELANOMA: PROGNOSTIC RELEVANCE FOR IMMUNOTHERAPIES. R. Dummer, JC Becker, E Schäfer, C Eilles, W Börner, G Burg
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From 14 patients with melanoma metastases, peripheral blood mononuclear cells (PBMC) were taken by leukapheresis and stimulated in vitro using 1000 I.U. recombinant interleukin 2 (IL-2)/ml to generate lymphokine-activated killer cells (LAK cells). Two-color immunofluorescence analysis demonstrated an IL-2 induced up-regulation of CD 25 on natural killer cells (CD 56 positive) as well as on T lymphocytes (CD 3 positive). After radiolabelling with Indium-111, the cells were retransfused.

Metastases could be demonstrated by gamma camera imaging in 8 out of 14 patients (57%). These cells can be produced in a fast and economic way. LAK-cells were able to image lymph node, bone, skin and gastrointestinal metastases but no parenchymatous metastases.

Since two patients gave informed consent to take biopsies of scintigraphically positive metastases, we analysed the peritumoral infiltrate using a panel of monoclonal antibodies (APAAP-staining of cryosections). Activated T helper lymphocytes (CD 3, CD 4, CD25 positive) were identified to be the main population of the reactive infiltrate in these two patients. No natural killer cells (CD 16-, or CD 56- positive) were found. This observation suggests that from the heterogeneous cell populations included in LAK-cells, only T lymphocytes but not natural killer cells are capable to migrate to tumor sites in melanoma patients. Probably, tumor recognition and traffic is restricted to specific T lymphocytes.

Out of the fourteen patients who received radiolabelled LAK-cells, five patients were treated in a clinical trial using dacarbazine and interleukin 2. From three scintigraphically positive patients, two responded to the therapy, whereas the two negative patients did not respond. We hypothesize that only metastases respond to immunotherapies using the "T-cell growth factor" interleukin 2, if activated T lymphocytes recognize tumor tissue, possibly in a HLA dependent manner. LAK-cell scintigraphy might be a fast and economic method to analyse the individual properties and might have prognostic relevance for immunotherapies using IL-2.

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ANALYSIS OF THE T CELL RECEPTOR REPERTOIRE IN TUMOR-INFILTRATING LYMPHOCYTES OF CUTANEOUS MELANOMA. Robert Strohal, Ljudomir Paucz, Josef Friedl, Hubert Pehamberger and Georg Stingl. Div. of Cutaneous Immunobiology, Dept. of Dermatology I, Univ. of Vienna Medical School, Vienna, Austria.

Lymphocytes infiltrating solid tumors (= tumor infiltrating lymphocytes = TIL) can be propagated in-vitro with IL-2 and are then capable of lysing autologous tumor targets in a class I restricted manner. As the specificity of these $CD8^+$ T-cells is determined by the T-cell receptor (TCR) configuration, we decided to define the Vα repertoire of TIL in-situ using the reverse polymerase chain reaction (PCR). We found that TIL in 30 different surgical specimens of melanocytic proliferations (5 nevi, 25 neoplastic lesions) predominantly used 5 (Vα1, Vα7, Vα13, Vα15, Vα16) of the 18 Vα families described. All other Vα gene families were represented in less than 40% of the melanomas or were totally absent. In contrast T-cells within normal human tissue employed 15 (lymph node) and 10 (dermis/epidermis) different Vα gene families, respectively. Collectively it is tempting to speculate that the local immune response within malignant melanoma is driven by a restricted i.e. oligoclonal population of intralesional TIL. Moreover, this technology should be useful for further analysis of different inflammatory as well as malignant skin diseases.

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EFFECTS OF MELANOCYTE-STIMULATING HORMONE AND DIVALENT IONS ON THE ATTACHMENT OF HUMAN MELANOCYTES TO EXTRACELLULAR MATRIX PROTEINS.

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Attachment of melanocytes to extracellular matrix (ECM) may be important in regulating their differentiation and melanogenic response. We have investigated the effects of melanocyte stimulating hormone (MSH) and divalent ions on the attachment of human melanocytes. Attachment of cells to tissue culture wells incubated with saline, bovine serum albumin (BSA), laminin (LM) or fibronectin (FN) was quantitated by pre-labelling the cells with ^{3}H-thymidine or staining with crystal violet. Assays, in triplicate, were performed in serum-free medium. In the presence of Ca^{2+} (1mM or 1.8mM), human melanocytes attached to all substrata: better to LM than BSA but with a propensity for FN. Two melanocyte cultures were incubated with 10^{-9}M MSH for 72 hrs: both showed increased attachment to LM and FN post-treatment. The effect of divalent ions was investigated on one of these cultures. Assays were performed in Tris-saline with: no added divalent ions; 1mM $CaCl_2$ or 1mM Mg Cl_2. In the absence of MSH, these treatments had significantly different effects on cell attachment to LM ($p<0.001$) by ANOVA. In the presence of MSH, the treatments maintained their different effects: LM $p<0.01$, FN $p<0.005$. MSH was able to increase attachment to LM in the presence of Ca^{2+} and Mg^{2+} (both $p<0.05$) and to FN in the presence of Ca^{2+} ($p<0.05$) and Mg^{2+} ($p<0.01$). In conclusion, we have shown that MSH and divalent ions influence human melanocyte attachment and we suggest that they may have a role in controlling melanocyte behaviour.

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GENE EXPRESSION AND GROWTH MODULATORY PROPERTIES OF TRANSFORMING GROWTH FACTOR BETA 1 (TGF-ß1) IN NORMAL AND MALIGNANT MELANOCYTES IN VITRO.

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TGF-ß1, a potent immunosuppressive cytokine, has been reported to be produced by melanoma cells in vitro, and on the other hand to inhibit melanoma cell growth. In order to understand the role of TGF-ß1 in human melanoma, we examined the TGF-ß1 gene expression in a panel of melanoma cells and in cultured normal human melanocytes in vitro and assessed proliferation rates after addition of exogenous TGF-ß1 protein.

12 melanoma cell lines and 8 melanocyte strains have been examined. Total RNA has been isolated by the LiCl/ureum method. Nothern hybridisations have been performed using ^{32}P cDNA fragments of TGF-ß1 and c-myc labeled with the random primer method. For proliferation experiments cells have been grown for 6 days in 96-multiwell plates in the presence of 0-5 ng/ml TGF-ß1. Cell proliferation has been assessed with a fluorometric microassay using 4-methylubelliferyl-heptanoate as substrate.

Northern hybridisation with TGF-ß1 probe has shown TGF-ß1 mRNA expression in 6/12 melanoma cell lines examined. TGF-ß1 mRNA was detected in 12-0-tetradecanoylphorbol-acetate (TPA) -stimulated normal human melanocytes cultures (2/4) but not in TPA-free cultures (0/2). TGF-ß1 inhibited proliferation of serum stimulated melanoma cells (max. 0-28% inhibition at 0.5 ng/ml) and more potently that of normal melanocytes (max. 55-59% at 0.5 ng/ml). In contrast to epithelial cells, the inhibitory effect of TGF-ß1 on human melanocytes was not mediated via suppression of c-myc proto-oncogene expression, as c-myc mRNA levels were not decreased in comparison to controls 24 and 48h after TGF-ß1 addition. Also, treatement of cultured melanocytes with the PKC activator TPA completely reversed the growth inhibitory effect of TGF-ß1 on these cells. TPA, however, did not affect the TGF-ß1 binding on the surface of melanocytes as shown by FACS analysis using FITC-labeled TGF-ß1.

In summary, our results show that cultured malignant melanoma cells and TPA-stimulated normal human melanocytes express TGF-ß1 mRNA. Exogenously added TGF-ß1 inhibited more potent the proliferation of normal melanocytes than that of melanoma cells. The reversibility of TGF-ß1 induced growth inhibition by TPA, whereby not affecting binding of TGF-ß1 on melanocyte surface, suggests an involvement of PKC in the intracellular signalling pathway of this process. The decreased sensitivity of melanoma cells to TGF-ß1 in comparison to their normal counterparts may play a role in the growth of human melanoma.

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STABILITY OF COLLAGEN mRNA IN FIBROBLASTS SEEDED INTO THREE-DIMENSIONAL DERMAL EQUIVALENTS.

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We are interested in characterizing fibroblast biosynthetic activity employing culture conditions which closely resemble the in vivo environment of the human dermis. Three-dimensional matrices reconstituted from type I collagen and contracted by dermal fibroblasts, represent a useful in vitro model for the investigation of connective tissue diseases.

We studied one of the most striking processes displayed by this system, the down-regulation of collagen I mRNA levels to less than 5% of the original level as a function of time of contraction. As we could show by nuclear run-on transcription, this reduction results in part from decreased de novo mRNA synthesis. Analysis of mRNA half-life by in vivo pulse-labeling with ^{3}H-uridine showed that in contracting dermal equivalents, the stability of collagen I mRNA is reduced by about 50%. In fibroblasts derived from healthy donors, these two mechanisms operate in concert, contributing to the observed low steady-state levels of collagen I mRNA.

Scleroderma fibroblasts, characterized by excessive up-regulation of collagen I mRNA, were studied in the same in vitro model. In comparison to control cells, mRNA for collagen I remains stable in the cells for a longer duration, as shown by increased RNA half-lives, and may therefore be subject to more initiation events by the translation apparatus. Similar to control cells, there are differences in mRNA stability depending upon the culture system used (monolayer vs. 3D-matrix).

Further studies analyzing the de novo mRNA synthesis by nuclear run-on transcription will demonstrate the importance of altered de novo synthesis of collagen mRNA in scleroderma fibroblasts with respect to the pathologically altered collagen metabolism.

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ELASTASE SPECIFIC INHIBITORS DERIVED FROM PSORIATIC SCALES.

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Recently we have isolated and characterized the protease inhibitor elafin from psoriatic scales, which seems to be involved in the protection of the epidermis against proteolytic degradation. It is a specific and powerful inhibitor for neutrophil derived broad spectrum proteases, such as the elastin degrading serine proteases human leukocyte elastase (HLE) and proteinase 3. From psoriatic scales, which were pooled from approximately hundred psoriatic patients, six additional elastase specific inhibitors could be purified to homogeneity by sequential HPLC. Isoelectric focusing showed them all to have highly cationic isoelectric points above pH 9.6 and size exclusion HPLC revealed for all inhibitors the 5 to 10 kD range. All inhibitors showed inhibitory activity towards HLE ($K_i < 10^{-10}$M) comparable with the originally described elafin. N-terminal amino acid sequence analysis of four of these inhibitors revealed elafin related molecules showing n-terminal elongation (19, 6, 4 amino acids) or deletion (5 amino acids). Therefore the active center of elafin seems not to be located on the N-terminus of the elafin molecule. Further investigations are necessary to decide whether the heterogeneity of elafin is related to the origin from different tissues, the origin from different individuals, or the elafin catabolism of the epidermis. Elastase specific inhibitors apparently not related to elafin have not been observed.

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EPSTEIN-BARR VIRAL GENOME IN CUTANEOUS LESIONS FROM PATIENTS WITH MYCOSIS FUNGOIDES AND SESARY SYNDROMA.

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Epidermotropic cutaneous T cell lymphoma (CTCL)[Mycosis Fungoides (MF) or Sesary Syndroma (SS)], is a neoplasic disorder of CD4, 4B4 T cell. Although the cause of this proliferation is unknown, it has been speculated that chronic antigenic stimulation might contribuate to the development of CTCL. Epstein-Barr virus (EBV) has a well known tropism for B lymphocytes. However, recently antibodies against EBV antigens have been found in high frequency and concentration in patients with CTCL, suggesting that EBV antigenic stimulation might be one possible factor which contribuates to the development of CTCL.

In this work, we studied the presence of EBV genome and EBV protein in tumoral cells from 25 CTCL (18 MF and 7 SS). Twelve benign cutaneous inflammatory lesions with CD4 infiltrate were used as control.EBV DNA was detected by in situ hybridation on frozen sections with biotinylated Bam HI-W probe and EBV protein (EBNA) by indirect immunofluorescence technique with double labelling (CD4 - EBNA monoclonal antibodies) to definite the tumoral phenotype of EBNA positive cells. At the same time, the sera of patients and controls were examined for antibodies to EBV antigens (EA, VCA, EBNA) using immunofluorescence and ELISA technique.

EBV DNA and protein were absent in cutaneous lesions of controls. In situ hybridation, was positive in 12 cutaneous lesions of CTCL (1SS, 11MF) showing that 10 to 50% of the tumoral cells had EBV DNA. EBNA protein was detected in 4 lesions (2 to 5 % of cells), dual staining confirming the CD4 phenotype of the EBNA positive cells. The serum levels of EBNA IgG and VCA IgG antibodies were high compared to controls, however EA antibodies were normal and no IgM antibodies were detected eliminating an active disease.

The presence and expression of EBV genome in lesions of CTCL associated with a high level of EBNA IgG and VCA IgG antibodies suggests that EBV might serve as a possible stimulus for the proliferation of CD4 tumoral cells.It could be an additional oncogenic factor in the multistep process tumor progression acting in association or after a retrovirus.

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DETECTION OF MONOCLONAL LYMPHOID SUBPOPULATIONS IN CLINICAL SPECIMENS BY PCR AND CONFORMATIONAL POLYMORPHISMS OF cRNA MOLECULES.

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The detection of monoclonal lymphoid subpopulations is of considerable interest in the diagnosis of lymphoma. We describe a new strategy for the rapid detection of monoclonal lymphoid cells, which is based on the conformational polymorphism of RNA, which strictly depends on the nucleotide sequence of the individual molecule. Highly variable junctional sequences of rearranged TCR-gamma genes of DNA from paraffin-embedded lesions of mycosis fungoides (MF), of lesions with benign polyclonal lymphoid infiltrations, and of peripheral blood cells of patients with leukemia were amplified by PCR. After transcription into cRNA the products were separated by PAGE. Samples with only polyclonal cells resulted in a broad smear, representing multiple conformations of different cRNA molecules. However, samples of MF or leukemia yielded a specific pattern of several distinct bands, representing cRNA conformations of the junctional sequence of the clonal subpopulation. This rapid assay requires only minute amounts of DNA and may provide an alternative to the detection of clonal cell populations by conventional and laborious Southern blot analyses.

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UV-INDUCED EPIDERMAL MACROPHAGES DERIVE FROM A SMALL DERMAL MACROPHAGE SUBSET WHICH UNDERGOES MARKED EXPANSION AFTER UV INJURY. L. Meunier, A. Gonzalez-Ramos, L. Oberhelman, C. Hammerberg, and K.D. Cooper. Immunodermatology Unit, Dept. of Dermatology, Univ. of Mich., Ann Arbor, MI., and Department of Dermatology, University of Montpellier, Montpellier, France.

Antigen-presenting, suppressor cell-inducing, CD1a$^-$DR$^+$CD36$^+$ macrophages appear in the epidermis 3 days following ultraviolet B radiation (UVB) exposure of human skin. To determine the origin and lineage of this population, we compared their phenotype to dermal cell subsets utilizing triple color flow cytometric analysis. Epidermal cell (EC) and dermal cell (DC) suspensions were obtained from non exposed (C-EC, C-DC) and UVB irradiated sites (3 days after 4 MED) (UV-EC, UV-DC) of 18 healthy human volunteers. The vast majority of the HLA-DR$^+$ UV-EC (75 $\pm$ 5%) were contained within a distinct subset that lacked the dendritic antigen presenting cell markers CD1a and CD1c, but which expressed the macrophage integrins CD11b and CD11c and which expressed high levels of Fc gamma RII, CD36 and CD45. This population is not present in C-EC where Langerhans cells (LC) (CD1a$^+$ CD1c$^+$ CD11b$^-$ CD11c^{dim} Fc gamma RII$^+$ CD36$^-$ CD45$^+$) comprise 100% of DR$^+$ C-EC. LC only comprised 7 $\pm$ 3% of HLA-DR$^+$ UV-EC. HLA-DR$^+$ C-DC from normal dermis were heterogeneous and contained both dermal equivalents of LC (dLC:CD1a$^+$CD1c$^+$) and dermal macrophage subsets (CD1a$^-$, CD1c$^-$), as well as endothelial cells. Normal dermis contained only a very small subset (1.5 $\pm$ 0.5% of total C-DC and 8 $\pm$ 4% of DR$^+$ C-DC) that is analogous to the macrophages in UV-EC characterized as DR$^+$ CD1$^-$ CD11$^+$ CD36$^+$ Fc gamma RII$^+$. After UV exposure this distinct subset of dermal macrophages became greatly expanded to comprise 10 to 15 % of total UV-DC. dLC were decreased in UV-DC. In conclusion, triple marker flow cytometry of epidermal and dermal cell suspensions identifies the human UV-induced epidermal macrophage as originating from a DR$^+$ CD1a$^-$ CD1c$^-$ CD11b$^+$ CD36$^+$ Fc gamma RII$^+$ population that undergoes a marked expansion after UV injury.

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THE 23 KDA COLLAGEN TYPE I SPLITT PRODUCT MODULATES THE PROLIFERATION, MATRIX PROTEIN SYNTHESIS AND INTEGRIN EXPRESSION OF HUMAN DERMAL FIBROBLASTS. [1]Herrmann K, [1]Münzberger Ch, [2]Krieg T, [1]Haustein UF, Department's of Dermatology, [1]Leipzig Univ., [2]Köln Univ., FRG

The connective tissue metabolism of systemic sclerosis (SSc) is characterized by an overproduction and accumulation of matrix proteins, especially of collagen. In recent studies we could demonstrate a 6-fold elevated neutral proteolytic activity and a 2-3 fold increase of the lysosomal ß-galactosidase in the involved skin homogenate of patients with SSc. Additionally, we could show the potency of such proteases to degrade collagen after its deglycosylation by ß-galactosidase. Therefore, it was the purpose of the present study to investigate the influence of collagen type I splitt products on the function of cultured fibroblasts. As we could show the 23 kDa degradation product of collagen type I is able significantly to stimulate the collagen synthesis on the protein as well as steady state mRNA levels. The quantification of collagen type I and III by interrupted electrophoresis shows a coordinative up-regulation of both types of collagen. Concerning its influence on the fibronectin synthesis we could demonstrate no differences on the protein and mRNA levels. Beside its stimulatory effect on collagen synthesis this collagen splitt product enhances also the proliferation of fibroblasts. Preliminary experiments give us the evidence for a modulation of the expression of various integrins on the surface of cultured fibroblasts. The investigations on integrin mRNA expression are in progress.

Our studies suggest the hypothesis that such matrix protein splitt products may be able to maintain the fibrotic process by enhancing collagen production in self perpetuation.

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CHARACTERISATION OF εBP (IGE-BINDING PROTEIN), AN ENDOGENOUS β-GALACTOSIDE SPECIFIC SOLUBLE LECTIN EXPRESSED ON THE SURFACE OF HUMAN LANGERHANS CELLS. A Wollenberg 1, H de la Salle 2, D Hanau 2, F T Liu 4, E Kolodziejczyk 3, T Bieber 1, 1 Department of Dermatology, University of Munich, FRG; 2 Centre Regional de Transfusion Sanguine, Strasbourg, F; 3 NESTEC Research Center, Lausanne, CH; 4 Scripps Clinic and Research Foundation, La Jolla, CA, USA

The IgE-binding capacity of human epidermal Langerhans cells (LC) is due to different structures; surface expression the high affinity receptor for IgE (FcεRI) as well as the low affinity receptor for IgE (FcεRII/CD23) has recently been demonstrated. In the present study, we document the presence of a third IgE-binding structure on LC, the β-galactoside specific lectin εBP.

Using a polyclonal antibody (εBP1) raised against recombinant εBP, flow cytometric analysis of freshly isolated, unfixed epidermal cells revealed the presence of εBP on the surface membrane of LC, whereas KC remained negative. By immunohistological staining on cryosections of normal human skin, we found strong cytoplasmic staining in keratinocytes (KC) and in acinous cells of eccrine sweat glands. In contrast, LC exhibited a cytoplasmic as well as a weak membranous staining. Furthermore, only LC, but not KC, bound murine IgE, as well as human myeloma IgE, on their surface. The binding of murine IgE was specific to εBP, since it was inhibited by preincubation with εBP1, or lactose, or A-tetrasacharide, known to specifically interfere with the murine-IgE-binding sites of εBP. In contrast, human myeloma IgE was not blocked under these conditions. Hence, εBP contributes to the IgE-binding capacity of LC. Molecular mass estimation by SDS-PAGE and Immunoblot analysis of cell lysates from purified LC or from KC disclosed a protein with an apparent molecular weight of 30kD consistent with εBP. Finally, specific mRNA-transcripts for εBP were detected in KC and purified LC implying an active synthesis of εBP by both cell types.

We conclude that εBp is actively synthesized by LC, exhibited on their cell surface and contributes to their total IgE-binding capacity.

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GENERATION OF B CELL LINES PRODUCING HUMAN ANTIBODIES AGAINST SKIN ANTIGENS. E. Peyron*, F. Rousset°, P. Roche*, V. Frances°, J. Banchereau°, D. Schmitt*, J. Thivolet*, J.F. Nicolas*.
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° SCHERING-PLOUGH, Laboratory for Immunological Research, Dardilly, France.

In order to better characterize the target antigens in auto immune skin disorders, attempts were made to generate human antibodies to skin antigens. We used a newly described method allowing a better efficiency in the generation of human antibodies producing B cell lines. This method consisted of a combination of Epstein-Barr virus infection and CD40 mediated proliferation of B cells. Using this system, we raised B cell lines from 5 patients with bullous pemphigoid, bullous systemic lupus erythematosus and pemphigus vulgaris. B cell culture supernatants were tested by indirect IF on normal human skin and positive wells were sub-cloned by limit-dilution techniques. Among the 450 B cell lines obtained from the 5 patients, 6 produced antibodies to skin antigens with 3 distinct reactivities : epidermal basal cells (3/6), basement membrane zone (2/6), whole epidermis (1/6). We subsequently derived from one B cell line 2 B cell clones, stable over 6 months, producing human monoclonal antibodies directed to components of epidermal basal cells. The monoclonal antibodies were both IgG1 Lambda with usage of a VH4 light chain gene. These antibodies will allow us to characterize the target antigens.

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ERROR-PRONE DNA JOINING IN CELLS FROM PATIENTS WITH WERNER SYNDROME (ADULT PROGERIA). Cathrin E. Bauer, Thomas M. Rünger, Department of Dermatology, University of Würzburg, Germany.

Werner Syndrome (WS) is a rare autosomal recessive disorder characterized by premature aging in young adults and an increased cancer risk. Cells from these patients demonstrate a chromosomal instability with a high rate of unusually large deletions (PNAS 86; p.5893-7, 1989). Elucidation of the underlying molecular mechanism in this genomic instability syndrome might provide further understanding of the molecular pathogenesis of aging and carcinogenesis. We reported earlier that cells from patients with the cancer-prone chromosome breakage syndromes demonstrate a reduced and hypermutable joining of DNA ends. Now, we have studied the *in-vivo* DNA joining process in WS cells. In our *host cell ligation assay* we transfect linearized plasmid pZ189 into lymphoblasts from WS patients and normal donors and measure the amount of plasmid rejoined by the host cells by its ability to transform bacteria. A mutagenesis marker gene close to the joining site allows to screen for mutations induced during the joining process (deletions, insertions, point mutations or more complex mutations), as verified by previous DNA sequencing. This provides information about the fidelity of the joining process at the base sequence level. The lymphoblastoid cell lines WS101, UJ, and KY were a gift from G. Martin, Seattle, WA. We found that WS cells were as effective as normal cells in re-joining DNA ends: The ligation rate was 3.2 ± 0.9 % (n=8) in WS101, 4.0 ± 1.7 % (n=9) in UJ, 3.3 ± 1.4 % (n=3) in KY, and 3.4 ± 1.6 % (n=7) in the normal cell line GM3715. Mutation analysis of 211 rejoined plasmids revealed, that the three WS cell lines introduced 2.2 - 3.8 fold more mutations than the normal cell line ($p \leq 0.002$) while joining the DNA ends: The mutation rate was 97.1 ± 5.6 % in WS101, 69.4 ± 6.4 % in UJ, 57.0 ± 16.3 % in KY, as compared to 25.7 ± 1.1 % in GM3715. The deletion was the predominant mutation in all cell lines. This error-prone DNA joining might be responsible for the genomic instability in WS cells and related to the aging process and high cancer risk in WS patients.

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OVEREXPRESSION OF CELLULAR RETINOIC ACID-BINDING PROTEIN TYPE II (CRABP-II) AND DOWN REGULATION OF CRABP-I IN PSORIATIC SKIN.

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CRABPs might play physiological roles by controlling the intracellular level of free retinoic acid (RA). We developped a new technique, PAGE-Autoradioblotting that allows for the first time the analysis of CRABP-I and -II in tissue extracts. These proteins were analyzed in normal skin, lesional (LPS) and non lesional (NLPS) psoriatic skin.Their respective genes were also studied by RNA-blot and in situ hybridization. We show that CRABP-I and -II differ in the dissociation constant with RA (Kd of 16.6nM and 50nM, respectively). We demonstrate that CRABP-II corresponds to the CRABP previously studied in epidermal cells and whose level is dramatically increased by exogenous RA.

We found that CRABP-II levels were 6-fold higher in LPS, 2.5-fold in NLPS as compared to NS. CRABP-I was detected in NS at levels similar to CRABP-II, whereas it was poorly expressed in NLPS and LPS. CRABP-I gene were underexpressed in both LPS and NLPS as compared to NS. CRABP-II mRNA were grossly overexpressed in LPS and in NLPS, but without a similar relative increase of the protein in NLPS.

These results indicate: (i) that CRABP-I and -II have distinct roles; (ii) a switch to the expression of CRABP-II gene in psoriasis which results in high levels of the protein in LPS; (iii) that CRABP-I decrease might be correlated with an alteration of the differentiation programme.

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HUMAN INTERLEUKIN 10 IS CHEMOTACTIC FOR T LYMPHOCYTES. Tan Jinquan, Christian Grønhøj Larsen, Kristian Thestrup-Pedersen. Department for Dermatology, Marselisborg Hospital, 8000 Aarhus C., Denmark.

Human interleukin (IL) 10 has recently been described as a cytokine synthesis inhibitory factor for IL1, IL6, TNF-alpha following LPS or IFN-gamma stimulation (Fiorentina et al., J.E.M. 1989; 170: 2081), as a down-regulator of antigen-specific T cell proliferation (Malefyt et al., JEM 1991; 174: 915), and as growth cofactor for mast cells and certain T lymphocytes (Zlotnik et al., Cytokine 1991; 3: 366). We have studied its effect in the 48-well microchemotaxis chamber technique using human T lymphocytes from healthy donors through monocyte depletion and nylon wool filtration. Checkerboard analysis showed that human IL10 induced a concentration gradient response (chemotaxis) as opposed to chemokinesis with the highest chemotactic index (CI) of 4.48 +/- 0.91). The chemotactic activity was dosage dependent (0.1, 1, 10, 100, and 1000 units/ml) with maximal CI at 100 units/ml (CI = 2.63 +/- 1.14; N = 8). The activity was similar to IL8 (CI = 2.07 +/- 0.31; N = 6). Murine Il10 was not chemotactic towards human T lymphocytes (0.93 +/- 0.25; N = 6). IL10 did not induce human chemotaxis for neutrophil granulocytes (0.86 +/- 0.37; N = 1) or monocytes (0.89 +/- 0.22; N = 1). It remains to be shown which subset of T cells are attracted the most. Its significance in skin inflammation is as yet unknown.

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KERATINOCYTE-INDUCED MODULATION OF IL-6 PRODUCTION IN FIBROBLASTS. Maria Ponec, Ingeborg Boxman, Lucien Aarden#, and Clemens Löwik*, Department of Dermatology and of *Endocrinology, University Hospital Leiden, and #Central Laboratory for Bloodtransfusion Service, Amsterdam, The Netherlands.

Interleukin-6 (IL-6) is known to play an important role in immune response and tissue repair. Although epidermal keratinocytes and dermal fibroblasts have been shown to produce IL-6, it is not known whether modulation of IL-6 production occurs upon interaction of these cells, the situation which may occur upon skin injury or tumor invasion. The present study aimed to investigate this issue. For this purpose normal human keratinocytes (NHK) or squamous carcinoma cells (SCC-4) were cultured alone or together with human foreskin fibroblasts (HFF) or murine 3T3 cells. IL-6 levels were determined either by the B9 bioassay or by ELISA specific for human IL-6. Furthermore, for studies on modulation of IL-6 expression at the mRNA level, Northern blot analyses were performed with cDNA probes specific for either human or murine IL-6.

All cells tested produced IL-6; the amount secreted into the medium was comparable for human (HFF) and murine (3T3) fibroblasts and for SCC-4 cells (approx. 2×10^4 U IL-6/mg protein during the 6-day period), whereas the amount produced by NHK was clearly lower ($\pm 10^3$ U IL-6/mg protein). The IL-6 levels were markedly increased in supernatants of co-cultures of SCC-4 cells or NHK with lethally irradiated 3T3 or HFF (approximately 10 to 30 times). The use of cells of murine and human origin in combination with a bioassay (species independent) or ELISA (detecting only human IL-6) together with Northern blot analyses (using cDNA probes specific for either human or murine IL-6) allowed us to establish which cell type was responsible for the observed effects. The results obtained with SCC-4/3T3 and SCC-4/HFF co-cultures revealed that the fibroblasts were responsible for the increased IL-6 production. The observed increase at both protein and mRNA levels also occurred when HFF or 3T3 fibroblasts were cultured in the presence of conditioned media derived from SCC-4 cultures. These results suggest that the keratinocyte-induced increase of IL-6 production in fibroblasts may play an important regulatory role during the repair of injured tissue or tumor invasion.

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RAPID INDUCTION OF ENDOTHELIAL CELL ADHESION MOLECULES BY NEUROPEPTIDES IN VIVO CH Smith, JNWN Barker, TH Lee, DM MacDonald. Laboratory of Applied Dermatopathology and Department of Allergy & Allied Respiratory Disorders, UMDS (Guy's Hospital Campus), London, UK

Granule membrane protein 140 (GMP 140) and endothelial leukocyte adhesion molecule 1 (ELAM-1) are expressed on activated vascular endothelium (EC) and mediate recruitment of neutrophils into tissue. To explore the role of neuropeptides in the induction of these molecules a study of normal human skin following intradermal injection of two neuropeptides implicated in cutaneous disease, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) has been performed. Intradermal injections (25ul) of VIP (2uM), CGRP (2uM) and phosphate buffered saline were given to healthy volunteers at times 0, 15 minutes, 1, 4 and 8 hours (n=3 for each neuropeptide studied at each time point). Staining was achieved using standard immunoenzyme and immunofluorescence techniques applied to 5uM cryostat sections.

CGRP and VIP induced rapid translocation of GMP 140 from cytoplasmic Weibel-Palade granules within dermal EC to plasma membrane at 15 minutes, and maximal at 1 hour; at 4 and 8 hours, EC were depleted of GMP 140. Marked upregulation of ELAM-1 was observed for both VIP and CGRP at 4 hours. These changes in adhesion molecule expression correlated with the time course of a prominent neutrophil infiltrate. Increased numbers of neutrophils confined to the vessel lumen were present within 15 minutes; maximal infiltrate occurred at 8 hours. An increase in EBM11 (macrophage/monocyte) positive cells occurred at 8 hours. Constitutive EC ICAM-1 expression was unchanged following CGRP or VIP. These findings demonstrate induction of GMP 140 and ELAM-1 expression coincident with rapid neutrophil recruitment into skin following intradermal VIP and CGRP. VIP and CGRP are predominantly found in autonomic and sensory afferents fibres respectively; their release in response to various stimuli may thus regulate acute inflammatory events.

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SPECIFIC BINDING OF SUBSTANCE P IN NORMAL HUMAN KERATINOCYTES. Birgit von Restorff, Lajos Kemény, Günther Michel and Thomas Ruzicka. Dept. of Derm. Univ. of Munich, FRG

Recently, substance P (SP) was found to increase the proliferation and cytokine synthesis of mouse keratinocytes acting via specific binding sites. We were interested whether normal human keratinocytes possess specific binding sites for SP, which would mediate the putative growth stimulating activity.

Human keratinocyte cell cultures were established from newborn foreskin. For receptor studies, standard radioligand binding assays were performed with 125J-SP. The effect of SP on cell proliferation was determined by counting cell number and 3H-thymidine incorporation.

In radioligand binding assays, we could identify specific binding sites for SP on cultured normal human keratinocytes. The analysis of binding data revealed single class of binding sites with a KD of 8 nM and Bmax of 46000 sites per cell. However, we failed to detect any significant growth promoting activity of SP in concentrations which should enable an optimal receptor occupancy.

Thus, cultured normal human keratinocytes possess specific SP binding sites which are likely to be involved in the effects of this neuropeptide in skin. However, the biologic effects other than direct growth stimulation need further investigations.

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Tumor necrosis factor alpha (TNFα) induces loss of immunogenic peptides from MHC class II molecules and thus interrupts the antigen presenting function of epidermal Langerhans cells. F. Koch, E. Kämpgen, B. Trockenbacher, Ch. Heufler, Peter Stöger, N. Romani, and G. Schuler, Department of Dermatology, University of Innsbruck, Innsbruck, Austria.

Recent in vitro and in vivo experiments suggest that TNFα disturbs the antigen presenting function of epidermal Langerhans cells (LC), and contributes to the well known immunosuppressive effect of low-dose UVB irradiation. We have now studied the effect of TNFα on murine LC in more detail.

Freshly isolated BALB/c LC actively processed soluble protein antigens into immunogenic peptide/MHC complexes as measured by peptide-specific class II-restricted T cell-hybridomas. LC lost their processing capacity upon culture (up to 72h) in GM-CSF, but retained the immunogenic peptides. Following culture in TNFα, however, LC progressively lost their capacity to stimulate the T-cell hybridomas. The addition of peptide to LC cultured in TNFα restored this capacity, indicating that LC had lost immunogenic peptides from their MHC class II molecules. This explains two seemingly contradictory and puzzling findings: 1) LC cultured in GM-CSF are extremely potent in the allo-MLR, whereas LC cultured in TNFα are inactive (immunogenic peptides needed for allostimulatory activity are lost!) 2) LC cultured in GM-CSF or TNFα are equally active in supporting the mitogenic response of T cells to anti-CD3 (which depends on the presence of Fc receptors, but not of peptide/MHC complexes!)

We have unravelled a novel immunomodulatory mechanism of TNF alpha, which will add to the understanding and possibly the therapy of (skin) diseases.

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EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF TUMOUR NECROSIS FACTOR-ALPHA (TNF-α) IN NORMAL HUMAN SKIN. RW Groves, E Ross, JNWN Barker, DM MacDonald. Laboratory of Applied Dermatopathology, St John's Institute of Dermatology, UMDS, Guy's Campus, Guy's Hospital, London, U.K.

TNF-α is a potent immunoregulatory cytokine produced by macrophages, keratinocytes, mast cells and Langerhans cells. To determine its potential role in inflammatory skin disease we have studied the effects of recombinant human (rHu) TNF-α in normal human skin.

rHuTNFα 100U (Gp A, n=3), 5000U (Gp B, n=3) or 100U daily for 5 days (Gp C, n=3) was injected intradermally to one buttock. Diluent was injected contralaterally as control. Biopsies were taken at 6 hrs (Gps A and B) or 6 hrs after the final injection (Gp C). Cryostat sections were labelled immunohistochemically with antibodies to inflammatory cell subsets and endothelial adhesion molecules. H & E sections were used to evaluate neutrophil infiltration. A marked inflammatory cell infiltrate occured: in Gps A and B this was predominantly neutrophilic (Gp A: 64±6; Gp B: 203±51 PMN/5hpf) whereas in Gp C most cells were Leu-1+ lymphocytes (PMN: 26±3; Leu-1+: 180±22/5hpf). In all cases an increase in EBM-11+ cells occurred, most markedly in Gp B (418±26; control 171±7/5hpf, $p<0.02$). There was a small but significant decrease in OKT6+ epidermal cells (Gp A: 7.1±0.4; control: 9.2±0.5/unit length epidermis, $p<0.05$). ELAM-1+, ICAM-1+ and VCAM-1+ positive vessels were increased in all groups; many interstitial dermal dendritic cells were also induced to express ICAM-1 and VCAM-1. Keratinocyte ICAM-1 was increased in Gps A and B but was less prominent in Gp C.

These findings suggest that TNF-α is a potent modulator of cutaneous immune function and may induce both an acute and chronic inflammatory cell infiltrate. TNF-α may be important not only in acute cutaneous inflammation but also in chronic inflammatory dermatoses.

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MEASUREMENT OF DNA-REPAIR IN CELLS FROM PATIENTS WITH SKIN CANCER-PRONE HEREDITARY DISEASES USING A PLASMID HOST CELL REACTIVATION ASSAY, T. M. Rünger, K. M. Möller, A. A. Hartmann, Department of Dermatology, University of Würzburg, Germany.

UV-damaged plasmid vectors have been used before to assess the ability of transformed human cells to repair uv-induced DNA-photoproducts. Now, we have established such a *host cell reactivation assay* as a diagnostic tool for the evaluation of DNA repair in patients. We irradiated the 5 kbp plasmid pRSVcat with 0.1 - 1.0 J/cm^2 UV-B and transfected it by means of electroporation into lymphoblastoid cells or lymphocytes from the following patients: normal donors (n=5), xeroderma pigmentosum complementation group A (XP-A; n=1), xeroderma pigmentosum-variant (XP-V; n=2), dysplastic nevus syndrome (DNS; n=3), basal cell nevus syndrome (BCNS; n=1), and Werner syndrome (WS; n=2). Cell lines were established in our laboratory or purchased from the cell bank in Camden, NJ, USA. Within the host cells the chloramphenicol-acetyltransferase-gene (cat) contained in the plasmid can only be expressed when the DNA damage is repaired by host cell enzymes. Thus, the CAT-activity in cell extracts after two days, as determined by a novel one-vial procedure, reflects the DNA repair capability of the host cells. We could confirm the DNA repair defect in XP-A cells by a 12- and 30-fold reduced CAT-activity after transfection of plasmids irradiated with 0.5 and 1.0 J/cm^2. The CAT-activity following transfection of irradiated plasmids into XP-V cell lines was 1.3 to 2.2- and 2.3 to 3.9-fold reduced at 0.5 and 1.0 J/cm^2, suggesting a slightly impaired excision repair in XP-V. The repair of uv-induced DNA damage was low-normal in DNS and normal in BCNS and WS. The presented *host cell reactivation assay* is a suitable tool for the assessment of DNA repair. It can be used in routine diagnostics of DNA repair defects in patients, as well as for further research on DNA damage processing in skin cancer prone hereditary diseases. It can easily be modified to investigate the processing of other kinds of DNA damage by transfecting differently damaged plasmids.

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DIFFERENTIAL EXPRESSION AND REGULATION OF THE *SPR* GENE FAMILY IN PRIMARY KERATINOCYTES AND SQUAMOUS CELL CARCINOMAS. Frans P. Lohman, Susan Gibbs, Maja Ponec*, Piet van de Putte and Claude Backendorf, Departments of Molecular Genetics and *Dermatology, University of Leiden, Leiden, The Netherlands.

Spr genes constitute a family of related genes which were first isolated in our department as genes which are strongly induced after irradiation of primary cultures of human keratinocytes with ultraviolet light. The same family was found to respond aswell to treatment with the tumor promotor TPA. In primary keratinocyte cultures, the different family members are, however, differently affected by these two external agents. Whereas *spr2* is induced by both UV and TPA, the expression of *spr1* is only affected by TPA and *spr3* does solely respond to UV irradiation. Besides of being responsive to environmental agents, *spr* genes were found to be induced during the normal process of keratinocyte differentiation both *in vivo* and *in vitro*. Regulation during differentiation seems to be stage specific since basal cells show no (or very low) expression, supra-basal cells high expression and in further differentiated cells expression decreases again. A very clear link between *spr* expression and differentiation is aswell found in a series of squamous cell carcinoma (SCC) cell lines. Here a correlation exists between basal levels of *spr* expression (non-induced by external agents) and the residual ability of these cells to differentiate in standard Ca^{2+} medium (Ker > SCC12F2 > SCC13 > SCC15 > SCC12B2 > SCC4). In primary keratinocytes induction by UV irradiation is a late response as it reaches a maximum after 24 to 48 hours after treatment. A similar late response is observed in SCC12F2 cells (intermediate differentiation potential). However in SCC4 cells (low differentiation potential) the UV reponse reaches a maximum much earlier (3 to 6 hours after irradiation). Additionally, the *spr1* gene appears to be inducible in SCC4 cells in contrast to the situation in primary keratinocytes (see above). Apparently, in cells with a high potential to differentiate (e.g. primary keratinocytes) an activity is present which counteracts the early induction of *spr* genes after UV irradiation. This activity seems to affect the expression of the different *spr* genes differently (cf. *spr1* and *spr2*). At present we have started to identify the molecular factors (cis-elements in the promoter region and the corresponding transcription factors) involved in the regulation of these genes. This analysis should result in a molecular description of the processes described above.

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LYSIS OF SKIN TUMORS BY ACTIVATED DENDRITIC EPIDERMAL T CELLS (DETC). **Marek J. Kaminski, Ponciano D. Cruz, Jr., John S. Gerometta, Paul R. Bergstresser and Akira Takashima, Department of Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas, USA.**

Based on our previous finding that γδ T cell receptor-bearing DETC can lyse the natural killer cell-sensitive YAC-1 target, we examined the capacity of DETC to lyse tumor cell lines derived from mouse skin. Two assays were employed: 51Cr release and visual assessment of target cell lysis. Each of the three long-term DETC lines tested lysed Pam 212 keratinocytes, SV40-transformed endothelial cells, two fibrosarcoma and five melanoma lines. Optimal lysis measured by 51Cr release was achieved after 18 h incubation at an effector to target ratio of 50:1. Spleen cells, by contrast, were markedly less efficient at lysing endothelial and fibrosarcoma lines, and more importantly, these control cells were incapable of lysing Pam 212 and melanoma lines. Lytic activity was not seen in freshly isolated DETC, but was detected in anti-CD3-activated DETC after 12 d culture in the presence of exogenous IL-2 (5 U/ml). By visual exam, binding of DETC to tumor cells was present as early as 15 min, loci of lytic activity by 4 h, and complete disappearance of tumor cells by 18 h. DETC did not lyse normal keratinocytes, indicating that cytotoxicity was tumor-directed. These findings suggest that activated DETC can serve as cytotoxic cells for skin cancers.

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TRANSCRIPTIONAL REGULATION OF RETROVIRAL LTR: POSSIBLE ROLE OF RETROVIRUSES IN HORMONAL CARCINOGENESIS. M. Dahm[1,5], P.E. Carreira[2], M. Matucci-Cerinic[4], S. Harris[4], T. Lotti[5] and J.S. Norris[1], Depts. of Medicine, Medical University of SC, Charleston, SC [1]; University of Texas, San Antonio Tx; Hospital 12 de Octobre, Madrid[2]; Depts. of Medicine IV [4]and Dermatology [5], University of Florence.

The reproductive tract of Syrian Hamsters has a well defined susceptibility for sex steroid induced carcinogenesis. From an estrogen/androgen-induced leiomyosarcoma, arising in the ductus deferens, the DDT_1 MF-2 cell line was derived (Cancer Res 1965 **25:**141). This cell line contains receptors for both androgens and glucocorticoids, and its proliferation is differentially sensitive to these classes of steroids: Androgens dramatically stimulate wild type cell growth and augment the level of intracellular androgen receptors, whereas glucocorticoids inhibit growth and prevent androgen receptor level augmentation. Previously, using a v-sis probe of simian sarcoma virus, a 4.2 kb cDNA was cloned out of DDT_1 tumor cells. Subsequently it was shown that the putative protein product of the v-sis oncogene was able to regulate growth of these cells by an autocrine pathway (Biophys Res Comm 1984 **122:** 124). The striking findings of rearrangements in the 3' portion of the v-sis cDNA clone and signs of recombination in the POL gene between SSV and MuLV have given rise to the hypothesis that hormones might induce tumors by regulating viral long terminal repeats (LTR) inducing infection, activation of oncogenes (v-sis) or retrotransposition, resulting in insertional mutagenesis and neoplasia. To begin to investigate this hypothesis we tested the hormonal responsiveness of the SSV-like LTR via transfection experiments and CAT (chloramphenicol acetyl transferase) assays. Briefly: The 3' LTR was isolated, cloned into a CAT reporter gene in both orientations and transfected into DDT_1 Clone 4 cell line via lipofection. CAT Assays performed after stimulation with steroids showed the retroviral LTR differentially regulated with CAT Activity resulting significantly increased by androgens, estrogens and retinoic acid, while glucocorticoids modest inhibitory. This data sustain our hypothesis and suggest that regulation of viral LTR's and associated genes should be investigated. Further questions arise from this observation in particular concerning the role that hormones may play in the expression of retroviral sequences and their consequent involvement in the pathogenesis of either cutaneous (melanoma) or systemic (prostate, breast) (J of Virology 1987 **61**: 2059) cancer in humans.

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T-CELL RECEPTOR Vß-FAMILY USAGE IN PRIMARY CUTANEOUS AND PRIMARY NODAL T-CELL LYMPHOMA. A.H. Preesman¶*, H.Z. Hu*, M.G.J. Tilanus†, R. Reitsma*, D.F. van Wichen*, H.J. Schuurman*, W.A. van Vloten¶ and R.A. de Weger*, Departments of Pathology (division Histo- and Cytochemistry & Electromicroscopy)*, Dermatology¶ and Molecular Genetics†, University Hospital Utrecht, the Netherlands.

To evaluate whether the expression of T-cell receptor (TCR) Vß families in 8 cases of T-cell neoplasia took place in a preferential manner, we analysed 4 cases of mycosis fungoides (MF), the most common form of primary cutaneous T-cell non-Hodgkin lymphoma (NHL) and 4 cases of primary nodal T-cell NHL. The usage of Vß-families in T-cell populations was investigated on mRNA which was transcribed to cDNA using a Cß-primer and reverse transcriptase. Subsequently, the specific usage of the families was analysed by polymerase chain reaction (PCR) using combinations of the selected Cß-oligonucleotide primer and one of the family-specific Vß primers. Peripheral blood lymphocytes of 4 healthy volunteers and 1 "reactive" lymph node were used as controls and expressed all 20 Vß-families. In T-cell lines, with restricted Vß expression, and in 3 advanced MF-lesions only one or two Vß-families were expressed at the mRNA level. In an early MF-lesion this clonal expression was absent: several Vß-families were expressed with a weak intensity. This may indicate either a polyclonal origin of MF, or that too few clonal neoplastic cells were present in the tissue specimen.

In the 4 nodal T-cell NHL, only one family could be clearly distinguished, whereas some of the other Vß-families showed only a weak expression. These latter families represent the reactive T-cell component in the T-cell NHL.

All T-cell lines, 3 cases of advanced MF and 3 cases of nodal T-cell NHL showed a re-arrangement of the TCR ß chain on DNA level. Both in T-cell NHL and MF there was no preferential expression of a particular Vß-family. There was a good correlation between PCR data and the expression of Vß-family protein products observed by immunohistochemistry on tissue sections of the T-cell neoplasia.

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CHARACTERIZATION OF NORMAL HUMAN CD7 NEGATIVE T LYMPHOCYTES

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CD7 is one of the earliest antigens of the T cell lineage and, therefore, is a useful marker for the diagnosis of T cell acute lymphoblastic leukemia. Several studies have shown that monoclonal anti-CD7 antibodies react with most human thymocytes and more than 85% of peripheral blood lymphocytes. However, CD7 neg. T cells have not been characterized yet. Our studies have shown that 8.0 + 3 % of circulating T cells of normal donors (n=38) expressed the CD4+CD7-CD45RO+CD45RA- phenotype. In contrast, human cord blood lymphocytes did not contain CD7 neg. T cells. Freshly isolated CD7 neg. T cells showed significant lower proliferation in response to IL-2, IL-4, IL-7, mitogens, and OKT3 as compared to CD7 pos. T cells. Furthermore, we have investigated the stability of CD7 expression vs. non-expression in CD7 pos. and neg. long-term cultured alloantigen-specific T helper cell clones. No phenotype change occured during a cultured period of more than 6 months. CD7 neg. T cell clones showed no cytoplasmic CD7 expression and absent CD7 gene transcription. Moreover, CD7 neg. T cell clones remained neg. for CD7 expression after stimulation with PHA, OKT3, and phorbolester for different periods of time. Recent studies indicate that CD7 neg. T cells may display skin tropism and may represent normal counterparts of malignant Sèzary cells. Studies are underway to further analyze whether absent CD7 expression is the result of late T helper cell differentiation or whether there is a distinct CD7 neg. lineage.

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ANTIBODIES TO RETROVIRAL PROTEINS IN CUTANEOUS AUTOIMMUNE PATIENTS IN RELATION TO CLINICAL DISEASE AND NUCLEAR AUTOANTIBODIES. Eija Stephansson, Pekka Kurki, and Annamari Ranki, Depts. of Dermatology and Serobacteriology, University of Helsinki, and Dept. of Dermatology, Karolinska Institute, Stockholm.

We have previously reported on antibodies cross-reacting with human retroviral proteins (mainly gag), in patients with cutaneous autoimmune diseases (Ranki et al., NEJM, 1987). We now present long-term follow-up studies on 45 patients with various immune connective tissue diseases and on 19 individuals with false positive Cardiolipin (CBFP). Antibodies to HTLV-I, HIV and HIV-nef and antibodies to nuclear antigens, RNP and histones were demonstrated by Western blot. Cross-absorption studies were performed both with viral and cellular antigens. Altogether 47% of the sera reacted with one or several of the retroviral proteins. HIV gag (core) proteins p24 and p55 were recognized most often. Antibodies cross-reacting with retroviral proteins (ARP) were found in 67% of 21 patients with SLE, in 75% of 8 patients with mixed connective tissue disease (MCTD) and in 63% of 8 patients with discoid LE. DLE patients typically reacted with HIV p55, and cross-reactivity to HTLV-I was mostly seen in the MCTD group. However, none of the 7 patients with SCLE and only 5 of 19 individuals with CBFP were ARP$^+$. No clear association of any nuclear or RNP antibody to the retroviral cross-reactivity was detected. During the follow-up, two ARP$^+$ individuals with CBFP developed 4 or more ARA criteria. Discoid LE lesions were more common (67% vs.21%) in the ARP$^+$ patients in all groups. Also, cutaneous vasculitis was seen in 27% of ARP$^+$ patients but only in 9% of ARP$^-$ patients. Most striking was the occurrence of recurrent infections (mostly pneumonia or bronchitis) in 43% of ARP$^+$ patients compared to 12% in the ARP$^-$ group. Pleuritis was seen twice as often in the ARP$^+$ group as in the ARP$^-$ group. Our results also showed that ARP$^+$ patients with discoid LE were more prone to develop a systemic disease.

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RECENT DEVELOPMENTS IN PEMPHIGOID (HERPES) GESTATIONIS. M.M. Black*, J.K.Shornick *, C.M. Artlett **, D.C. Briggs **, K.I.Welsh **, M. Garvey *, and S.E. Kelly ***. * Institute of Dermatology, UMDS, London. ** Tissue Typing Laboratory, Guy's Hospital. London. *** Royal Infirmary,Edinburgh.

Pemphigoid gestationis (PG) is a rare auto-immune bullous disease associated with pregnancy or the puerperium, characterised by a dense linear deposition of complement along the cutaneous basement membrane zone. Based on a total of 74 immunofluorescence proven cases of PG we have further evaluated the fetal risks, auto-immune associations and both MCH class II and MCH class III profiles. The obstetric history of 74 women with PG were analysed by comparing involved to uninvolved pregnancies, plotting birth weight versus gestational age on standard growth curves. A clear tendency for premature delivery was associated with PG, but only a slight tendency for small for dates infants in PG pregancies. Auto immune profiles to organ specific auto-antibodies were performed in 72 patients with PG. An increased frequency of Graves' disease and PG was confirmed (11%) and a higher incidence of gastric parietal cell antibodies was also found. Restriction fragment length polymorphism of DQA, and sequence specific oligo nucleotide probing of DQB and DRB1 were performed in 48 cases of PG. The results confirmed a definite association between PG and DRB1 * 0301 (DR3) and DRB1 * 040X (DR4). Class III antigens C4A, C4B1, C3 and factor B were investigated in 38 cases of PG. 90% of PG cases carried a C4 null allele. No statistical association was seen with C3 or factor B alleles. The C4 null allele may be the primary genetic association in PG and could possibly explain the impaired handling of circulating immune complexes and auto-antibodies.

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IN VITRO RELEASE OF GM-CSF BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN REACTION TO BORRELIA BURGDORFERI (BB) AS A DIAGNOSTIC MARKER FOR BB-INFECTION M. Owslanowski[1]; C. Schempp[1]; R. Lange[2]; H. Bocklage[3]; F.M. Schaart[1]; R. Soehnchen[1]; C.E. Orfanos[1] and H. Gollnick[1] [1]Department of Dermatology, University Medical Center Steglitz, and [2]Department of Neurology, University Medical Center Rudolf Virchow, The Free University of Berlin, FRG. [3]Institute for Hygiene and Microbiology, University of Würzburg, Germany.

In order to investigate cellular parameters as diagnostic makers in Bb-infection peripheral blood mononuclear cells (PBMC) of erythema chronicum migrans (ECM)-, acrodermatitis chronica atrophicans (ACA)-, morphaea-, lichen sclerosus et atrophicans (LSA)-patients and controls were isolated and 10^6 cells/ml were cultured for 2 and 7 days after stimulation with ultrasonicated Bb-organisms or control-stimuli. After two days supernatants were harvested and levels of granulocyte-macrophage-colony stimulating factor (GM-CSF) were estimated by an in house - Elisa; after 6 days cell proliferation was determined by ^{3}H-thymidine-incorporation. We found that 1. all seropositive probands (ECM, n=4 and ACA, n=4) showed a high GM-CSF-secretion (8.5 ± 4.7 U/ml) and lymphocyte transformation (LT) (SI 7.6 ± 3.0) in response to Bb-ultrasonicate whereas none of the controls (n=14) responded (GM-CSF: 0.4 ± 0.7 U/ml; SI: 0.9 ± 0.45). 2. the seronegative ECM-patients (n=2) could also be stimulated by Bb. (GM-CSF: 10.4 U/ml; SI: 8.8) 3. all six morphaea-patients were seronegative (Elisa and western blot). Two of them showed a strong reaction to Bb-ultrasonicate (GM-CSF: 25.8 U/ml; SI: 6.2). Moreover, investigation of the affected skin by PCR and immunhistology performed in one case revealed Bb specific DNA and flagellin antigen in the lesion. 4. LSA-patients (n=5) were seronegative. A cellular immune response to Bb was found in 2 cases. (GM-CSF: 9,5 U/ml; SI: 7.9) 5. after antibiotic therapy the investigated seropositive patients (ACA, n=3) as well as a seronegative mophaea-patient showed no longer a significant GM-CSF-secretion (11.0 ± 6 versus 0.5 ± 0.4 U/ml) and only a reduced lymphocyte proliferation (SI 8.2 ± 3.2 versus 2.5 ± 0.5) in response to Bb-antigen although IgG antibodies against Bb were found in the serum of these treated ACA-patients.

We conclude that cellular parameters such as antigen-specific LT and, in particular, GM-CSF-secretion are beneficial in the diagnosis of Bb-associated diseases as well as in the evaluation of therapeutic results especially in seronegative cases.

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EVIDENCE FOR LOCALIZED SPIROCHETAL INFECTION IN MORPHEA WITH ABSENCE OF SYSTEMIC ANTIBODY RESPONSE. Schempp C[1], Bocklage H[3], Lange R[2], Owsianowski M[1], Gollnick H[1], Orfanos CE[1]. 1Department of Dermatology, University Medical Center Steglitz, and [2]Department of Neurology , University Medical Center Rudolf Virchow, The Free University of Berlin, FRG. [3]Institute for Hygiene and Microbiology, University of Würzburg, Germany.

A spirochetal origin of morphaea is still contraversally discussed. It was demonstrated that some types of morphaea can be associated with a Borrelia burgdorferi infection, confirmed by enzyme linked immunosorbent assay (ELISA) and western blot analysis (Aberer et al. 1991). In contrast others found no correlation between Borrelia burgdorferi-infection and localized scleroderma in 26 patients (Lupoli et al. 1991).

We present a 16-year-old female with an abdominal skin affection which was clinically diagnosed as morphaea (circumscribed scleroderma). The skin lesion developped after a tick bite 10 years ago. Histologic examination of lesional skin showed characteristics of scleroderma and necrobiosis lipoidica as well. Warthin-Starry staining revealed no spirochetes on investigated sections. IgM and IgG antibodies directed against Borrelia burgdorferi could not be detected in the peripheral blood neither by an enzyme linked immunosorbent assay nor by Western blot analysis. Serologic tests for Treponema pallidum were negative. Investigation of the affected skin by a nested Polymerase-chain-reaction (PCR) using primer pairs specific for the flagellin gene (fla) revealed Borrelia specific DNA. Furthermore, a strong and reproducible lymphocyte proliferation in reaction to ultrasonicated Borrelia burgdorferi organisms and, even stronger, to Treponema denticola was found. These results were confirmed by increased amounts of granulocyte-macrophage-colony-stimulating factor (GM-CSF) secreted by the patients peripheral blood mononuclear cells (PBMC) in reaction to Borrelia burgdorferi and especially to Treponema denticola in vitro. A rapid progressive parodontitis (RPP) which is supposed to be caused by Treponema denticola was excluded in our patient.

In this case PCR analysis and cellular immune response indicate a possible spirochetal etiology of morphaea although no antibodies against spirochetal antigens could be detected.

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STRUCTURAL FEATURES AND SITE OF EXPRESSION OF A NEW MURINE 65kD AND 48kD"HAIR-RELATED" KERATIN PAIR,ASSOCIATED WITH PARAKERATOTIC EPITHELIAL DIFFERENTIATION.

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In the course of studies on local keratin phenotypes in mouse epidermis,we have identified a new 65kD and 48kD keratin pair.In skin,these keratins are only expressed in suprabasal cells of the parakeratinizing tail scale epidermis.Another site of expression is found in differentiating cells of the likewise parakeratinizing posterior unit of the filiform tongue papillae.The causal relationship between the expression of the 65kD/48kD keratins and the formation of a non-pathological parakeratosis is shown by the complete suppression of the mRNAs of the two keratins during retinoic acid mediated orthokeratotic conversion of tail scale epidermis.Apart from tail scale epidermis and the posterior unit of the filiform papillae,the two new keratins are,however,also co-expressed with"hard"α-keratins in suprabulbar cells of hair follicles and the central core unit of the filiform papillae.

The non-α-helical domains of the 65kD/48kD keratins are rich in cysteine and proline residues and lack the typical subdomains into which the head and tail portions of "soft" keratins can usually be divided. The structural resemblance of the two new keratins to "hard"α-keratins is confirmed by comparative flexibility calculations for their non-α-helical domains.Phylogenetic investigations clearly reveal that the 65kD and 48kD keratins have evolved together with "hard" α -keratins. During evolution,they have,however,diverged from these and constitute an independent pair of "hair-related" keratins.

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MECHANISMS OF HAIR GROWTH CONTROL: THE ROLE OF SUBSTANCE P. Ralf Paus, Thomas Heinzelmann, Jörg-Andreas Böttge, Ruth Pliet, Jens Furkert*, Klaus Fechner*, Beate M. Czarnetzki, Dpt. of Dermatology, University Hospital R. Virchow, Freie Universität Berlin, D-1 Berlin 65, *Academy of Sciences, D-1136 Berlin

Clinically, stressful life events can be associated with the onset of hair loss (e.g. in alopecia areata). Yet, the neural mechanisms of hair growth control are entirely obscure. In the light of our observation that mast cell degranulation may be an important component of hair growth induction (Maurer et al., this meeting), we have begun to study the role of substance P (SP) in hair growth regulation: this neuropeptide stimulates mast cell degranulation, is abundant in sensory nerves of the skin, and is released during stress responses. Using the C 57 Bl-6 mouse model for hair research (Br J Derm 122:777, 124:415) we injected capsaicin or SP i.c. into mice in the telogen phase of the hair cycle. While capsaicin, which is thought to deplete sensory neurons of neuropeptides, induced anagen hair growth at the site of injection, SP alone or controls did not. However, when SP was administered in the form of slow-release polymer-complexes implanted s.c., there was significant induction of anagen follicles in telogen skin after 12 days. In organ culture of telogen mouse skin, SP significantly stimulated the proliferation of epidermal and hair bulb keratinocytes as compared to controls at doses as low as 0.01 nM ($p < 0.01$), while SP inhibited hair bulb keratinocyte proliferation at 1uM ($p < 0.05$). Histologically, degranulation of dermal mast cells could be seen in those skin punches incubated with SP. In vivo, mouse skin exhibits significant, hair cycle-dependent fluctuations of the SP concentration: we found 446 fmol SP/g tissue weight in telogen skin, but 894 in early anagen and 260 fmol SP/g in late anagen skin (measured by RIA, HCl extraction, 60% recovery, $p<0.05$). During anagen development in mice, the controlled release of SP from the perifollicular nerve plexus may serve regulatory functions by directly stimulating keratinocyte proliferation or by triggering the release of growth-modulatory mast cell products. Growth-inhibitory perifollicular concentrations of SP may contribute to the development of stress-related alopecia in man.

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ADHERENS JUNCTIONS AND DESMOSOMES: TWO TYPES OF CELL-CELL ADHESION STRUCTURE IN EPIDERMIS. Ed J. O'Keefe, Hans W. Kaiser, Volker C. Hinz, Friedhelm Achenbach and Hans W. Kreysel, University of North Carolina, Chapel Hill, NC, USA; and University of Bonn, Bonn, Germany.

The adherens junction, or zonula adhaerens, is a vinculin-containing actin microfilament-associated structure extensively studied in gut epithelium. Previous studies have demonstrated adherens junctions and focal adhesions in epidermal keratinocytes in vitro. In order to test for the presence of these structures in epidermis, we used antibodies to vinculin in immunofluorescence and immunoblotting experiments, and we examined epidermis for adherens junctions by electron microscopy. Antibodies to vinculin which demonstrated characteristic structures in vitro and immunoprecipitated a 130 kDa protein from extracts of cultured keratinocytes stained both the periphery of cells in the epidermis and also the dermal-epidermal junction. Immunoblots performed with this antibody showed that a 130 kDa protein was present in extracts of epidermis. Electron microscopy showed in addition to desmosomes regions of apposition of cell membranes consistent with adherens junctions. α-actinin and actin, other proteins of adherens junctions were also present in epidermis. Adherens junctions which may be involved in wound healing and morphogenesis, may complement the structurally important desmosome in maintaining the integrity of the epidermis.

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CHARACTERIZATION OF ANNEXINS IN HUMAN SKIN AND RECONSTITUTED EPIDERMIS. N.Basset-Séguin*, J.F. Culard*, B.Calas **, F.Martin **, J.J.Guilhou *. * Laboratoire de Dermatologie Moléculaire, **UPR-CNRS 8402-INSERM U249, Montpellier, France.

Annexins represent a widespread family of Ca $^{2+}$ dependent phospholipid binding proteins. Although their precise functions are still unknown, they probably play an important role in cell regulation as they are major substrates for various growth receptor kinases. Using three different antisera raised against annexin II, annexin V and against a synthetic peptide covering the consensus sequence of all annexins, we have characterized the presence of these proteins in normal human skin (NHS) and in reconstituted skin (RS) in culture by SDS-page electrophoresis and immunoblot analysis. Identity of these proteins as annexins was further confirmed by their ability to bind phosphilipids in the presence of calcium and by determination of their isoelectric points by 2-di gel electrophoresis. According to these criteria, annexin I, II, V, VI and VII were detected in NHS and all, except VI and VII could be seen in RS in vitro. The cellular distribution of annexin in NHS was determined by immunofluorescence with anti-annexin II and anti-annexin V antibodies. Labelling with both antibodies was observed predominantly at the cell membrane. This staining was seen in all epidermal layers but decreased in the granular layer and disappeared in the phospholipid-deprived stratum corneum. Specificity of these observations was confirmed by the absence of staining using pre-immune sera or after absorption of the antibodies with their corresponding antigens. In conclusion, NHS contains a variety of annexins. The RS culture system should further serve as a model for studies of the expression and regulation of these proteins in human skin.

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HIGHLY AGGRESSIVE HUMAN MELANOMA CELL LINES REORGANIZE COLLAGEN I FIBRILS BY UTILIZING THE $\alpha_2\beta_1$ COLLAGEN RECEPTOR OF INTEGRINS. C.E. Klein, D. Dressel, Th. Steinmeyer, R. Schirmbeck, C. Mauch*, B. Eckes*, Th. Krieg*, and L. Weber, Department of Dermatology, University of Ulm, and University of Cologne*, F.R.G.

The ability of remodelling connective tissue may provide substantial advantages for tumor cells during tissue invasion. We have tested human melanoma cell lines for their ability to reorganize and contract collagen I gels and investigated the role of integrin receptors. 9 human melanoma cell lines and 4 normal melanocyte cultures were tested. 5 melanoma cell lines contracted the gels whereas melanocyte cultures did not. 2 highly aggressive melanoma lines (MV3 and BLM) most efficiently contracted gels. In these cells, synthesis of 7 integrin receptors was assessed after metabolic labeling and subsequent immunoprecipitation with specific mAb's. Only synthesis of $\alpha_2\beta_1$ was upregulated in cells (4- to 8-fold) when seeded into the gels. Moreover, function blocking anti-α_2- in conjunction with anti-β_1-chain mAb's completely inhibited gel contraction. It was remarkable that the inhibition lasted for several days after a single administration of mAb's. To further investigate this, we compared the synthesis of $\alpha_2\beta_1$ in gel cultures with or without inhibiting mAb's. The presence of function blocking mAb's prevented the upregulation of $\alpha_2\beta_1$-synthesis. This was confirmed by western blotting. Our results show that interference by mAb's with this receptor which favors the formation of metastases (Chan et al., Science 251: 1600-02, 1991) results in a long lasting downregulation of its expression.

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REGULATION OF HUMAN KERATINOCYTE TUMOR NECROSIS FACTOR RECEPTOR (TNFR) EXPRESSION AND FUNCTION BY ULTRAVIOLET (UV) LIGHT: EVIDENCE FOR AN AUTOCRINE LOOP. U Trefzer, M Grewe, M Brockhaus*, H Lötscher*, F Parlow, A Budnik, A Kapp, E Schöpf, TA Luger*, and J Krutmann, Dept. of Dermatology, Univ. of Freiburg and *Münster, F.R.G.; +PRCB, F. Hoffmann-LaRoche LTD, Basel, Switzerland.

Ultraviolet radiation previously was found to induce binding of endogenous TNFα to the 55 kd TNFR on the surface of cultured human keratinocytes (KC), indicating that UV-induced TNFα may exert autocrine regulatory effects. In order to test this hypothesis, TNFR surface (binding of ^{125}I-TNFα and/or anti-TNFR mAb) as well as mRNA (55 kd TNFR cDNA) expression was studied in long term cultured, normal human KC. Binding of UV-induced TNFα to KC TNFR occurred in a time dependent manner with a maximum at 6 h post irradiation, and was followed by a biphasic modulation of KC TNFR expression. Accordingly, 12 h post irradiation, KC TNFR surface and mRNA expression was markedly reduced. In contrast, 24-48 h post irradiation, TNFR reexpression could be observed. Most remarkably, at this later time point, TNFR expression exceeded that observed in KC prior to UV exposure. UV-induced TNFR expression was inhibitable by cycloheximide and associated with a marked upregulation of TNFR mRNA expression. Most important, this biphasic modulation could be mimicked by stimulating unirradiated KC with rh TNFα. To determine whether UV-induced TNFα mediates autocrine effects besides modulation of KC TNFR expression, the role of endogenous TNFα in UV-induced KC prostaglandin synthesis was assessed. Accordingly, UVB-irradiation induced a 3-4 fold increase in KC PGE_2 synthesis, which could be mimicked by stimulating unirradiated cells with rh TNFα. The time course of TNFα binding and increased PGE_2 synthesis was consistent with a cause-effect relationship, and UV-induced PGE_2 synthesis was markedly reduced, if irradiated KC were cultured in the presence of an anti-TNFα Ab. These studies demonstrate that binding of endogenous TNFα to KC TNFR leads to autocrine regulatory events including the biphasic modulation of TNFR expression and the induction of PGE_2 synthesis.

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STRUCTURE AND FUNKTION OF THE HUMAN CD58 GENE. M. Roux (1,2), M. Albert-Wolf (2), T.Dengler (2), J. Hoffmann (2), S. Meuer (2) and R. Wallich (2), Department of Dermatology, University of Cologne, FRG (1) and Department of Applied Immunology, German Cancer Research Center, FRG (2)

The CD58 molecule (formerly LFA3) is a glycosylated cell surface structure which is broadly distributed in the human and shows marked upregulation in inflammatory diseases. CD58 serves as a ligand for the T cell restricted CD2 molecule. The interaction between both molecules is implicated in antigen restricted primary T cell responses as well as in T cell triggering via the "alternative pathway of T cell activation".

Since the CD2/CD58 interaction represents an important step in the control of T cell activation during inflammation we elucidated the structure-function relationship in CD58 binding to CD2. Both cosmid and lambda clones hybridising to a CD58 cDNA probe were isolated from human genomic libraries. Exons were identified by hybridisation to the CD58 cDNA. The exon-intron organisation was determined by DNA sequencing using a series of synthetic oligonucleotides. The canonical 5'- and 3'-splice donor and acceptor sites were determined. A leader exon contains the 5' non-translated part and most of the nucleotides coding for the CD58 signal peptide. The extracellular part of the CD58 protein is encoded by two separate exons. By comparing the CD58 and CD2 genes, the exon boundaries are situated within similar regions (<20bp) of the genes, supporting the suggestion that CD2 and CD58 could be derived from a common anchestral gene by gene duplication.

Individual exons coding for the extracellular part of the protein were fused to synthetic leader sequences and expressed in a baculovirus system. These proteins were used to study their functional properties in CD2 - CD58 interaction in human T cell activation. Furthermore, the splicing mechanism between a transmembrane and a phosphatidylinositol linked form of CD58 is described.

The mechanisms of regulation of CD58 gene expression could provide important insights into the control of the inflammatory response in many disease processes.

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IN VITRO REGULATION OF THE EXPRESSION OF THE HIGH AFFINITY RECEPTOR FOR IGE (FcεRI) ON HUMAN LANGERHANS CELLS Thomas BIEBER*, Henri de la SALLE+, John HAKIMI§, Richard CHIZZONITE§, Daniel HANAU+, Corinne de la SALLE+, Andreas WOLLENBERG*. *Dept of Dermatology, University of Munich, Germany; +C.R.T.S. Strasbourg, France; §Hoffman-Laroche, New Jersey, USA.

Very recently, we demonstrated that human Langerhans (LC) cells express the high affinity receptor for IgE FcεRI. We fully characterized its tetrameric structure (α, β, 2γ) and showed its identity with the FcεRI present on basophils and mast cells. With regard to the profound phenotypic and functional alterations of cultured LC (cLC), we now investigated the fate of the FcεRI expression on LC in vitro. Freshly isolated LC (fLC) and cLC (36 h) were first compared for their ability to bind human myeloma IgE protein by double labeling and flow cytometric analysis. Thus, while approximately 50% of fLC bind IgE, this property was nearly completely lost by cLC. Similar results were obtained by staining with anti-FcεRI antibodies. Most important, using specific primers for the α- and the γ-chain and reverse transcriptase polymerase chain reaction (RT-PCR) technique, we observed a complete disappearance of the transcripts for both subunits within the first 8 h of culture. We then tested whether cytokines may affect the disappearance of FcεRI on LC. The addition of GM-CSF, IL-1 or TNF-α to the culture medium did not affect this phenomenon. However, upon the addition of IL-3, cLC sustained their IgE-binding capacity. Similarly, anti-FcεRIα reactivity was maintained in this condition. Finally, transcripts for the α- and to a lesser extent for the γ-chain were also further detectable by RT-PCR. Our results clearly indicate that FcεRI expression on LC is lost in culture and can therefore not be considered as a stable mark for human LC. However, this expression is regulated by IL-3 known to exert a similar effect on basophils, mast cells and their precusors.

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PARTIAL cDNA SEQUENCE OF THE NEW BASEMENT MEMBRANE PROTEIN NICEIN (BM-600) REVEALS DISCRETE HOMOLOGIES WITH HUMAN LAMININ.

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Isolation by affinity chromatography of the 600 kDa new basement membrane component Niceine (formely BM-600), allowed preparation of antibodies directed against each of the three protein subunits. Using these antibodies for the screening of a human epidermal λgt11 cDNA library, a 1404-bp cDNA clone (PCR1.3) encoding for 468 amino acids was obtained, which is related to the 100 kDa Nicein subunit. By northern-blot, PCR1.3 hybridizes three major keratinocyte mRNA (3.7, 4.8, 5.3 kb) showing either a multigene family or alternative splices of a precursor transcript. In situ hybridization of PCR1.3 to metaphase chromosomes establishes the 100 kDa Nicein subunit gene located in the q25-q31 region of chromosome 1.

Computer analysis of the PCR1.3-deduced protein sequence confirms that Nicein is an original protein that presents discrete homologies with human laminin, an ubiquitous basement membrane component. The most similar sequences are in the laminin B2 chain. As for the B2 chain, the 468 amino acid part of the 100 kDa Nicein subunit possesses cystein-rich EGF-like repeats immediately followed, towards the C terminal part, by an α–helical domain. Since the homologies are quite high for the cystein-rich repeats (71-76 % identity) and elevated for the α-helical secondary structure (25-50 % identity), it could be hypothesized that Nicein and laminin are related molecules. The confirmation that Nicein could be an iso-laminin will be of interest to better understand the cell-adhesion and basement membrane cohesion defects that occurs in lethal junctional epidermolysis bullosa, a genodermatosis where Nicein is specifically impaired.

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MCP-1 mRNA EXPRESSION IN BASAL KERATINOCYTES AND PDGF mRNA EXPRESSION IN DERMAL PAPILLAE OF PSORIATIC LESIONS.

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In order to better understand psoriasis (PS) pathogenesis, it is necessary to analyze the factors responsible for the abnormal cutaneous topobiology of infiltrating leukocytes, in particular the juxtaposition of proliferating basal keratinocytes (KC) to immunocompetent cells.Suprisingly, our immunohistological analysis of psoriatic lesions (n=11) revealed that as oppossed to neutrophils and T cells, basically mobil dermal macrophages/dendrocytes (DM/DD) are almost exclusively restricted to the dermal compartment with only few cells penetrating the basal membran. They are encountered in the papillary dermis and are frequently arranged along the basement membran of the cubbing rete ridges, thus having the closest spatial proximity to proliferating KC. Recently, the potent monocyte chemoattractant protein-1 (MCP-1) was isolated and sequenced. Since T-cells, fibroblasts, endothelial cells, smooth muscle cells and KC have the potential to secrete MCP-1 in vitro and all cell types are integral parts in lesions of PS we have performed in situ hybridizations studies with ^{35}S-labelled MCP-1 RNA probes to detect and localize MCP-1 mRNA expression. Using MCP-1 antisense but not sense probes, we consistenly detected highly abundant silver grain precipitates along the basal epidermal layer of the tips of the rete ridges and to a lesser extend in cells residing in the papillary dermis. In contrast, all suprabasal epidermal cells and (except few single cells) the resident and passenger cells in the dermal compartment were quiescent. Thus, the highest concentration of MCP-1 is most probably achieved at the dermal-epidermal junction and may explain the particular distribution of DM/DD in PS. It suggests that a dialogue between proliferating KC and DM/DD, mediated by MCP-1, is one of the dominating regulatory events in PS pathogenesis.
Furthermore, platelet derived growth factor B (PDGF-B) mRNA expression in the papillae, as detected by in situ hybridization, could account for the induction of MCP-1 in this area as well as for the growth of papillary vessels. Our in situ microanatomic analysis may explain the accumulation of DM/DD and angiogenesis in PS.

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DEMONSTRATION OF HISTAMINE RELEASING AUTOANTIBODIES AGAINST THE HIGH AFFINITY IGE RECEPTOR (FcεRI) IN CHRONIC URTICARIA.

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Histamine releasing anti-IgE autoantibodies have been demonstrated previously in chronic urticaria (CU)[1]. Here we describe anti-FcεRI autoantibodies in 4 CU patients who gave wheal and flare responses to intradermal autologous serum injection (wheal volumes:18.1-97.6mm^3). All 4 patients' sera provoked histamine release (mean 15%) from basophils in mixed leukocyte preparations (BL) from a healthy donor who has a low level of serum IgE (<20IU/ml). The histamine release was abolished (0%) by passive sensitisation of BL by myeloma IgE and restored (17%) following removal of IgE with lactic acid treatment. A monoclonal antibody (6F7), which recognises the IgE binding region of FcεRI, gave the same pattern of histamine release. Protein G affinity purified IgG from the 4 patients' sera gave histamine release with dilution curves similar to those of whole sera and 6F7. Dose related inhibition of histamine release by the 4 patients sera and 6F7 was obtained using the recombinant extracellular domain of the FcεRI α-subunit. These results demonstrated that IgG autoantibodies directed against the IgE binding region of FcεRI are present in some CU patients and are functionally active in triggering histamine release from basophils and probably cutaneous mast cells.

1.C.E.H.Grattan, et al 1991, Clin.Exp.Allergy, in press.

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P53 MUTATIONS IN HUMAN EPITHELIAL SKIN TUMORS.

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P53 mutations are among the most frequent genomic alterations found in human cancers. Their incidence is quite high in squamous-type oesophagus tumors. Using a combination of PCR and SSCP (single strand conformation polymophism) technics, we searched for P53 gene mutations in 38 epithelial skin carcinomas. These tumors comprised 19 basal cell carcinomas (BCC), 13 squamous cell carcinomas (SCC) and 6 bowen diseases (BwD). Genomic DNAs were amplified by PCR using a set of primer pairs covering exons 2, 5 ,6 ,7 , 8 and 9 of the P53 gene. When shifted bands were observed, a new round of PCR-SSCP was realised for confirmation. Confirmed bands were then excised and sequenced.

Results showed an elevated incidence (48%) of P53 gene mutation in BCCs contrasting with a low incidence (8%) or no mutation observed in SCC and BwD respectively. No preferred transition (3 C -> T and 1 T -> C) or transversion (3 C -> G and 1 G -> T) were observed, excluding a dominant mode of mutation in these cancers. Most mutations were seen in the evolutionary conserved domain of the P53 gene with two hot mutational spots in exons 5 and 8.

These data indicates that among the various tumors samples tested, dramatic difference in P53 gene abnormalities are seen between BCCs and SCCs supporting independant pathogenetic pathways for these tumors.

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TREATMENT OF PSORIASIS VULGARIS WITH A CHIMERIC MONOCLONAL CD4 ANTIBODY.

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Predominance of T cells in psoriatic lesions, HLA-association and response to Cyclosporin A indicate that Psoriasis vulgaris (PV) is an immunologically mediated disorder. Based on these observations we employed a chimeric monoclonal CD4 antibody (mAb), cM-T412 (Centocor Inc., Malvern, PA, USA), for the treatment of three patients with generalized pustular psoriasis (GPP, n=2) or severe chronic PV (n=1). The patients received 1 or 2 one week-cycles of i.v. infusions of a total of 120mg to 400mg mAb. All patients responded to therapy with a clear improvement of symptoms. 2 patient (1 GPP, 1 chronic psoriasis) had a long lasting remission, while the other GPP patient showed a relapse immediately after termination of mAb-infusion. The response was reflected by a decrease in C reactive serum protein, respectively. Despite a decrease in the CD4/CD8 ratio, no clinical signs of immunosuppression became obvious. Immunohistological studies demonstrated a loss of IL-2 receptor- and HLA-DR-expression from the inflammatory infiltrate. Collectively, the use of CD4 mAb as a new auspicious therapeutic approach for PV stresses the (auto-?)immune nature of this eminently chronic disorder.

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REDUCTION OF SUNBURN CELL FORMATION BY INJECTION OF A TNFα-ANTISERUM.

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The mechanisms involved in the formation of sunburn cells (SC) found in UV-exposed epidermis are not clear. Since keratinocytes are known to release tumor necrosis factor α (TNFα) upon UV-exposure and to express TNFα receptors the role of TNFα in the pathogenesis of SC was studied. C3H/HeN mice were exposed to UVB-light, immediately thereafter a TNFα-antiserum (anti-TNF) was injected into the irradiated skin, 24 hrs later biopsies were taken and SC counted. While injection of PBS or a rabbit controlserum did not affect the number of SC, application of anti-TNF resulted in a significant reduction of SC. This effect was dose dependent and maximally pronounced upon immediate injection of anti-TNF after UV-treatment. When anti-TNF was preincubated with recombinant murine TNFα and then injected into UV-exposed skin, the SC reducing effect was not observed. In contrast, injection of TNFα alone into normal skin was not able to induce SC. Since SC are regarded as apoptotic cells induced by DNA fragmentation, DNA was extracted from murine skin and run on a 1% agarose gel. While DNA from normal skin did not enter the gel due to DNA integrity, DNA from UV-exposed skin revealed the typical ladder pattern due to DNA fragmentation. This pattern was less pronounced in DNA from UV-exposed mice treated with anti-TNF. In summary, the present study demonstrates that TNFα plays an important role in the pathogenesis of SC. However, since TNFα alone is not able to induce SC, TNFα seems to be necessary, but not sufficient for the formation of SC.

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THE TYPE VII COLLAGEN GENE: INTRON-EXON ARRANGEMENTS, CHROMOSOMAL MAPPING, AND EVIDENCE FOR RESTRICTION FRAGMENT LENGTH POLYMORPHISM. L.C. Chung-Honet, A. M. Christiano, A. Hovnanian, M. Ryynänen, and J. Uitto, Jefferson Medical College, Phila., PA; and INSERM U91, Creteil, France.

Recent genetic linkage analyses have identified COL7A1 as the candidate gene in families with the dystrophic forms of EB, with a highly informative PvuII RFLP in COL7A1. Towards elucidation of the underlying mutations, we have initiated characterization of normal COL7A1, which we have recently mapped to 3p21. A human λFIX genomic DNA library was screened with a type VII collagen cDNA, K-131. Seven overlapping clones, varying from 13 to 23 kb in size, were isolated and characterized. The region of genomic DNA corresponding to the 1.9 kb cDNA was 5.3 kb in size, predicting an intron-exon ratio of 1.8:1. Nucleotide sequencing of these clones, in comparison to cDNA sequences, indicated that K-131 consisted of 21 separate exons. The 5' non-collagenous domain consisted of 10 exons with the average size of 130 bp, while the 11 exons encoding the collagenous 3' segment were only 53 bp on the average. The intron-exon ratios in the non-collagenous and collagenous segments were 1.2:1 and 3:1, respectively. Exon-intron borders demonstrated characteristic splice site consensus motifs. Most of the exons in the collagenous region were multiples of 9 bp, and began with a complete Gly codon. Unlike most previously characterized collagen genes, the junction between the distinct non- collagenous/collagenous segments was encoded by two discrete exons. These results indicate that this segment of COL7A1 is unusually compact and the collagenous and non-collagenous domains demonstrate distinct and unusual exon-intron organizations.

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MELANOCYTIC NEVUS (MN) MATURATION BEGINS WITH LOSS OF VLA 2 AND 3 INTEGRIN ADHESION MOLECULE (IAM) EXPRESSION AND CONTINUES WITH DE-NOVO EXPRESSION OF VLA 1 AND 4 BINDING TO EXTRACELLULAR MATRIX (ECM) COMPONENTS. C. Garbe, U. Homann, D. Schuppan, C.E. Orfanos. Department of Dermatology and Department of Gastroenterology, Steglitz University Medical Center, The Free University of Berlin, Berlin, Germany

The development of MN from junctional to compound and dermal types is still controversial and some authors interprete this process as "dropping off" and "maturation" whereas others postulate different origins of epidermal and dermal nevus cells. The present study was planned to examine the relationship of nevus cells to ECM components and to investigate the modulation of IAM during the process of downmigration of nevus cells. Additionally, the modulation of IAM expression in relationship to ECM-components and to growth was studied in MN cell culture.

Snap frozen tissue sections of 4 junctional, 15 compound and 10 dermal MN were stained with monoclonal antibodies against IAM CDw49 a-f (VLA 1-6), CD51 (aV-chain), CD29 (ß-chain), CD61 (ß3-chain) (APAAP-staining) and with polyclonal antibodies against the ECM-proteins collagen I, III, IV, V, VI, laminin and fibronectin (immunofluorescence). Nevus cell cultures were grown in MCDB-153 supplemented with insulin, transferin, BPE, bFGF and 1 % FCS. Cultured nevus cells were grown on culture dishes coated with different ECM-components and on uncoated control dishes and proliferation (^{3}H thymidine) and IAM expression (FACS analysis) were assessed.

ECM components were absent in junctional MN portions. They were in part expressed in the upper dermal and intensively stained in the deep dermal MN portions. Predominantly the basal membrane proteins collagen IV, laminin and fibronectin were detected. They encapsulated MN cells mostly in a pericellular and sometimes in a septal pattern. IAM expression differed between junctional and dermal MN portions with presence of VLA 2 and 3 in junctional and of VLA 1 and 4 in deep dermal portions. IAM expression in MN cell culture was dependend on cell culture growth phases with enhanced expression of VLA-2 and VLA-3 in proliferating cells and of VLA-1 in resting cells, whereas the contact to ECM proteins hardly modulated IAM.

A dynamic concept of nevus maturation is suggested: (a) IAM VLA 2 and VLA 3 are expressed in proliferating nevus cells. (b) These molecules mediate cell-cell and cell-basal membrane contacts and thereby support nesting. (c) VLA 2 and 3 decrease in resting MN cells and thus nesting is reversed and downmigration is supported. (d) VLA 1 and 4 expression is enhanced in resting MN cells. (e) These molecules bind to the encapsulating ECM proteins (laminin, collagen) and thereby contribute to the downmigration of MN cells.

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Cytokine gene expression in murine epidermal Langerhans cells: MIP-1α is a major product and inhibits the proliferation of keratinocyte stem cells

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Epidermal Langerhans cells (LC) are considered precursors of the dendritic cells in the draining lymph nodes *in vivo*. *In vitro* freshly isolated murine LC develop into potent immunostimulatory dendritic cells upon short term culture.

By using reverse transcriptase PCR analysis for screening (17 cytokines) followed by Northern blotting for selected analysis we have determined the hitherto unknown cytokine gene expression profile of LC. Of the 4 cytokines that were expressed by LC [Macrophage inflammatory proteins (MIP)-1α and -2, Interleukin-1 β and - 6] MIP-1α proved to be the most interesting one. LC were the only source of MIP-1α amongst epidermal cells. MIP-1α mRNA was downregulated in cultured LC. We tested the effect of MIP-1α on keratinocytes in a standard clonogenic assay (culture in low calcium medium) as MIP-1α functions as a stem cell inhibitor in the bone marrow. MIP-1α was found to act as a reversible, cell-cycle specific inhibitor of keratinocyte proliferation.

Our findings suggest that LC in addition to immune and inflammatory functions regulate keratinocyte growth. LC may play an important role in diseases like psoriasis, where LC appear abnormal in that they resemble cultured LC, and may no longer produce MIP-1α and inhibit epidermal stem cells.

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PMA-INDUCED UPREGULATION BY HUMAN KERATINOCYTES (KC) OF THE B7 ACTIVATION ANTIGEN IS ASSOCIATED WITH THEIR CAPACITY TO TRIGGER T-CELL PROLIFERATION. M.Augustin[1], P.S.Linsley[2], J.A.Ledbetter[2], C.Prinz[1], A.Kapp[1], E.Schöpf[1] J.Krutmann[1] and J.C.Simon[1], [1]Dept. Dermatol., Freiburg, FRG, [2]Oncogen, Seattle, WA, USA.

In previous studies human KC treated with PMA were shown to induce proliferation of allogeneic and autologous T cells via a MHC/Ag-independent mechanism that was mediated, in part, by PMA-induced ICAM-1 on KC. Recently, the interaction of the B7 activation Ag on APC with its ligand CD28 on T cells has been shown to deliver an activation signal(s) distinct from that transduced via interaction of MHC/Ag with the T cell receptor. These findings led us to determine whether B7-transduced signals play a role in T cell proliferation induced by PMA-treated KC. We first examined B7 expression on KC expanded from normal human skin, by staining with the anti-B7 mAb BB-1 and then analyzing by flow cytometry. Untreated KC expressed little B7 on their surface. By contrast, PMA-treated KC clearly demonstrated a dose-dependent upregulation of B7 expression. We also tested the effects of the mAb BB-1 on the capacity of PMA-treated KC to stimulate proliferation of allogeneic peripheral blood mononuclear cells (PBMC). The anti-B7 mAb BB-1 inhibited PBMC proliferation induced by PMA-treated KC in a specific and dose-dependent manner. We conclude that PMA-treated KC upregulate B7, which may contribute to their capacity to induce MHC-independent T cell proliferation. We speculate that "activated" KC in inflammatory skin diseases such as psoriasis may stimulate T cell proliferation through a similar mechanism.

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CLONING AND EXPRESSION OF HUMAN HAIR KERATIN GENES. Paul E. Bowden, Sandra Hainey, Anne P. Withers, *Gillian Parker and *Malcolm B. Hodgins, Depts. of Biological Sciences, Dundee University, and *Dermatology, Glasgow University, UK.

The expression of low sulphur hair keratins is a marker of hair growth and differentiation. Sheep wool and mouse hair keratins have been cloned and are related to the IF multigene family (type II basic and type I acidic keratins) but do form a distinct sub group. We now report the cloning of PCR amplified DNA fragments of human hair-specific keratin genes.

Synthetic oligonucleotides were made on the basis of published sequences and used to prime PCR amplification of hair keratin sequences from human genomic DNA. PCR fragments were cloned into a bacterial vector (pGEM-3Z) and sequenced. Labelled riboprobes from cloned PCR fragments were used for *in situ* hybridization on human skin. Labelled hair keratin DNA probes were used to screen a human cosmid library and clones containing hair keratin genes were characterized by southern hybridization and sequencing.

Three human hair-specific keratin clones were obtained from the PCR reactions. Two were type I hair keratins (hHaKA1-b2, 386 bp; hHaKA1-XH1, 1214 bp) and both covered C-terminal and 3'nc regions, while the larger included some 2B helix and one intron. They were 64.5% homologous to the mouse equivalent (82% in C-terminus and 90% in 2B-Helix). The other clone (hHaKB2-1, 829 bp) covered the 2B-helix and C-terminal regions of a type II hair keratin, showing 95% homology to the mouse, and contained two introns. *In situ* hybridization with the hHaKA1-b2 probe on human skin sections showed a reaction with the precortical cells of the hair follicle and no reaction with the epidermis or outer root sheath.

A human cosmid clone (40 Kb) was isolated with the hHaKB2-1 probe and preliminary characterization has indicated this clone contains at least one type II hair-specific keratin gene. These human hair-specific keratin gene clones represent valuable tools for the study of hair growth regulation and differentiation.

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THE ANONYMOUS MARKER D3A2 CLOSE TO THE COL7A1 LOCUS IS TIGHTLY LINKED TO THREE BRITISH FAMILIES WITH AUTOSOMAL DOMINANT DYSTROPHIC EPIDERMOLYSIS BULLOSA. L. Al-Imara[1], A.J.Richards[1], R.A.J.Eady[2], I.M.Leigh[3], M.Farrall[1], F.M.Pope[1] [1]MRC Clinical Research Centre, Northwick Park Hospital, Harrow; [2]Dept of Experimental Pathology, Institute of Dermatology, St.Thomas' Hospital, London; [3]Institute of Cancer Research Skin Tumour Laboratory, London.

Recently the gene for collagen VII together with its partial gene sequence and chromosomal location have been identified[1]. A single Finnish family shows tight linkage to a collagen VII RFLP[2]. As COL7A1 markers were unavailable we studied the segregation of anonymous markers close to the collagen VII locus at 3p21[3]. We chose D3S2 which was PCR amplifiable and could be conveniently tested in blood, hair and saliva samples. With the collaboration of the Institute of Dermatology Epidermolysis bullosa clinic and the Dystrophic Epidermolysis Bullosa Research Association (DEBRA) we have ascertained 3 large affected British families containing 67 affected and 101 unaffected individuals. The disorder segregated with the +allele with a combined lod score for the D3S2 marker of 6.75 at θ=0. This provides over a million to one probability of linkage and both confirms the American findings and indicates that collagen VII mutations are causative in these three families.

1. Parente MG et al (1991) Proc Natl Acad Sci, 83 6931-8
2. Riyynanen M et al (1991) Amer J Hum Genet 49, 797-803
3. Ganly P et al (1991) Nucl Acids Res 19, 3761.

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ADDUCIN: AN ERYTHROCYTE MEMBRANE SKELETON PROTEIN, WHICH PROMOTES BINDING OF SPECTRIN TO ACTIN AND IS REGULATED BY CALMODULIN AND PROTEINKINASE C, IS PRESENT IN EPIDERMIS. Hans W. Kaiser, Winfried Ness, Ed J. O'Keefe, Andrea Balcerkiewicz, Hans W. Kreysel, Departement of Dermatology, University of Bonn, Germany, Departement of Dermatology, University of North Carolina, Chapel Hill, N.C.

A dense protein filament matrix required for maintenance of the biconcave shape of erythrocytes and known as the membrane skeleton contains spectrin and actin, proteins which have also been detected in keratinocytes and fibroblasts. Although spectrin and actin bind to each other with low affinity, adducin, a newly described protein of the erythrocyte membrane skeleton, stabilizes the association of spectrin and actin and promotes binding of additional spectrin molecules to the ternary complex. This function of adducin is regulated by calmodulin and proteinkinase C. Immunoblot analysis with affinity purified anti-erythrocyte adducin antibodies showed that human epidermis contains an adducin-like protein of 103 kDa, consistent with the α-subunit of the heterodimeric erythrocyte protein. Immunofluorescence of human epidermis showed intense staining in basal cells and moderate staining of suprabasal layers. In cultured human keratinocytes adducin was diffuse in the cytoplasm at 0.1 mM Ca^{++} but was localized at cell-cell contact sites at 1.0 mM Ca^{++}. These studies indicate that regulatory microfilament-associated proteins are present in keratinocytes and may be involved in formation of intercellular junctions.

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PSORIATIC KERATINOCYTES LACK INTRINSIC HYPERPROLIFERATION OR ALTERED DIFFERENTIATION, BUT DISPLAY INCREASED SUSCEPTIBILITY TO GROWTH STIMULATION BY IL 6 AND PSORIATIC SERUM, WHICH IS INHIBITED BY ANTI-IL 6 ANTIBODY AND ANTIPSORIATIC DRUGS. Michael Detmar, Constantin E. Orfanos, Dept. of Dermatology, Steglitz Medical Center, The Free University of Berlin, Berlin, Germany.

In the present study we investigated whether psoriatic keratinocytes (KCs) are characterized by an intrinsically altered differentiation or hyperproliferative behaviour, or by functional abnormalities under stimulation by distinct cytokines, using isolated KCs cultured in serum-free medium.

6mm punch biopsies were obtained from lesional (PP) and nonlesional (PN) skin of 8 chronic plaque-type psoriatics and from normal skin (NN) of 8 age- and sex-matched healthy volunteers, and KCs were cultured using serum-free KGM (Clonetics). 2nd passage KCs were treated with the recombinant human cytokines interleukin 1-alpha (IL 1a), IL 1b, IL 2, IL 3, IL 6, GM-CSF, IFN-gamma, TNF-alpha (all 1-1000 U/ml), EGF or TGF-alpha (0.1-10 ng/ml), or with 10% human serum from psoriatics (n=20), eccema patients (n=16) or healthy controls (n=20) either alone or in combination with neutralizing antibodies (NAb) against several cytokines or with the antipsoriatic drugs acitretin, cyclosporin A, or calcitriol (10^{-5}-10^{-9}M). Proliferation was assessed by cell counts, thymidine incorporation, and by a fluorescence assay using 4-methylumbelliferyl heptanoate. KC differentiation was studied by immunocytochemistry, electron microscopy, and SDS-PAGE of extracted cytokeratins.

No differences of growth or differentiation between normal and psoriatic KCs were observed in 1st and 2nd passage cultures. IL 1a, IL 1b, IL 3, IL 6, GM-CSF, EGF, and TGF-alpha were identified as cytokines inducing a dose-dependent growth stimulation of PP KCs. Psoriatic KCs showed a significantly increased susceptibility to growth stimulation by IL 6 and TGFa, as compared to normal controls, but not to the other examined cytokines. Psoriatic serum induced a greater growth stimulation of PP KCs than normal or eccema serum, and PP KCs displayed a significantly increased susceptibility to this growth stimulation, which was partly abrogated by combination with a NAb against IL 6. All tested antipsoriatic drugs inhibited the cytokine-induced hyperproliferation of psoriatic KCs in a dose-dependent manner.

These data demonstrate that there is no intrinsic hyperproliferation or disturbed differentiation of isolated psoriatic KCs under serum-free conditions. The increased susceptibility of psoriatic KCs to the growth stimulation by IL 6 and psoriatic serum, together with the reported elevation of IL 6 serum levels suggest a major role of IL 6 in the pathogenesis of epidermal hyperplasia in psoriasis. In addition, the antipsoriatic activity of calcitriol, cyclosporin A, and calcitriol may be partly explained by their inhibition of cytokine-induced epidermal hyperplasia.

P1

ANTI-EPIDERMAL ANTIBODIES IN PATIENTS WITH HIV INFECTION. J.A.Stefan, I.Böhm, R.Bauer, H.W.Kreysel. Department of Dermatology, University of Bonn, Germany.

The pathomechanisms of the manifestation of noninfectious dermatoses in HIV patients are still unknown. Because this skin lesions often show the histologic characteristics of an interface-dermatitis, we investigated immunologic phenomenons in 34 patients with HIV in different stages suffering of noninfectious dermatoses by using the indirect immunofluorescence technique. In 26/34 serum samples we could detect anti-epidermal-antibodies. 19 out of 34 sera recognized only antigens within the basal cell layer. Antibody binding could be observed on the cell surface as well as within the cytoplasma. Anti-basement-membrane-zone antibodies could not be detected. In three sera (9%) the fluorescence microscopic pattern were similar to that in pemphigus. Moreover, in four cases (12%) we found antinuclear antibodies against epithelial nuclei, whereas Hep2-cells using the same sera were negative. We believe that anti-epidermal-antibodies in patients with HIV are involved in the pathogenesis of noninfectious dermatoses, since the origin of hyperproliferative skin diseases (i.e. psoriasis, seborrhoic eczema, drug eruption etc.) can not be explained by the progressive immunodeficiency in patients with HIV.

P2

EPITOPES WITHIN THE Ig-LIKE DOMAIN OF THE CD4 MOLECULE ON CD8+ LYMPHOCYTES IN HIV-PATIENTS. I.Böhm, R.Bauer, H.W.Kreysel Department of Dermatology University of Bonn, Germany.

The pathomechanisms which lead to a rise of circulating CD8+ cells in HIV patients remain unclear. Recent studies demonstrating CD8+ cells as targets for HIV infection too, may provide important clues to solve the questions. In the present study, we investigated epitopes of the CD4- molecule on CD8+ lymphocytes in patients with HIV (n=31) and healthy controls (n=37). CD8+ cells were incubated with two different PE-labelled antibodies (13.B.8.2 and SK3) directed against different sites within the Ig-like domain V1 of the CD4 molecule. After incubation the cells were washed twice, resuspended in cold PBS and analyzed in a flow cytometer.
CD8+ cells in patients with HIV were found to bind to the anti-CD4-antibody 13.B.8.2, whereas CD8+ substes of controls did not. However, we could not detect an attachment of anti-CD4-antibodies derived from the SK3 clone to CD8+ cells. The expression of CD4-epitopes on CD8+ cells was an inconstant finding ranging from 10% to 80%. A correlation of this cell subset to the stage of HIV infection, HIV-associated diseases, risk factors and/or other immunologic abnormalities could not be found. The binding site for gp120 to the CD4 molecule has been identified within the Ig-like domain V1. Our results suggest that CD4- epitopes on CD8+ lymphocytes can be considered as targets for HIV as well.

P3

ANTILYMPHOCYTE ANTIBODIES IN HIV-INFECTED PERSONS PREFERENTIALLY REACT WITH THE CD3+ HLA-DR- SUBSET OF T-LYMPHOCYTES C. Müller, S. Kukel, R. Bauer, Department of Dermatology, Bonn Univ., Germany.

More than 60% of patients with HIV infection develop autoantibodies against lymphocytes, but specifity, etiology and possible role in the pathogenesis of AIDS are still unclear. We determined the specifity of lymphocyte reactive antibodies by a method based on three-color flow cytometry. To prevent false positive results caused by Fc-receptor bound immunoglobulines (Ig) and antiviral antibodies we developed an indirect detection system. To minimize the detection of antibodies reacting with antigens expressed not only on lymphocytes but also on platelets, red blood cells and granulocytes the experiments were performed with whole blood. Samples of whole blood from a healthy person (blood group 0) were incubated with plasma of different HIV-infected patients. Bound Ig was detected by a polyclonal antibody. Subsequently the samples were stained with monoclonal antibodies against different lymphocyte subsets and analysed. Antilymphocyte antibodies could be detected in 65% (30/46) of the HIV positive plasms (0/15 in the control group). The strongest reaction was observed against T cells. B cells were stained positively only in a few cases, NK cells with weak intensity in 17% (5/29). There was a trend to lower CD4 cell numbers in the blood of patients with antibodies to T-cells. HLA-DR- T cells showed significant ($p<0.01$) more abnormal bound Ig than HLA-DR+ T cells. The correlation between abnormal bound Ig on T and B cells was weak ($r=+0.35$). We were able to show a predominant reaction of the antilymphocyte antibodies with T cells and this is the first time that a preferentially reaction with non activated (HLA-DR-) T cells could be demonstrated.

P4

ANTIGEN-KERATINOCYTE INTERACTION. THE EFFECTS OF POTASSIUM BICHROMATE. C Marchese, M Picardo*, C Zompetta, F Ameglio*, B Santucci*. Dep Exp Med Uni Rome, *San Gallicano Derm Inst., Rome, Italy.

Recently, increased attention has been focused on the role of antigen-keratinocyte interaction in the pre-immunological phase of contact dermatitis. We have examined the effects of different concentrations of potassium bichromate (BK) on viablity, growth rate, cytokine production and intercellular adhesion molecule-1 (ICAM-1) expression on a keratinocyte derived cell line. The effects of BK were compared with those produced by dinitrochlorobenzene (DNCB), a strong sensitizer. Concentrations from 5 to 20 μg/ml, depending on the period of treatment (3-48h), induced a significant cytotoxicity. A stimulation of IL1α and GC-CSF secretion was observed following 24h and 48h incubation of keratinocytes with concentrations of BK between 0.5-5 μg/ml, while non relevant effects were observed on TNFα and IL6 production. Following 3-6 h treatment ICAM-1 expression was detectable by FACS analysis and T-blast adherence assay. Similar effects were observed with DNCB at concentrations from 0.1 to 0.5 μg/ml. The results support the hypothesis that the antigen keratinocytes interaction, with the consequent production of inflamatory mediators, may be the first step in the sensitization process.

P5

ANTIGEN-KERATINOCYTE INTERACTION. THE EFFECT OF PARA-PHENYLENEDIAMINE. C Zompetta, M Picardo*, C Marchese, F Ameglio*, C De Luca*, B Santucci*. Dep Exp Med, Uni "La Sapienza", *San Gallicano Inst, Rome Italy.

Recently it has been shown that paraphenylendiamine (PPD) and its oxidation products are able to intefere with the growth rate and viability of human keratinocytes in culture and induce the expression of intercellular adhesion molecule 1 (ICAM-1). We have examined the effect of PPD on the production of epidermal cytokines in vitro. After 24h incubation, with concentrations from 0.5 to 5.0 μg/ml, a significant stimulation of ILlα, TNFα and IL6 secretion was detected by immunoassay. 48h treatment produced an increase of the cytokine secretion. Since after 24h incubation in culture medium PPD was completely oxidized, the results suggest that also its oxidation products posses biological activities. The cytokine production was associated with a lipoperoxidative damage of polyunsaturated fatty acids of membrane phospholipids. The results indicate that PPD is able to induce an oxidative stress on human keratinocytes interfering with their biological activities, can stimulate ICAM-1 expression and the production of inflammatory cytokines. In vivo, these phenomena may facilitate the sensitization process by the recruitment of cells of the immune system.

P6

SPECIFIC IMMUNITY TO PLATINUM COMPOUNDS IN MICE. H.-Chr. Schuppe, Chr. Lerchenmüller, J. Kulig, A. Lübben, U. Kloeters, E. Gleichmann*, P. Kind, Dept. of Dermatology and *Med. Institute of Environmental Hygiene, Heinrich-Heine-University, Düsseldorf, FRG.

Halide salts of platinum (Pt) are potent occupational sensitizers. More than 50% of Pt refinery workers may develop symptoms of immediate type allergy. In contrast to specific skin tests, attempts to demonstrate Pt-specific IgE in vitro remained inconclusive. In the present study, we established a mouse model to investigate whether or not exposure to halide Pt salts generates specific lymphocytes.

Immunogenicity of halide Pt salts was demonstrated by means of the popliteal lymph node (PLN) assay. In various mouse strains except athymic nude mice single injections of $Na_2[PtCl_6]$, $(NH_4)_2[PtCl_6]$, or $Na_2[PtCl_4]$ into one hind footpad induced a dose-dependent increase in PLN weight and cellularity (up to 10-fold). Peak reactions were obtained around day 6 after administration of 90-180 nMol Pt. For systemic sensitization, C57BL/6 mice were repeatedly injected with $Na_2[PtCl_6]$ (3x2μg/animal weekly; 20 weeks; s.c., i.p.). Up to 22 weeks after cessation of treatment, spleen cells of these mice were able to elicit specific anamnestic PLN responses when adoptively transferred into the hind footpad of untreated syngeneic recipients. In contrast spleen cells from saline-treated controls or unrelated compounds did not induce significant PLN reactivity.

In conclusion, nanomolar amounts of halide Pt salts elicit T-cell-dependent primary immune reactions in mice. Persistent Pt-specific immunity can be induced by chronic low-dose treatment. It is therefore suggested that Pt-specific T cells play a central role for the induction of Pt-related immunopathological reactions.

P7

MANIFOLD INCREASE OF TNFalpha AND IL-6 LEVELS IN HUMAN SKIN LYMPH DURING THE EARLY PHASE OF AN IRRITANT CONTACT DERMATITIS. Thomas Hunziker[1], Christoph U. Brand[1], Alexander Kapp[2], Ernst R. Walti[1], Lasse R. Braathen[1], [1]Dermatological Clinic and [1]Institute of Pathology, Univ. of Berne, Switzerland, and [2]Dept. of Dermatology, Univ. of Freiburg, Germany.

A superficial peripheral lymph vessel draining the skin of the upper and medial part of the foot was cannulated on the lower leg of 6 healthy human volunteers. After two days an irritant contact dermatitis was induced by application of 10% sodium lauryl sulphate to the drained skin area. Three days later the spontaneously regressing skin reaction was treated with clobetasol propionate.

Lymph was collected twice daily for 7 days, and the levels of various cytokines (IL-1alpha, IL-1beta, IL-2 and soluble IL-2 receptors, IL-6, IL-8, TNFalpha, GM-CSF) were determined by ELISA technique. In the majority of the volunteers all cytokines examined were detected in several lymph samples, with the exception of IL-1alpha and IL-8. In parallel to the clinical symptoms of the contact dermatitis the levels of IL-6 and TNFalpha increased 8- to 10-fold, whereas for IL-1beta, IL-2, IL-2 receptors, and GM-CSF there was a delayed, 2- to 3-fold increase.

These results suggest that cytokines, in particular IL-6 and TNFalpha, may actively participate in the immunological reactions in the skin and in the regional lymph nodes during contact dermatitis.

P8

PHOSPHATIDYLSERINE INDUCES AUGMENTATION OF CONTACT HYPERSENSITIVITY REACTIONS IN MICE

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Phosphatidylserine (PS) is a membrane phospholipid which has been shown to be modulator of immune responses in vitro, including antibody production and macrophage functions. The objective of the present study was to evaluate the effects of PS and other phospholipids on the development of contact sensitivity (CS) reactions in mice.

BALB/c mice (6-8 wks) painted with PS (0.18-1.5 mg) at the time and on the same site of sensitization, exhibited a dose-dependent augmentation (>80-150%) of CS reactions to DNFB. Lyso-PS and phosphatidylethanolamine also induced enhanced CS responses, whereas phosphatidylcholine had no effect. Increased (>30%) CS reactions were also observed when PS was injected intraperitoneally during DNFB sensitization. On the other hand, PS treatment did not modify irritant reactions to DNFB. Immunization with DNFB-derivatized skin grafts treated with PS resulted in enhanced (>30-50%) CS responses, suggesting that PS effects can be due to an action at the skin level. Immunization by i.v. injection of epidermal cell suspensions subjected to density gradient centrifugation, treated with PS (10-100 μM, 30 min, 37°C), and then haptenized with DNBS (1 mg/ml, 30 min, 37°C), resulted in increased (>65%) CS responses, indicating Langerhans cell and/or dendritic epidermal T cell as possible targets of PS activity.

We conclude that PS and neutral or negatively, but not positively charged phospholipids are capable of up-regulating the induction of CS responses in mice presumably through an action on bone marrow-derived epidermal cells.

P9

A model of chronic eczema in the hairless mouse.

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Most of the animal models of contact eczema mimic acute human pathology. In the present study, it is demonstrated that once daily topical applications of low dose of oxazolone (O.3%) on the dorsal skin of hairless mice induce an acute contact eczema which develops into a chronic state. After one week of treatment, the animals appeared to be sensitized to the hapten. Macroscopical and histological studies and measurements made with the MOP-Videoplan system at different stages during the treatment, ranging from one week to 11 weeks, showed that during the second week, an acute lesion develops, which is characterized by extensive desquamation, erythema, spongiotic vesicles, and increase of epidermal and dermal thickness. At later stages, this acute lesion changes to a chronic state characterized by a decrease in desquamation and spongiotic vesicles, maintenance of erythema, hyperplasia, and dermal oedema, and presence of dermal infiltrates. Numeration of dermal mast cells indicated that their number began to increase during the third week and continued to increase constantly during the following weeks becoming 5 times higher than control at 11 weeks. Topical treatment with 2 NSAIDs (parfenac and piroxicam) or 3 steroids (hydrocortisone, Betamethasone-17-valerate (BMV), or dermoval) of the acute lesion, or of the chronic lesion, indicated that only the two steroids (BMV, or dermoval) were active on both types of established lesions. They reduced hyperplasia, dermal oedema and dermal infiltrates. In conclusion, the present data describe a model of chronic eczema in mouse which demonstrates numerous similarities with the human pathology, such as the mode of induction, numerous morphological characteristics, and its pharmacological response. As the mode of action of hapten, such as oxazolone is to form a complex with an epidermal protein and to induce an immune mediated response to this complex, it is proposed that such a model could also serve to mimic certain aspects of auto-immune diseases, such as atopic dermatitis or psoriasis.

P10

ULTRASTRUCTURAL STUDIES BEARING ON THE MECHANISM OF UVB-IMPAIRED INDUCTION OF CONTACT HYPERSENSITIVITY TO DNCB IN MAN.AM Mommaas, AA Mulder, M Vermeer, BW Boom, C Tseng, BJ Vermeer and # JW Streilein. Dept of Dermatology, University Hospital Leiden, the Netherlands, and # Dept of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida, USA

During both induction and elicitation phases of allergic contact dermatitis (ACD), Langerhans cells (LC) present antigen on their surface in the context of HLA class II molecules. During primary sensitization, UVB radiation can impair ACD induction. At the light microscopic level, both UVB and epicutaneous dinitrochlorobenzene (DNCB) have been shown to reduce the density and alter the morphology of HLA class II$^+$ Langerhans cells. To determine more precisely the effects of these agents on LC, an immunoelectron microscopic technique has been used with gold-labelled anti-HLA DR antibodies. Following DNCB application (2000 μg/100μl in acetone on a 1.8 cm diameter skin area) alone to human skin, HLA class II molecules migrated form intracellular to surface expression on epidermal LC, but the density of LC was only slightly reduced. Following four daily exposures of skin to 144 mJ/cm^2 UVB radiation, there was a marked reduction in LC, and the surviving LC showed extensive cell damage. Epicutaneous DNCB after UVB treatment caused no further reduction in LC density. When epidermis from UVB-resistant and UVB-susceptible subjects was exposed to UVB and/or DNCB, changes in LC density and morphology were virtually identical. These findings indicate that (a) DNCB enhances HLA-DR expression on LC via redistribution of molecules from cytoplasm to the surface, (b) reduction in HLA class II$^+$ LC following UVB radiation is due to cell destruction rather than down-regulation of HLA-DR expression, and (c) these effects are similar whether the subject proves to be UVB-resistant (develops CH) or UVB-susceptible (fails to develop CH).

P11

INCREASED ADHERENCE OF *STAPHYLOCOCCUS AUREUS* TO CORNEOCYTES IS NOT SPECIFIC TO THE ATOPIC FORM OF DERMATITIS.
REA Williams and R Donegan. Department of Dermatology, University of Glasgow, Glasgow, UK.

Staphylococcus aureus (*S.aureus*) to corneocyte adherence was assessed in 10 patients with atopic dermatitis (AD) and 6 patients with allergic contact dermatitis (ACD). Separate assays were carried out using corneocytes derived from clinically involved skin and corneocytes from non-involved skin. Adherence was also assessed in 10 normal subjects and in 10 patients with asthma or allergic rhinitis but with no history of dermatitis. Corneocytes were obtained by simple swabbing of the skin with a rayon tipped swab moistened with 0.15M phosphate buffered saline (PBS). They were then suspended in PBS at a concentration of 10^4 corneocytes per ml and incubated with *S.aureus* (10^8 cells per ml) at 37°C for 90 minutes. The suspension was filtered onto a 12μ polycarbonate membrane and the *S.aureus* stained with crystal violet. Numbers of bacteria adhering to each of 120 corneocytes were counted by direct vision (x100 oil immersion).

A one-way analysis of variance of the mean numbers of bacteria adhering per corneocyte indicated increased staphylococcal adherence to cells from the involved areas of both AD (mean=43 bacteria per cell) and ACD (57 per cell) ($p<0.001$). No differences were shown in adherence between *S.aureus* and corneocytes from either normal individuals (25 per cell), non-involved areas in patients with AD (32 per cell) and ACD (30 per cell) or from the skin of patients with asthma or allergic rhinitis but no history of dermatitis (26 per cell). These results suggest that the increased staphylococcal to corneocyte adherence, previously reported as being specific to AD, is likely to be a feature of the non-specific dermatitic changes of the skin rather than the atopic state per se and thus the increased adherence may also be seen in other dermatitic conditions such as ACD.

P12

IgE-BINDING ANTIGENS OF *PITYROSPORUM OVALE* AND *CANDIDA ALBICANS*
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The lipophilic yeast *Pityrosporum ovale* is a usually non-pathogenic member of the normal skin microflora of most healthy adults. In atopic eczema, however, IgE- and/or T cell-mediated anti-*P.ovale* reactions may be important pathogenic factors. Since the main features of the involved antigens are largely unknown, we started investigations on the antigenic composition of *P.ovale*. Extracts were fractionated by Con A chromatography, gel filtration, and chromatofocusing, and IgE-binding activity was monitored by ELISA, using human sera with a known IgE-reactivity to whole *P.ovale* extracts. Practically all (>90%) IgE-binding components were recovered in mannoside-eluates after binding to Con A-Sepharose, and appeared to be very heterogeneous with respect to molecular size and pI: IgE-binding activity was demonstrated at MW 's from 50,000 up to $2x10^6$ after gel filtration, and in the whole pH range from 7.0 to 4.0 after chromatofocusing. A substantial (30%) contribution of highly acidic (pI<4.0) components to the total IgE-binding activity was noted.

IgE-binding components of *Candida albicans* showed very similar chromatographic behaviour, in accordance with the reported cross-reactivity with *P.ovale* allergens. This cross-reactivity was confirmed by inhibition ELISA with isolated pools and fractions from both yeasts. Surprisingly, components with clearly different MW or pI were capable of complete reciprocal inhibition, and all could completely block IgE-binding by the whole extracts. This indicates that IgE-binding structures in *P.ovale* and *C.albicans* extracts are a restricted number of epitopes expressed on a range of mannans or mannoproteins of different MW and pI, and thus might be repetitive elements of protein-sugar conjugates with a variable degree of polymerization.

P13

INCREASED IMMUNOREACTIVE NERVE FIBRES IN ATOPIC DERMATITIS.
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The skin receives a rich supply of nerve fibers. Altered patterns of innervation have been reported in many inflammatory dermatoses. We studied merkers for sensory nerve fibres (neurofilaments, substance P and calcitonin gene-related product), noradrenergic innervation (neuropeptide Y and tyrosin hydroxylase), and neuron-specific protein 9.5, by immunohistochemistry on frozen tissue sections. The material studied included normal skin (n=33), and skin manifesting urticaria (n=6), leukocytoclastic vasculitis (n=4), lupus erythematosus (n=23), and atopic dermatitis (n=40, out of which 16 from lesions after allergen provocation). In normal skin, immunoreactive nerve fibers expressing neuron-specific protein 9.5 were observed in the epidermis, dermis and around blood vessels. For the other markers, immunolabeling was mainly observed in the dermis around blood vessels. Neurofilaments were scarcely present in normal skin epidermis, and were manifest in higher density in the epidermis of affected skin in all disease conditions. Immunoreactive nerve fibres in the dermis and around the vasculature were increased in biopsies from atopic dermatitis. In this group, biopsies after allergen provocation showed an enhanced density of fibers labeled by antibody to neuron-specific protein 9.5, and a lower density in labeling for tyrosin hydroxylase. The data indicate a potential role of innervation and neuropeptides in atopic dermatitis, and form a first indication of the involvement of sensory nerves in the epidermis in the etiology and pathogenesis in immunologically-mediated skin disorders.

P14

EFFECT OF CONTACT SENSITIZER DNFB ON HUMAN EPIDERMAL LANGERHANS CELLS IN SKIN ORGAN CULTURE
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Most of our knowledge concerning the role of Langerhans cells (LC) in the induction of contact sensitivity has been obtained on the basis of animal experiments, but relatively little is known in the human system. As an alternative to in vivo animal experiments we have established a short-term human skin organ culture as a model system for the in situ studies of LC. Using this system in which most of the LC preserved their characteristics up to 3-4 days in culture, we studied the effect of contact sensitizer 2, 4 dinitrofluorobenzene (DNFB) on human LC in cultured skin explants. After direct epicutaneous application of preevaluated non toxic concentration of DNFB, randomly selected skin explants were taken out from the culture system at various time intervals and cryostat sections of these explants were analyzed by immunoenzyme single and double staining using unlabeled or FITC-labeled anti CD1a and anti HLA-DR MAbs. Significant decrease of HLA-DR expression on epidermal LC was observed in skin explants after 18 to 24 h upon DNFB application whereas CD1a expression pronouncely decreased only after 48 h as compared to control experments which include the application of the solvent of DNFB (acetone/olive oil). Similar decrease of the intensity of CD1a/HLA-DR double+ epidermal LC was found after 48h and more significantly after 72h of exposure to DNFB. In these experiments we found no change in cell number of either single + ve CD1a, HLA-DR or double +ve CD1a/HLA-DR epidermal LC. Moreover, no significant effect was detectable on CD1a positive LC as well as on other HLA-DR + cells in the dermal compartment. Interestingly, after 2 days of DNFB application a definite migration of both CD1+ and HLA-DR+ LC towards the basal cell layer and in some cases migration to the upper dermis could be seen as compared to the controls. Our results indicate that DNFB modulates not only the MHC class II molecule but also the CD1a expression on human epidermal LC and further substantiate the notion that DNFB induces the migration of human epidermal LC.

P15

CULTURE OF CD1a+ LEUKEMIC MONOCYTIC CELLS, PUTATIVE PRECURSORS OF LANGERHANS CELLS.
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Langerhans cells (LC) and monocytes may share a common stemm cell. Their precursors can become leukemic cells. We searched for the presence of CD1a antigen, specific for LC and precursors on leukemic monocytic cells, with monoclonal antibodies by immunofluorescence (IF) and immunoelectron microscopy (IEM). CD1a antigen was found present in 11/38 cases of monocytic or myelomonocytic leukemia cells and never found expressed on cells from patients with other types of acute leukemias. Coexpression of monocytic markers was showed by double staining and in 3 cases non specific enolase and S-100 protein were also identified. Cultures of these CD1a + cells were maintained during 4 weeks and CD1a antigen was present as far as day 17, when cells were grown in absence of added cytokine. Interleukine 1 or 3, gamma-IFN did not change the percentages of CD1a + cells. GM-CSF and TPA decreased these percentages. TNF alpha inhibited CD1a expression. Cell sorting allowed an enrichment of 98 % of CD1a + cells with an output of 40 %. These cells shared many morphologic features with LC but did not possess typical Birbeck granules. Taken together, these results indicate that monocytic leukemic cells express CD1a antigen and can be cultured in vitro for a longer time than LC without a rapid loss of CD1a. They could be used for the analysis of the induction of Birbeck granules and our comprehension of LC lineage.

P16

LANGERHANS CELLS MIGRATION: VALIDATION OF A NEW IN VITRO ASSAY AND EFFECT OF CYTOKINES.
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Langerhans cells (LC) are a mobile population of cells which are derived from bone marrow precursors, migrate to the skin and are able to migrate from the skin to regional lymph nodes following antigenic challenge. The factors that influence LC migration are unknown and it was the purpose of this study to develop a technique to examine LC migration or chemotaxis in vitro and to investigate cytokines that could influence this.

Two methods were examined - 1) a modifyed Böyden chamber technique in which partially purified LC (10-15% by sedimentation on ficol/hypaque gradients) were used with PVP free polycarbonate filters; 2) whole epidermal explants prepared by dispase digestion of fresh human skin which were laid over Sartorius filters which were placed on filter pads containing the factor to be tested. Following incubation at 37°C in 5%CO2 and 95% air, the filter and the epidermal explant were stained with anti-CD1 mAb using a peroxidase technique. The factors tested were zymozan treated plasma (ZP), SVK14 cell (human keratinocyte cell line) conditioned medium, MLR supernatant, IL3, GM-CSF, TGFβ1 and RPMI medium as a negative control. Incubation times varied from 15 mins to 48 hrs, and preparations were read by direct visualisation counting the number of CD1+ cells in the polycarbonate filter or the Sartorius filter and the depletion of these cells from the epidermal explants.

The chamber technique gave variable results. There was migration to the RPMI, but much greater migration with ZP (peak concentration of 10^{-2}) and SVK14 medium (peak at 10^{-2}). Checkerboard analysis of ZP showed this to be chemotactic rather than chemokinetic. As the results were variable with low reproducibility, other factors were not tested in this system.

In the explant system, enhanced migration into the filter was observed with MLR supernatants, ZP, SVK14 conditioned media, IL3 and GM-CSF. All factors showed a time (of incubation) and dose dependant response. The greatest responses were observed with GM-CSF (1μg/ml) and IL3 (4μg/ml).TGFβ1 used at up to 1μg/ml showed no response. Counting cells migrating into the filter was more sensitive than counting the depletion of CD1+ cells from the epidermis.

The explant system is thus a sensitive new method for assessing chemotaxis of LC and using this method we have demonstrated the potent chemotactic effect of GM-CSF and IL3 on LC.

P17

INHIBITION OF LANGERHANS CELL ALLOANTIGEN PRESENTATION BY CULTURED KERATINOCYTES. J Morris, M Aliabac and AC Chu. Unit of Dermatology RPMS London UK

Langerhans cells (LC) the immunocompetant dendritic cells of the epidermis present alloantigen to T cells, which can be demonstrated using the mixed epidermal lymphocyte reaction (MELR). Previously it has been impossible to accurately assess the contribution to this reaction by contaminating keratinocytes since it was not possible to purify LC to a sufficient extent. However using Dynal beads and Detachabead it is now achievable to obtain LC of 99% purity.

LC purified in this way from epidermal cell suspensions prepared from skin removed at cosmetic operations, that had been cultured for 3 days , showed >95% viability and purity of 98.2 ± 1.93% using CD1a as a marker and 97.8 ± 3.2% using HLA DR as a marker.

These purified LC were used in a MELR using allogenic peripheral blood mononuclear cells (PBMC). LC were used at 1000 cells/well and were incubated with PBMC from 3-6 healthy volunteers. Basal keratinocytes, autologous to the LC were then added at cell numbers from 780-10^5 cells/well with or without indomethacin.

Results showed a dose dependent reduction in LC alloantigen presenting capacity by keratinocytes with a 50% reduction in function when 10^4 keratinocytes were added. Indomethacin at 1mg/ml didnot affect this reduction in LC function.

In summary, we have used purified LC to examine the contribution of cultured basal keratinocytes to the MELR, cultured basal keratinocytes exert a potent immunosupressive effect on LC function, which is not mediated by prostaglandins. Our results demonstrate the importance of using purified LC when investigating the functional activity of these cells and point to an important immunoregulatory role of keratinocytes.

P18

BIOCHEMICAL AND FUNCTIONAL STUDIES ON THE SURFACE ATPase OF EPIDERMAL LANGERHANS CELLS

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The neutral surface ATPase of epidermal Langerhans cells is used as a histochemical marker of these cells since 1967. Its biochemical type and physiological role are unknown. To investigate this question we tested the influence of biochemically defined inhibitors on LC ATPase activity. Most potent inhibition was achieved with lanthanum compounds, representing broad specific ATPase inhibitors, and calmodulin antagonists (chlorpromzine, trifluoperazine). Blockers of Na+/K+-, or Ca++/Mg++- transport-ATPases had no effect, inhibitors of vacuolar proton transport ATPases inhibited the LC enzyme, but only at high concentrations, indicating that the LC ATPase may participate in the acidification of endosomes during antigen processing. The LC ATPase showed a broad substrate specifity and reacted with all nucleotide triphosphates tested (ATP, GTP, CTP, TTP, UTP), but among nucleotide diphosphates only with ADP. No reactivity with organic or inorganic phosphates was observed. The LC ATPase fulfils all biochemical criteria for an ecto-enzyme. A pretreatment of the skin with lanthanum compounds led to an inhibition of contact sensitization to dinitrofluorobenzene and to the generation of antigen specific T suppressor cells capable of transferring specific unresponsiveness to syngeneic recipients. These data show that the LC ATPase plays a role in the antigen presenting function of LC (contact sensitization) and that it may be a target for dermatological therapy.

P19

THE APPLICATIONS OF CONFOCAL SCANNING LASER MICROSCOPY IN THE STUDY OF EPIDERMAL LANGERHANS CELLS. RCH Yu, D Abrams[1], M Alaibac, L Lemieux[1], DR Springall[2], JM Polak[2] and Chu AC. Unit of Dermatology, [1]Dept of Medical Physics and [2]Dept of Histopathology, Royal Postgraduate Medical School, London.

The confocal scanning laser microscope (CSLM) produces high resolution images from fluorescein-stained biological tissue blocks of up to 100µm thick, this allows 3-dimensional quantitative and qualitative analyses of biological structures using stereological and computer techniques. In this study, epidermal sheets (ES) were obtained from operative human skin following overnight dispase digestion at 4°C. The ES were then fixed with acetone and stained with an indirect immunofluorescence (IF) method with mouse anti-human monoclonal antibody against CD1a and fluorescein-conjugated F(ab')$_2$ fragment of rabbit Igs to mouse Igs. The ES was then viewed using the Bio-Rad MRC-600 Confocal Imaging System. Data obtained from the imaging system was volumetrically rendered and quantitatively analysed using ANALYZE TM 5.0.

For the first time, we are able to visualise noise-free images of LC within the 3-D matrix of the epidermis. Epidermal LC are large flat cells with between 5 to 7 long dendritic processes per cell. The dendritic processes between cells do not to make direct contact with each other. LC form a network across the epidermis, being only about 2% of the epidermal cell population, they cover between 30 to 58% of the total surface area of the epidermis. Useful quantitative data of LC was calculated, they include the mean cell volume density 3.1%, mean cell volume, 213µm^3 and mean cell density per unit volume, 1.6x10^5 cells per mm^3.

CLSM, in conjunction with ANALYZE TM 5.0, provides a powerful tool for visualising LC within the epidermis. Future works will concentrate on the interactions between different cell populations using double IF staining in normal and diseased skin. This technology also allows the introduction of the fourth dimension of time in living cells, hence integrating structure and function as never before.

P20

ALLO-ANTIGEN PRESENTING CAPACITY OF LANGERHANS CELL HISTIOCYTOSIS CELLS. RCH Yu, J Morris and AC Chu Unit of Dermatology, Royal Postgraduate Medical School, London.

Langerhans cell histiocytosis (LCH) cells resemble normal Langerhans cells (LC) histologically and immunocytochemically, furthermore, both cell types possess a unique cytoplasmic organelle, the Birbeck granule. So far, there has been no reported study examining the functional activity of LCH cells as antigen-presenting cells. We report the unique opportunity of studying the functional activity of LCH cells derived from a patient with fatal multisystem LCH. Involved and uninvolved skins were obtained at the post-mortem of an infant who died within hours of multisystem LCH. LCH and normal LC cells were treated separately and used as stimulator cell populations in a 6-day allogeneic mixed cell reaction. Peripheral blood mononuclear cells from 3 volunteers were used as responder cells.

Indirect immunofluorescence of the cell suspensions and APAAP staining of cryostat sections both demonstrated high concentrations of CD1a and HLA-DR positive cells in the dermis clinically affected by LCH. The epidermis overlying the LCH cells showed a normal expected density of LC. LCH cells showed minimal functional activity on a per cell basis when compared to normal epidermal LC from the patient and the positive control. Epidermal LC from uninvolved skin of the patient demonstrated near-normal functional activity, whereas, the epidermal LC overlying the involved skin showed an augmented activity when compared to the positive control.

Our findings strongly suggest two important facts about LCH: 1) that LCH is not a disease caused by an inherent defect of LC stem cells, and 2) that the LCH cells are functionally defective and this defect may be the primary cellular abnormality resulting from the disease process rather than a secondary phenomenon due to suppressive cytokines.

P21

Monocyte derived Langerhans cells depend on IL-6 autocrine stimulation

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Epidermal Langerhans cells live in an environment enriched in IL-6 both by local production and by accumulation. Although they are producers and targets of IL-6 the role of the cytokine for their development is unknown.

We have developed an in-vitro system (IMDM, 2% FCS) which allows the differentiation of Langerhans cells from peripheral blood monocytes. "MoLC" are characterized by high expression of HLA-DR, mannose receptors and CD1 and decreasing CD14, low phagocytic and high antigene presenting activity. MoLC development is strictly dependent on their own supernatant. Cytokine analysis revealed significant production of IL-6 with points of 80 ng/ml. IL-6 release started immediately after adherence being detectable within 1 to 2 hours, and it reached its maximum after 12 hours. The highest concentration maintained a plateau for the following two days, indicating that an equilibrium was established between release and binding of IL-6. From scatchard plot analysis with ^{125}J IL-6, 3.000 high affinity receptors (Kd 62,5 nM) and 21.000 low affinity receptors (Kd 760 nM) were deduced. rh IL-6 was the only monokine which solely guaranteed the survival and CD1 expression of MoLC after complete medium exchange. These data suggest that MoLC development depends on an IL-6 autocrine loop.

P22

EVIDENCE FOR THE HANDLING OF SPECIFIC MYCOBACTERIAL PROTEINS BY LANGERHANS CELLS IN THE SKIN LESIONS OF LEPROSY REVERSAL REACTION

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Leprosy reversal reaction is known to be due to a clinical manifestation of the delayed type hypersensitivity (DTH) reaction to *Mycobacterium leprae* antigens. Langerhans cells (LC) represent the primary immunocompitant cells that are involved in the induction of DTH reaction in general. Although accumulation of LC has been shown in leprosy granuloma little is known about the role of LC in the cutaneous pathology of leprosy. Since Mycobacterial 30-kDa region proteins, the major secreted proteins of mycobacteria, are known to be involved in the pathogenesis of mycobacterial diseases, we investigated the *in situ* localization of these proteins and their epitopes in skin lesions of leprosy patients across the clinical spectrum using a panel of 12 newly prepared MAbs to mycobacterial 30-kDa region proteins in immunohistochemistry. We found that only one of the MAbs designated as 3A8 showed a preferential expression of mycobacterial 3A8 epitope in all lesional skin biopsies from leprosy patients with varying degrees as compared to the lesional biopsies from non leprosy patients. Interestingly, in patients with reversal reaction the 3A8 epitope expression was found more pronouncely in association with cells of the epidermis which are morphologically similar to dendritic LC. Immunoenzyme double staining using FITC-labeled MAb 3A8 and unlabeled anti CD1a (OKT6) we clearly demonstrated the expression of mycobacterial 3A8 epitope on CD1a positive LC both in the epidermis and in the dermis. 3A8/CD1a double positive LC in the epidermis were only found in patients with reversal reaction whereas the number of dermal 3A8/CD1a double positive LC were found significantly increased in the lesions of reversal reaction as compared to other forms of leprosy. Double staining of parallel sections from lesions of reversal reaction with anti CD1a /CD3, anti CD1a/CD4 showed that 3A8 positive LC are found in juxtaposition with the infiltrated CD3 and CD4 positive T cells both in the epidermis and in the dermis. Most of these LC were also found to be HLA-DR positive. Our results indicate that epidermal LC and the perivasculary located CD1a positive dermal dendritic LC carry the mycobacterial 3A8 epitope, and may serve as mycobacterial antigen presenting cells for T cell mediated immune response at the lesional site of leprosy reaction.

P23

WILL A COMBINATION OF ZINC-ERYTHROMYCIN TREATMENT PREVENT THE SELECTION OF ERYTHROMYCIN RESISTANT PROPIONIBACTERIA?

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In the last decade the use of topical antibiotics has increased with erythromycin being used extensively. As a result erythromycin-resistant cutaneous staphylococci and now *Propionibacterium acnes* have been isolated this has been associated with therapeutic failure (BJD, 121, 51, 1989). The purpose of this study was to determine the effect of zinc upon erythromycin-resistant (E^R) and erythromycin-sensitive (E^S) strains of *P. acnes* when grown in mixed culture in the presence of zinc. The bacteria were isolated from patients, grown in liquid media under anaerobic conditions, mixed in various proportions and these mixtures used to inoculate media containing various concentrations of erythromycin with and without zinc. E^S *P. acnes* outgrew E^R *P. acnes* in the absence of erythromycin and zinc. At low levels of erythromycin, E^S outgrew E^R cells, whilst the addition of 600 pg ml^{-1} zinc further reduced the growth of E^R compared to E^S cells. Growth of E^R and E^S cells were similar at erythromycin concentrations near the MIC of E^S cells. Concentrations above the MIC for E^S cells inhibited E^S but not E^R cells. At the higher concentrations of erythromycin, the addition of 96 ng ml^{-1} zinc delayed the growth of E^R cells, whilst the addition of 300 μg ml^{-1} prevented the growth of E^R cells. The combination of erythromycin and zinc, at appropriate concentrations, thus inhibits both E^S and E^R cells. These results strongly indicate that *in vivo* treatment with a zinc erythromycin combination would have therapeutic advantages because it would be predicted that E^R *P. acnes* would not be selected during treatment.

P24

THE INTERACTION OF *PROPIONIBACTERIUM ACNES* WITH LIPIDS *IN VITRO*.

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Propionibacterium acnes is the predominant microbial resident within the pilosebaceous follicles of sebum-rich areas of human skin. The importance of these lipids to colonisation of the follicles by *P. acnes* is uncertain. The effects on the physiology of *P. acnes* caused by some lipid components of human sebum were investigated. *Propionibacterium acnes* was grown under anaerobic conditions in continuous culture in a chemically defined synthetic medium comprising eight amino acids, at 33°C with pH maintained at 5.6. Micronised stock solutions of lipids were aseptically pulsed into continuous cultures in the presence and absence of glucose, and nutritional effects monitored. The range of lipids assayed were based on the C_{18} mono-unsaturated fatty acid, 9-cis-octadecenoic acid (oleic acid). None of the lipids had any effect on the growth of *P. acnes*, either in terms of maximum specific growth rate or final yield. A striking observation was the adherence of cells to one another and to the lipid, the extent of which varied with the lipid used and was maximum with the free fatty acid. This observation was confirmed by transport experiments using radiolabelled (^{14}C) oleic acid. Sebaceous triglycerides are hydrolysed to free fatty acids by the lipase of *P. acnes*. It is proposed that while the production of extracellular lipase *in vivo* may provide *P. acnes* with carbon-energy sources from other lipid species, an equally important function may be the provision of a means to achieve follicular colonisation through adhesion to lipid components such as oleic acid. This novel observation could have important therapeutic potential.

P25

Skin surface and follicular concentrations of azelaic acid after a single topical application.

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Topical azelaic acid (AA) has been used successfully in the treatment of acne and compares favourably with established therapies in clinical trials (Acta Derm Venereol Suppl. **143**, 40, 1989 & **143**, 45, 1989). *Staphylococcus epidermidis* and *Propionibacterium acnes* are the predominant microorganisms both on the surface and in the follicles of acne-affected skin and the antibacterial action of AA *in vitro* is directed primarily at protein synthesis (J.Appl.Bact. **64**, 497, 1988; JID **92**, 446, 1989). The purpose of this study was to determine the follicular concentration of AA after the application of topical AA, in order to establish whether the antimicrobial effects observed *in vitro* are relevent *in vivo*. Pre-weighed amounts of 20% (w/w) AA cream were applied over defined areas on the forehead and back of 9 young adults (5 male, 4 female; mean age 24.1y, range 19.7-29.3). Samples were taken over a period of up to six hours. AA was removed from the skin surface by washing with acetone and follicular casts were collected using a cyanacrylate gel method. The casts were homogenized in acetone. The washings and homogenates were centrifuged to remove particulate matter, and the supernatants derivatized for analysis by HPLC. The results showed wide-ranging variability although it was apparent that the follicular concentration increased as the surface concentration declined. The mean maximum follicular concentrations attained were 23.7±9.7 and 11.6±6.2 ng(μg of follicular casts)$^{-1}$ in casts taken from the back and forehead respectively. Assuming an average density of follicular material of 0.9 gL^{-1}, the mean maximum follicular concentration attained on the back was 114mM (range 36-251) and on the forehead was 56mM (range 3-112). Therefore, the concentrations of azelaic acid attained in follicular casts *in vivo* are greater than the concentrations required to inhibit the growth of *S. epidermidis* and *P. acnes in vitro*.

P26

PURIFICATION AND CHARACTERISATION OF TWO KERATINASES FROM *MICROCOCCUS SEDENTARIUS*.

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Micrococcus sedentarius has been implicated in the condition pitted keratolysis which presents as pitting of the stratum corneum of load-bearing sites of the foot. The bacterium produces two proteases which are capable of degrading both callous skin and extracted keratins and, therefore, are likely to be important in the condition. The aim of this investigation was to characterise the proteases. Growth of *M. sedentarius*, at 34°C, occurred between pH 6.3 and 10.2 with an optimum at pH 8.4. Maximum protease activity was produced when the organism was grown at pH 8.0. The two proteases, P_1 and P_2, were purified from *M. sedentarius* culture supernatant fluids by anion exhange-, hydrophobic interaction- and affinity-chromatography, and then characterised. The molecular masses of P_1 and P_2 were 30.3 and 50.0 kDa, respectively and the isoelectric points were 4.6 and 2.7, respectively. Degradation of callous skin by P_1 was observed between pH 5.9 and 8.0, with an optimum at pH 7.1, whereas degradation by P_2 occurred between pH 4.3 and 10.0, with two peaks of activity at pH 4.9 and 7.5. Both enzymes were more active at increased ionic strength and in the presence of divalent cations. Enzyme stability was enhanced by $CaCl_2$. Activity was unaffected by cysteine and inhibited by EDTA and PMSF. Keratin polypeptides, extracted from finely ground callous skin, were proteolytically cleaved by P_1 and P_2 as indicated by the appearance of lower molecular weight proteins on sodium dodecyl sulphate polyacrylamide gels. The above data implicates both proteases as important factors produced by *M. sedentarius* which account for the symptoms of pitted keratolysis.

P27

PARTIAL PURIFICATION OF EXTRACELLULAR PROTEASES PRODUCED BY *BREVIBACTERIUM EPIDERMIDIS*, A SKIN COMMENSAL ASSOCIATED WITH INTERDIGITAL INFECTIONS.

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Evidence suggests that wound infections initiated by dermatophyte fungi are colonised by skin commensal microorganisms including *Brevibacterium* species which release extracellular proteolytic enzymes capable of further damaging the stratum corneum (Semin. Dermatol. **1**, 149-152, 1982 and J. Am. Acad. Dermatol. **22**, 578-82, 1990). This is supported by evidence that *B. epidermidis* can breakdown keratin (Infec. Immunity, **55**, 621-25, 1987). To further investigate these findings, a partial purification and characterisation of the extracellular proteases of *B. epidermidis* has been undertaken. A strain of *B. epidermidis* was isolated from the skin of a patient's foot and protease was produced by growing this strain in a complex medium to stationary phase. A partial purification of the proteases was achieved by anion exchange- and hydrophobic interaction-chromatography. The number of proteases in the supernatant fluid was determined by combined isoelectric focusing and electrophoresis. The molecular weights of the proteases were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Isoelectric focusing was used to determine isoelectric points. Two peaks of activity were eluted from anion exchange-chromatography, designated P1 and P2. Three polypeptides were detected in P1 with molecular weights of 42, 45 and 48 kDa. Two of these polypeptides have been shown to have pI values of 3.6 and 4.4. The proteases present in P1 have activity over a broad pH range from 5.5 to 10.0, whereas P2 has only been detected at pH 5.5. This study has shown that there is a complex array of proteases. It remains to be determined whether all or some of these proteases are important in the pathological processes and/or the colonisation of normal skin by the organism.

P28

LACK OF NORMAL CONTROL OF HMG-CoA REDUCTASE AND LDL RECPTOR FUNCTION IN HUMAN SEBACEOUS GLANDS.

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An understanding of the control of sebaceous lipid synthesis may lead to novel therapeutic agents for diseases such as acne. Cellular sterol and isoprene synthesis is normally controlled through sterol-mediated feedback, however, sebaceous glands produce large amounts of squalene but little cholesterol. In order to investigate sterol mediated control in human sebaceous glands (HSG), we have examined the effects of overnight pre-incubation with 25-hydroxycholesterol (HOC), Simvastin (SIM) and low density lipoprotein (LDL) on 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity determined in HSG homogenates. In skin fibroblasts, pre-incubation with SIM or lipoprotein-deficient serum (LPDS) induces HMG-CoA reductase, whereas LDL and HOC both repress its synthesis. In HSG after 15-20h culture with foetal bovine serum (FBS)-containing medium, HMG-CoA reductase activity was 167±71 pmol mevalonate/min/mg protein (mean±SD; 8 individuals). This was not altered when the glands were pre-incubated for 15 hours with 20µg/ml human LDL or when 5 mg/ml LPDS was substituted for the FBS. However, pre-incubation with very high concentrations of LDL (675µg protein/ml) gave slight inhibition of HMG-CoA reductase activity. We have detected activity of the LDL receptor (304 ± 83 ng degraded/mg cell protein) in HSG after 15h culture with FBS medium. Only 3% of control of LDL receptor activity was detected when human LPDS replaced the FBS in the medium. In conclusion, we have demonstrated for the first time that HMG-CoA reductase and LDL receptor activity are present in human sebaceous glands and that they are not regulated in the same way as in skin fibroblasts and other cell types.

P29

PROLIFERATION AS WELL AS DIFFERENTIATION OF HUMAN FOLLICULAR OUTER ROOT SHEATH CELLS IS SUBSTANTIALLY MODULATED BY COCULTURED MESENCHYMAL CELLS IN VARIOUS ORGANOTYPICAL ARRANGEMENTS. Alain Limat, Thomas Hunziker, Lasse R. Braathen, Dermatological Clinic, Univ. of Berne, Switzerland.

Depending on environmental influences, follicular outer root sheath (ORS) cells in vivo can differentiate either towards interfollicular keratinocytes or hair-matrix cells. Crucial regulators of both proliferation and differentiation are the mesenchymal cells of the respective tissues. In a two-chamber model, human dermal fibroblasts (HDF) as well as hair papilla cells (HPC) equally and markedly enhanced ORS-cell proliferation. This stimulation was more prominent with mesenchymal cells in the postmitotic than in the mitotic state, but reduced when ORS-cells and mesenchymal cells were separated by a medium layer. In addition, close association of ORS-cells and mesenchymal cells resulted in increased motility of ORS-cells. When ORS-cells were grown on HDF- or HPC-populated collagen gels at the air-medium interphase, a stratified epithelium developed which was largely reminiscent of epidermis both in terms of tissue organization and differentiation markers (e.g. suprabasal keratins, filaggrin, involucrin, basement membrane components). In the absence of HDF, stratification and epidermal differentiation remained poor. In an attempt to mimic the follicular environment, ORS-cells alone cultivated inside extracellular matrix components (Matrigel) formed large spheroids of inward-directed growth and differentiation positive for epithelial membrane antigen. Addition of HDF markedly sustained both cell growth and epidermis-type differentiation. No obvious differences were seen when HDF were replaced by HPC.

P30

THE B-FRINGE OF THE HAIR FOLLICLE - DEFINITION OF A NEW STRUCTURE BASED ON MORPHOLOGICAL AND KERATIN IMMUNOHISTOCHEMICAL CRITERIA

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The human hair follicle is classically divided into infundibulum, isthmus, and the large inferior portion. This crude classification is insufficient for precise localization of the various differentiation steps in the follicle, most of them occurring in the inferior portion.

We therefore define as B-fringe ("below Adamson's fringe") a horizontal line through the follicle where Henle's layer of the inner root sheath (IRS) looses its trichohyaline granules. At this site, besides the cornification of Henle's layer, the outer root sheath (ORS) proliferates to become a thick stratified epithelium, the cuticle of the IRS acquires trichohyaline granules, and the hair cortex enters the keratogenous zone, as reflected by its cells becoming eosinophilic and fusiform, and by its discreet birefringence at examination in polarized light.

The physiological relevance of this line is corroborated by the results of our immunohistochemical investigations on keratin distribution; these studies confirm in part and extend the results of other investigations. Using monospecific monoclonal antibodies we demonstrate at the B-fringe the onset of expression of keratin 14 in the entire ORS, and of keratin 7 and keratin 8 in its basal layer. In contrast, immunostaining of keratin 7 and keratin 13 in the IRS started at the follicle base, and that of keratin 18 in Huxley's layer started above the tip of the papilla. Keratin 19 staining of the ORS was variable and could be found as early as in the bulb; however, immunopositive cells were preferentially located in the bulge area. Anti-keratin 1 stained exclusively the suprabasal layers of the infundibulum, where, different to the ORS, keratin 14 was located in the basal layer only.

The B-fringe, similar to Adamson's fringe, is readily recognized by standard stainings or by immunohistochemistry; it serves as an intelligible morphological subdivision of the inferior portion. Physiologically it reflects an important step in all three differentiation pathways of the follicle (ORS, IRS, and hair).

P31

THE CONTINUING EVOLUTION OF ANTIBIOTIC RESISTANT PROPIONIBACTERIA: AN EMERGING CLINICAL PROBLEM. Joanne L. Tipper, Christopher E. Jones, Jonathan H. Cove, E. Anne Eady & *William J. Cunliffe, Dept. of Microbiology, Univ. of Leeds and *Dept. of Dermatology, Leeds General Infirmary, Leeds, U.K.

A decade ago antibiotic resistance in cutaneous propionibacteria was practically unheard of despite >20 years of intensive and prolonged use of antibiotics in acne therapy. During the 1980s, resistant strains were reported in the USA, UK and Japan. We decided to re-examine the extent of propionibacterial resistance in the UK at the present time since it is clear that failure to respond to antibiotic therapy is a growing clinical problem. Two hundred and eighty eight acne patients were sampled between March and November 1991. The patients' skin was sampled with a moistened swab which was then used to immediately inoculate a series of plates containing different selective antibiotics. The plates were examined for growth after 7 days anaerobic incubation at 37°C. One in four patients was found to carry propionibacteria resistant to one or more of the antibiotics used in acne therapy. Resistance to erythromycin was the most common with 18.4% of patients carrying erythromycin resistant strains. In the majority of cases (42/53) the isolates were cross resistant to clindamycin. Preliminary evidence suggests that some of the propionibacterial erythromycin resistance determinants hybridise in dot blots to ribosomal methylase genes from *Bacillus* and *Bacteroides* spp. Eight per cent of patients carried tetracycline resistant propionibacteria which were cross resistant to doxycycline but not minocycline. Thirty-one patients (10.8%) carried strains with reduced sensitivity to trimethoprim. Significantly, a number of isolates (26) were multiply resistant and two strains were resistant to five of the six therapeutically useful antibiotics for the treatment of acne. The levels of antibiotic resistance encountered in this study are alarming and suggest that mobile genetic elements are spreading resistance determinants throughout previously sensitive cutaneous propionibacterial populations.

P32

TETRACYCLINE RESISTANT PROPIONIBACTERIA FROM ACNE PATIENTS ARE CROSS RESISTANT TO DOXYCYCLINE BUT SENSITIVE TO MINOCYCLINE. E. Anne Eady, Christopher E. Jones, Joanne L. Tipper, Jonathan H. Cove & *William J. Cunliffe, Dept. of Microbiology, Univ. of Leeds and *Dept. of Dermatology, Leeds General Infirmary, Leeds, U.K.

Tetracyclines are still used extensively in the treatment of acne vulgaris because of their ability to inhibit the growth of cutaneous propionibacteria. We have already shown that *in vivo* the anti-propionibacterial activity of minocycline is greater than that of tetracycline. Increasing numbers of patients are failing to respond to antibiotic therapy (currently 25%) and propionibacterial resistance has been shown to be a contributory factor. Therefore, we determined the minimum inhibitory concentrations of tetracycline, doxycycline and minocycline for 46 resistant propionibacterial strains by agar dilution and the results are shown below.

Antibiotic	MIC μg/ml	
	Range	Mean±95% confidence limits
Tetracycline	2-64	20.61±4.56
Doxycyline	1-32	9.70±2.03
Minocycline	0.25-4	1.95±0.35

Levels of resistance to tetracycline and doxycycline are significantly much higher than levels of resistance to minocycline. Phenotypically, the strains resemble staphylococci containing *tetK*, which confers resistance to tetracycline but not minocycline by encoding a tetracycline efflux pump. We have recorded serum levels up to 2 μg/ml in acne patients treated with 100mg/day of minocycline or 500mg b.d of tetracycline. Thus, at therapeutically achievable serum concentrations, all the propionibacterial strains would demonstrate clinical resistance to tetracycline but the majority (37/46) would be sensitive to minocycline. These results suggest that patients with tetracycline resistant propionibacteria should respond to minocycline therapy.

P33

EXPRESSION AND FUNCTIONAL STUDIES OF MSRA, AN ABC TRANSPORTER MEDIATING ANTIBIOTIC RESISTANCE IN STAPHYLOCOCCI. Jeremy I. Ross, Jonathan H. Cove & *William J. Cunliffe, Dept. of Microbiology, Univ. of Leeds and Dept. of Dermatology, Leeds General Infirmary, Leeds, U.K.

ABC transporters such as the multidrug resistance protein of cancer cells operate in concert with membrane proteins or possess hydrophobic domains essential for solute transport. We have already shown that MsrA, a plasmid encoded ABC transporter mediating erythromycin resistance in staphylococci, does not possess membrane spanning domains. Furthermore, the protein functions in a heterologous host (*S. aureus*) and in the original host (*S. epidermidis*) in the absence of additional plasmid-encoded products. Therefore, MsrA must interact with and confer antibiotic specificity upon chromosomally encoded transmembrane efflux complexes of staphylococcal cells. The determinant has been located by dot blot hybridisation in all commonly isolated cutaneous staphylococcal species and also confers resistance to azithromycin. Staphylococci with a similar phenotype have now been isolated from patients on CAPD and from the blood of premature neonates. In common with other ATP-binding transport systems, the active efflux of erythromycin from staphylococcal cells containing *msrA* could be prevented by inhibitors of proton-motive force (CCCP & DNP) and compounds which reduced the intracellular ATP concentration (arsenate). MsrA could be expressed in *E. coli* mini-cells and located on SDS-PAGE gels. However, it conferred no resistance in *E. coli* or *B. subtilis*. These results indicate a requirement for additional proteins not normally produced by these organisms. MsrA could be visualised on SDS-PAGE gels of crude membrane preparations from resistant *S. aureus*. NTG treatment resulted in the production of mutants in which *msrA* did not confer resistance despite the apparent overexpression of the MsrA protein. The basis of these mutations is under investigation. They may represent isolates in which the membrane proteins have been inactivated.

P34

A NEW APPROACH FOR TREATMENT OF HYPERHIDROSIS USING ALTERNATING CURRENT. Stephen Reinauer, Ansgar Neußer, Gunnar Schauf, and Erhard Hölzle, Department of Dermatology, Heinrich-Heine-University, Düsseldorf, Germany.

Tap water iontophoresis (TWI) using direct current (DC) has been widely adopted as the treatment of choice for palmoplantar hyperhidrosis. Shortcomings of this method are discomfort with burning and tingling, skin irritation including iontophoretic burns, and complicated safety measures to prevent electric shock. Our goals were to minimize side effects and to increase technical and safety standards of TWI.

In a controlled blind study treatment of the hands by alternating current (AC) was compared to the conventional DC-method (n=20). A newly developed apparatus generated AC of 8-12 mA effective amperage with a maximum voltage of 8 V and a frequency of 5,1 kHz. The DC-method of TWI employed 10-25 mA and 30-50 V.

Palmar hyperhidrosis was completely controlled after an average of 12 treatments for 30 minutes once daily in both groups as revealed by quantitative gravimetric and hygrometric measurements of sweat secretion. No side effects were seen using AC. In addition to equal efficacy and lack of dermal side effects and discomfort, the AC-method requires much less safety measures; the possibility of electrical shock when submerging or removing hands from the water baths is excluded.

Therefore, AC-treatment should become the treatment of choice for palmoplantar hyperhidrosis in the future.

P35

IMMUNOCYTOCHEMICAL DEMONSTRATION OF NEURAL AND MATRIX MARKERS IN DEVELOPING RAT SKIN AND HAIR FOLLICLES. Mari-Outi Lehtimäki, Annikki Vaalasti and Leena Rechardt, Department of Biomedical Sciences, Univ. Tampere, Tampere, Finland

Immunoreactivities (IR) against neurofilament (NF), neuron specific enolase (NSE), tyrosine hydroxylase (TH), 5-HT, neuropeptide Y (NPY), S-100, vimentin (VN), fibronectin (FN) and tenascin (TEN) were demonstrated in the skin of 14,15,16,18-day-old embryos and newborn Wistar rats by using Coon's indirect immunofluorescence method. The skin samples were excised from the frontal scalp areas.

Intense NH-IR was seen in dermal nerve trunks and fine nerve networks around the hair follicles in 18-day-old embryos and newborn rats, while only few fibres were stained in 14,15,16-day-old embryos. No immunoreactive cells were seen. NSE-IR correlated to NF-IR in the oldest age groups, but in 14,15,16-day-old embryos a row of regularly arranged cell groups in dermis showing NSE-IR was identified. TH-IR was seen only in the newborn rats in the nerves around blood vessels and in arrector pili muscles. Large cells with processes exhibiting NPY- and S-100-IR were localized in the hair matrix of newborn rats, and in wandering cell clusters in the dermis of 14 and 15-day-old embryos. Intensity of extracellular FN-IR was found to decrease with the embryonal age, while the staining with tenascin antibody increased, respectively. VN- and 5-HT-IR were not detected.

Our findings suggest that the cells which participate in the formation of hair pulp express neural markers and thus possibly originate from the neural crest. Especially fibronectin might be involved in the guidance and differentiation of the migrating cells.

P36

STIMULATION OF HUMAN HAIR GROWTH IN VITRO BY CYCLOSPORIN A. M. Taylor, A.T.T Ashcroft, A.G. Messenger, Department of Dermatology, Royal Hallamshire Hospital, Sheffield, U.K.

Cyclosporin A (CyA) causes hypertrichosis but its mode of action on hair growth is unknown. We have investigated the effect of CyA using a recently described model (Philpott et al, J Cell Sci 1990: 97, 463) in which isolated human hair follicles are grown in organ culture.

Human hair follicles were obtained by microdissection and cultured individually in 24-well plates in serum-free Williams E medium supplemented with antibiotics, insulin and hydrocortisone. CyA was added to the culture medium on Day 0. Sets of 6 follicles from each biopsy (n=7) were used for each concentration of CyA (10^{-7}M-10^{-9}M) and control. Follicle length was measured at intervals over 15 days.

In both control and CyA-treated cultures, follicle length increased by approximately 0.3mm/day over the first 5 days. In control cultures, linear growth then declined and only 2% of follicles were still growing at 15 days (final mean length increase = 1.81±0.08mm). In follicles treated with CyA 10^{-7}M, follicle growth and morphology was maintained for longer with 43% of follicles still growing at Day 15 (mean increase in length = 2.57±0.27mm $p<0.01$ cf control). There was a smaller, non-significant response to lower concentrations of CyA.

These results confirm the validity of this model for studying pharmacological stimulators of human hair growth. They show that CyA maintains follicle growth for longer (?prolongs anagen) rather than increasing linear growth rate and that this is a local effect on the hair follicle.

This work was supported by Unilever Research.

P37

Differential expression of gap junction proteins in human hair follicle. Elisabeth Masgrau, Solange Vischer, Jean-Hilaire Saurat, Denis Salomon, Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Genève 4, Suisse

The hair is a complex structure comprising the outer root sheat (ORS), the inner root sheat (IRS) the hair shaft and the hair bulb. Each of these compartments originates from the hair bulb (matrix + dermal papillae) and undergoes a specific differentiation process. Electron microscopy has revealed the presence of gap junctions in both ORS and IRS. We have assessed by immunofluorescence the distribution of three gap junctions proteins named connexins (Cx) : Cx43, Cx32 and Cx26 in the human skin. We found that Cx32 is not expressed in human skin, Cx43 is expressed abundantly in the epidermis and adnexae, and Cx26 is expressed only in the hair and sweat duct. The distribution of Cx43 and Cx26 in the hair was as follows : ORS : Cx43 : +++, Cx26 : ++, the labeling for both Cx remain unchanged in the totality of the ORS; IRS: Cx43 : ++, Cx26 : ++, the Cx labeling disappeared in parallel with the occurence of the IRS cell keratinization ; hair shaft : cortex Cx43 : ++, Cx26 : +, again the Cx labeling disappeared with keratinization; hair bulb : matrix : Cx43 : +, Cx26 : - ; dermal papillae : Cx43 : +, Cx26 : -.

These results indicate that 1) hair follicle keratinocytes express several types of Cx in contrast to epidermal keratinocytes ; 2) both in hair follicle and epidermis Cx expression is low in the proliferative compartment, abundant in the differentiative compartment and disappears with full keratinization ; 3) Cx26 expression in keratinocytes is related to the process of differentiation specific to epidermal adnexae.

P38

ANDROGEN RECEPTORS AND REGULATION OF HAIR GROWTH IN ORGAN CULTURE OF HUMAN HAIR FOLLICLES. Rukhsana Choudhry, and Malcolm B. Hodgins, Department of Dermatology, University of Glasgow, United Kingdom.

Immunohistochemical staining with a specific androgen receptor monoclonal antibody demonstrated that in the human hair follicle, during anagen or telogen, androgen receptors (AR) are restricted to cells of the dermal papilla (DP). Of 163 DP sectioned (from 21 skin specimens) 44% stained AR-positive. Both AR-positive and AR-negative DP were found among large anagen hair follicles in male beard and genital skin. Organ culture of dissected follicles (male beard & groin, female axilla) in serum free DMEM was used to study the regulation of AR in DP. In culture, the hair shaft continued to increase in length for up to 12 days before the DP collapsed and the matrix formed a keratinized club-like structure. 47% of DP from freshly isolated follicles stained positively with the AR monoclonal antibody. The percentage of AR positive DP fell to 20% after 1 day, 18% after 2 days and 9% after 6 days in culture. Addition of the synthetic androgen Mibolerone (3nmol/l) did not affect AR staining or the rate or duration of hair shaft growth.

This study confirmed that AR immunoreactivity is restricted to the dermal papilla in human hair follicles and suggests that variation in AR staining between large anagen follicles in androgen dependent sites could reflect a decrease in AR during late anagen. In culture large androgen dependant anagen hairs continue to grow in the absence of exogenous androgens or serum factors.

P39

DO MAST CELLS PLAY A ROLE IN HAIR GROWTH REGULATION? Marcus Maurer, Ralf Paus, Susanne Daum, Matthias Lüftl, Thomas Heinzelmann, Andrzej Slominski, Beate M. Czarnetzki, Dpt. of Dermatology, University Hospital Rudolf Virchow, Freie Universität Berlin, D-1000 Berlin 65

Based on the observation that surprisingly many mast cells (MC) can be found in routine sections of normal scalp skin as well as in scarring alopecias, we are examining the potential physiological role of MC in hair growth regulation. Using the C 57 Bl-6 mouse model for hair research, we had previously found that the MC product heparin can modulate murine hair growth patterns in vivo and in vitro (BJD 124:415). We have now characterized cycle-associated MC changes during the C 57 Bl-6 mouse hair cycle, using light and electron microscopy. Hair growth (anagen) was induced in telogen mice by depilation (BJD 122:777) or by i.p. administration of cyclosporine A (CsA) (Lab Invest 60:365). Two days after anagen induction (anagen I-II), the number of of Giemsa- or Toluidin-stainable dermal cells with characteristic MC morphology and metachromasia decreased significantly as compared to telogen skin ($p<0.001$), while stainable MC numbers in mid anagen (IV) rised slightly above telogen values. A similar, though less pronounced decrease in the number of stainable MC was found in CsA-induced or spontaneous anagen. Giemsa stains and TM-EM revealed that as early as one hour after anagen induction by depilation there is significant MC degranulation, partially in the form of "piecemeal" degranulation, which likely explains the reduction of stainable MC. In skin organ culture of mouse skin (BJD 122:777), the MC products heparin, heparansulphate, serotonin and histamine altered epidermal and/or hair bulb keratinocyte proliferation, dependent on dose, administration schedule, length of incubation and whether telogen or anagen skin was used. Administration of two IgE-R-independent endogenous MC-degranulatory agents, substance P or NGF, to telogen mouse skin in organ culture resulted in MC degranulation (as verified by histology). We suggest that MC may have important regulatory functions during the hair cycle and that, under physiological circumstances, MC participate in tissue remodelling and growth control.

P40

GRANULOMATOUS REACTIONS FOLLOWING HERPES ZOSTER CONTAIN VARICELLA-ZOSTER GLYCOPROTEIN gpI. A.F. Nikkels, C. Sadzot-Delvaux, J.M. Cloes, B. Rentier, G.E. Piérard. Depts of Dermatopathology and Microbiology, Univ. of Liège, Belgium.

Granulomatous reactions at the sites of herpes zoster (HZ) are rather common. Their etiology has never been elucidated and DNA of varicella-zoster virus (VZV) has not been disclosed by polymerase chain reaction. We explored the possible presence of other VZV antigens as the cause of HZ related granulomas. We developed a monoclonal mouse antibody (VL8), IgG1 Kappa isotype, directed to the envelope glycoprotein gpI of the VZV. The VL8 ab is specific for VZV without cross reactivity with the other herpes viruses as asseessed by EIA and cytofluorimetric analysis. Biopsies of HZ (5), herpes simplex I and II (8) and appropriate controls (10) were formalin fixed and paraffin embedded. Immunostaining with ABC method was performed with VL8 ab and Herpes I and II ab (Dakopatts).

No cross reactivities were noted. VL8 ab labelled keratinocytes at the site of HZ vesicles and in some hair follicles. A positive immunostaining was also disclosed at the site of inflammatory reactions in the deep dermis as well as in neurovascular plexuses and perineural cells.

We conclude that the presence of viral envelope antigens in the deep dermis is likely responsible for the HZ related granulomatous reactions.

P41

ERYTHROMYCINE - DEMETHYLATION ACTIVITY IS PRESENT IN MURINE SKIN AND INDUCIBLE BY DEXAMETHASONE. Frank K.Jugert, Klaus Bolsen, Günter Goerz, Hasan Mukhtar and Hans F.Merk, Departments of Dermatology, Universitys of Cologne, Düsseldorf, Germany, and CWRU Cleveland, USA.

The skin functions as an important interface of the body with the environment. Beside its barrier functions it has been shown that the skin possesses metabolic activities capable of altering the profile of substances entering the body by percutaneous absorption. The cytochrome P450 (P450) isoenzymes are the first receptors of many xenobiotics and drugs. Glucocorticoids are the most widely used topical drugs in dermatology which are known to be metabolized by P450 III A dependent erythromycine-demethylase (EMDM) in the liver. However there is no information whether this particular P450 isoenzyme is also present in the skin. In this study, female NMRI-mice were topically treated with 0.1% or 1% aequeous solutions of dexamethasone, pregnenolone-16-a-carbonitrile or cyclosporine for two days. EMDM activities were then measured in murine skin and liver microsomes. In skin and liver microsomes dexamethasone induced the EMDM activity 2.2-fold as compared to controls (Skin: 2.4. vs. 1.1, liver: 12.1. vs. 5.6). Immunoblots with murine liver microsomes using an antibody against P450 III A showed staining-intensity areas corresponding to the measured EMDM-activities. In murine skin microsomes P450 III A was detected after 0.1% dexamethasone pretreatement.

P42

STUDIES ON HUMAN HAIR FOLLICLES IN CULTURED FULL THICKNESS SKIN EXPLANTS: PRELIMINARY RESULTS. PB Nanninga, WJ Menko, PK Das, JD Bos, W Westerhof, Department of Dermatology, Univ. of Amsterdam, Academisch Medisch Centrum, Amsterdam, The Netherlands.

Isolated human hair follicles can be maintained and grown in culture for up to 14 days (Philpott et al. J Cell Sci 97,1990). By isolating the follicles from their natural surroundings, deep in the dermis, they are no longer under the influence of all the possible growth regulating factors. Moreover, the possibility of damage is always present. Therefore, a full thickness skin explant model has been developed, to culture whole skin biopsies, hair follicles included. The method has first been tried on pig skin biopsies and a medium has been developed in which all cell types grew well. Hereto, 3 mm full thickness skin biopsies were placed in a Transwell (Costar) culture system. The upper well has a microporous bottom and is filled with an agar gel to support the biopsies. This well is placed in a larger one, filled with culture medium. The nutrition of the biopsies takes place by diffusion through the porous bottom and the gel. The epidermal side of the biopsies is exposed to air and culturing took place by 37°C and 8% CO_2 in air. Viability of the culture was assessed by routine histology and by a bromodeoxyuridine (BrdU) staining procedure. At regular intervals the biopsies were harvested, frozen, sectioned and stained with HE, DOPA (active melanocytes), NKI-beteb (active and inactive melanocytes) and *OKT-6* (Langerhans cells).

In all the biopsies cultured, pig skin as well as human skin, the epidermis and skin adnexae were left intact and showed outgrowth as assessed by BrdU incorporation and seen in the HE sections. Apart from some dermal edema and collagen changes the morphology was preserved. Epidermal melanocytes decreased in numbers during the culture period as was observed with the DOPA and NKI-beteb staining. Follicular melanocytes however, remained viable during the entire experiment (14 days). Langerhans cells were still present after 2 weeks and were even seen in the epithelial outgrowth.

This method enables us to study hair follicles (melanogenesis, tyrosinase, melanocyte stimulation) in vitro, with all the beneficial influences of the surrounding epithelial and dermal components.

P43

CHARECTERIZATION OF HUMAN NAIL MATRIX CELLS IN VITRO M Picardo, C Marchese*, C Zompetta*, N Cameli**, P Fanti**, A Tosti**. San Gallicano Derm Inst, Rome, * Dep Exp Med Uni "La Sapienza", Rome, * Dep Dermatol Uni Bologna, Italy.

An in-vitro model to study the biological properties of nail matrix cells (NMC) was established. Human nail matrix was obtained by biopsies from patients affected with ingrown toenails. After trypsinization nail matrix cells (NMC) were cultured on a feeder layer of mitomycin-C treated 3T3 in Keratinocyte Growth Medium. Up to 7 subcultures were performed. Growth rate, ultrastructural examination and integrin expression were perfomed in comparison with normal human keratinocytes. Morphologically nail matrix cells appeared larger than keratinocytes with little evidence of stratification or squamous differentiation. The growth rate was higher than that of keratinocyte with a calculated doubling time of 18h. VL1 and VL5 were significantly expressed on the cell surface in this cultural condition. Polyacridamide gel electrophoresis of NMC revealed the presence of both soft and hard keratinis with a pattern comparable to those extracted from nail. The results indicate that this in vitro system may be a useful model to study the biological characteristics of NMC in normal and possibly in pathological conditions.

P44

GLUCOCORTICOID RECEPTOR EXPRESSION IN HUMAN EPIDERMAL CELLS.

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Glucocorticoids are widely used in therapy of inflammatory skin diseases. Recent studies have shown that their main effect is mediated by a down-regulation of several genes involved in cell proliferation and inflammation particularly in the cytokine production. A direct interaction by protein complex with the glucocorticoid receptor was demonstrated at the AP1 site implicated in cell regulation.

In this study, we examined which epidermal cells express the glucocorticoid receptor (GR) and how its expression is modulated in pathological cases such as psoriasis compared to normal skin. This work was also done on fresh isolated and cultured epidermal cells. Immunostainings and FACS analysis were performed with polyclonal anti-human GR antibody (from Dr. Vale, Salk Institute USA). The results showed that more than fifty per cent of freshly isolated epidermal cells express the cytoplasmic form ot the GR which was identified by Western Blot as a protein of 94 kD. Double immunofluorescence stainings with an anti-CD1a antibody showed that GR is not detected on $CD1^+$ Langerhans cells (LC). In vivo, on frozen normal skin sections the expression of GR is mainly located in basal and spinous layers. In psoriasis, the same staining distribution with an enhancement of GR expression was observed. Keratinocytes cultured in defined serum free medium express also the GR. The interactions of glvcocorticoids with keratinocytes was emphasized by the expression of a constitutive lipocortin I, a protein induced by glvcocorticoids. Lipocortin I was detected both by Western Blot and immunostainings using an anti-lipocortin I antibody (Dr Russo-Marie, Paris) in keratinocytes but not in $CD1^+$ LC. A higher expression of this protein was also observed in psoriasis compared to normal skin.

These data show that the main target of glvcocorticoids in epidermis are keratinocytes. The regulation of the expression of lipocortin I and GR is under investigations.

P45

INABILITY OF TOPICAL GLUCOCORTICOID TO INCREASE EPIDERMAL LIPOCORTIN-1 CONCENTRATION IN NORMAL HUMAN SKIN. Jonathan Dixey, Christopher R. Lovell* and Nicolas J. Goulding, Bath Institute for Rheumatic Diseases, Bath BA1 1HD and *Royal United Hospital, Bath, UK.

Lipocortin-1 (Lc-1) belongs to a family of proteins which bind phospholipid in the presence of calcium. It has anti-inflammatory properties in animal models and is glucocorticoid-inducible in peripheral blood monocytes.We have demonstrated Lc-1 in the basal layer of normal epidermis and have determined epidermal concentrations by a specific ELISA. In a double-blind study we sought to determine whether Lc-1 can be induced by topical glucocorticoid. Nine volunteers applied 1% hydrocortisone cream and the vehicle base to two defined areas of the forearm. Three applications were made, 24, 12 and 2 hours before raising suction blisters at each site. Blister roofs were weighed, homogenised in a buffer containing 10 mM EDTA and stored at -20°C before analysis by ELISA.

A mean value of 4.0 ng Lc-1 per mg tissue (wet weight) (range 1.4 - 7.5) was detected in steroid-treated epidermis and 4.4 ng/mg Lc-1 range (1.3 - 10.1) in placebo-treated epidermis. Changes were not statistically significant. (Wilcoxon signed rank test p=0.44).

This study failed to demonstrate quantitative differences in EDTA-extractable Lc-1 between topical application of hydrocortisone or placebo over a 24 hour period, suggesting that Lc-1 production by epidermis is not under the control of glucocorticoid hormones.

P46

TOPICAL CORTICOSTEROID AND RELEASE OF PLATELET ACTIVATING FACTOR (PAF) IN HUMAN SKIN. F. Lawlor, R.M. Barr, M.R. Judge, A.I. Mallet, P.F. Courtney, R. Barlow & M.W. Greaves. St. John's Institute of Dermatology, London, UK.

PAF is synthesised by hydrolysis of the sn-2 arachidonyl residue from alkylcholine phospholipids to give lyso-PAF which is acetylated to PAF. Anti-inflammatory properties of corticosteroids are attributed to inhibition of phospholipase A_2 (PLA_2) mediated arachidonate release and reduced eicosanoid synthesis. We assessed a potent corticosteroid for inhibition of PAF and lyso-PAF release in human skin.

Clobetasol propionate (CP), 0.05%, and its base were applied with occlusion, at 24h intervals for 72h, to 10 cm squares on either thigh of 8 healthy volunteers. Suction blister exudates were analysed for PAF and lyso-PAF by GC-MS using deuterated analogues as internal standards.

Lyso-PAF concentrations were lower in exudates from CP treated skin than from control skin for all subjects, mean concentrations $\pm$ sem 152 $\pm$ 24 and 230 $\pm$ 42 nM ($P<0.05$), respectively. PAF levels were lower in exudates from CP treated than control skin for 6 of 8 subjects, but the difference between the mean concentrations, 1.06 $\pm$ 0.36 and 1.97 $\pm$ 0.52 nM PAF (n=8) respectively, was not significant.

Clobetasol propionate, applied with occlusion to healthy skin, inhibited release of lyso-PAF, the initial product of PLA_2 catalysed alkylphosphocholine hydrolysis, but did not produce a consistent reduction in the release of PAF.

P47

POLYCYCLIC AROMATIC HYDROCARBON INDUCED DNA DAMAGE IN HUMAN SKIN. M.J. Edwards, P.J. Dykes and R. Marks, Department of Dermatology, Univ. of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN.

Genotoxic compounds which may be present in the general environment pose a risk to humans which is quite difficult to assess. The aim of the present study has been to develop a technique that can detect minor degrees of DNA damage after exposure of human skin to test compounds *in vivo*. We have utilised the ^{32}P-post labelling method of DNA-adduct detection to investigate the incidence of bulky-polycyclic aromatic hydrocarbon induced DNA-adducts in skin samples from human skin explants or calf thymus DNA following their exposure to 2 μM 1,6-dinitropyrene in 1% DMSO for 24 hrs at 37°C in an atmosphere of 5% CO_2 in air. Cellular DNA was extracted in butanol and purified by reverse phase chromatography. The DNA-adducts were 5'-phosphorylated with T4 polynucleotide kinase and [γ-^{32}P]-ATP. The radiolabelled DNA-adducts were purified with a C18-reverse phase column and analysed by reverse phase high pressure liquid chromatography or two dimensional PEI-cellulose thin-layer chromatography and autoradiography. One major and two minor DNA-adducts were detected in skin explants and calf thymus DNA which had been treated with 1,6-DNP. The major 1,6-DNP induced DNA-adduct has been shown to be C-8 deoxyguanosine adduct. Using the same DNA-adduct detection system we detected bulky DNA damage in skin biopsies taken from the arms of a number of normal individuals. These DNA-adducts did not co-elute with any of the 1,6-DNP induced DNA-adduct standards. These DNA-adducts were probably formed by the incidental exposure of the individuals to environmental carcinogens. Therefore the procedure provides a means of monitoring the exposure of humans to environmental carcinogens and it may be of value in human risk assessment.

P48

RETINOIC ACID-INDUCED INHIBITION OF K1735-M2 MELANOMA CELL INVASION IN VITRO. Christine Helige, Josef Smolle, Esther Hartmann+, Regina Fink-Puches, Helmut Kerl, and Helmut A. Tritthart*, Department of Dermatology and Venerology, +Department of ophtalmology and *Department of Medical Physics and Biophysics, University of Graz, Austria.

Tumor cell invasion in vitro can be evaluated by using a recently developed computerized image analysis method (J. Invest. Dermatol. 94, 114-119, 1990). Immunohistochemically stained cryostat section from confrontation cultures of K1735-M2 melanoma cells with rounded fragments of embryonic chick heart tissue, which represent a stroma analogue, were used for measurement. 1 μM retinoic acid (RA) considerably impaired melanoma cell invasion as demonstrated by various measuring parameters. TUMAREA, expressing the amount of tumor tissue, indicates an antiproliferative effect. The parameter showed no change instead of a significant increase in the control experiment. Invasion parameter STRCSTR, expressing host tissue disintegration and degradation by invasion, indicates that after treatment with RA the stromal component was more preserved than in untreated controls. Beside the inhibitory effect of RA on melanoma cell invasion the compound modulated the attachment abilities of melanoma cells to the extracellular matrix components type I collagen and laminin. Pretreatment of the cells with 1 μM RA for 48 or 96 h resulted in significantly faster adhesion to both substrates, compared with control cells. However, melanoma cell directional migration, which might also play a role in invasion, was only moderately inhibited by RA analogue, using tpye I collagen - coated surfaces.

In conclusion, our data demonstrate that 1 μM RA showed a pronounced antiproliferative- as well as antiinvasive effect on high metastatic melanoma cells in vitro. Impairment of invasion might be due to an inhibitory effect of the compound on host tissue degradation and on altered adhesion dynamics. In addition, the antiproliferative activity may in part be responsible for inhibition of invasion.

P49

INFLUENCE OF COMPOUNDS INTERFERING WITH SIGNAL TRANSDUCTION PATHWAYS ON MELANOMA CELL DIRECTIONAL MIGRATION. R. Fink-Puches, Ch. Helige, H. Kerl, H.A. Tritthart, J. Smolle, Department of Dermatology and Institute of Medical Physics, University of Graz, Graz, Austria.

Signal transduction pathways as the Calcium-calmodulin-proteinkinase C cascade, G-protein-dependent processes and transmethylation reactions are supposed to play a role in tumor cell motility. In this study we examined the antimigratory effect of drugs interfering with steps of these pathways. The radiary migration of the murine melanoma cell line K 1735-M2 was evaluated by cumputerized image analysis. Untreated K 1735-M2 controls showed a migration rate of 377 +- 22 um/day. The calcium channel blockers verapamil and devapamil showed a slight reduction of motility. The calmodulin antagonists flunarizine and W-7 exhibited more effect on directional migration. 2uM of the PKC-inhibitor dequalinium decreased the migration rate to 154+-7um/day, 5 uM tamoxifen to 106+-15um/day. Similar results were found for H-7. 40uM of the G-protein antagonist L651582 showed a marked inhibition of motility (73+-11um/day). The transmethylation inhibitors dzADO and dzARI showed a dose dependent, but moderate inhibition of directional migration: 321+-15um/day, using 10 uM and 196+-17um/day applying 100 uM dzARI. The conclusions derived from this study are: 1) At least three signal transduction pathways are involved in the regulation of K 1735-M2 melanoma cell motility. 2) Pharmacological inhibition of these pathways leads to an inhibition of directional migration. 3) The assay of directional migration combined with computer assisted image analysis may be used for the screening of potential antiinvasive drugs.

P50

INVESTIGATION OF AUTO-ANTIBODIES AGAINST ANNEXINS IN SERA OF PATIENTS WITH SKIN DISORDERS. Nuß B, Kraus M+, Römisch J+, Haubitz I*, Pâques EP+, Hartmann AA, Bastian BC, Dept. of Dermatol. and * Dept. of Statistics, University of Würzburg, + Behringwerke AG Research Laboratories, Marburg, Germany.

Annexins/Lipocortins (AX) are a group of structurally related, calcium and lipid binding proteins which have been implicated as mediators of the anti-inflammatory action of glucocorticosteroids. Auto-antibodies (AAb) against AX-1 have been found in association with systemic lupus erythematodes and rheumatoid arthritis and their appearance has been discussed in the context of prolonged steroid medication. We therefore studied IgG-and IgM-AAb against AX-1 to AX-6 in sera of 212 patients with skin disorders and 117 healthy blood donors with newly established ELISAs, which in contrast to former studies enable a quantitative determination of each AAb tested. Patients were clustered into 8 groups according to their diagnosis as follows: autoimmune diseases (n=27), psoriasis (20), vascular diseases (27), acute dermatitis and eczema (45), malignant tumours (44), benign tumours (9), infectious diseases (28), miscellaneous (12). In all groups it was evident that antibodies could be present against every AX. Furthermore the level of IgM-AAb displayed a highly significant decrease with rising age, whereas IgG-AAb showed a less stronger tendency to rise with age. Females had an overall higher level of IgM-AAb than males. Patients were also clustered according to their drug history of corticosteroids. We could demonstrate a significant decrease of IgM-AAb and IgG-AAb against AX-3, -5 and -6 with rising doses of corticosteroids. The analysis of variance (ANOVA) of the different diesease groups investigated revealed no significant difference in their pattern of AX-AAb. We furthermore studied 27 female patients for the association of AX-AAb with anti-nuclear AAb (ANA). The 15 patients who had positive ANA had overall higher IgM- and IgG-AAb than the 12 ANA-negative patients.

This study demonstrates that for the investigation of AX-AAb not only AAb to AX-1 but AAb against all AX should be considered. In contrast to other studies which had been done only for AX-1-AAb our data does not show rising levels of any AX-AAb in association with increasing doses of corticosteroids. Furthermore we could not detect any disease specific pattern of AX-AAb in the disease groups studied.

P51

THE INHIBITION OF HUMAN SKIN PHOSPHOLIPASE A_2 BY ANNEXINS DEPENDS ON SUBSTRATE CONCENTRATION. Sellert C, Römisch J*, Pâques EP*, Bastian BC, Dept. of Dermatol. University of Würzburg, *Behringwerke AG Research Laboratories, Marburg, Germany.

Proteins of the annexin/lipocortin family have been claimed to mediate the anti-inflammatory action of glucocorticosteroids by the inhibition of phospholipases A_2 (PLA_2). This hypothesis has been challenged by the finding that annexins (AXs) do not interact directly with the enzyme in a classical enzyme/inhibitor behaviour but more probably block the access of the PLA_2 to its substrate by binding to phospholipids. Because former studies with skin PLA_2 suggested a direct inhibition of AX-1 (Cartwright et al, 1989 Br. J. Dermatol. 121(2):155-60), we now wanted to investigate the substrate dependence of this effect. For this purpose PLA2 enriched fractions of human epidermis and corium homogenates were tested with various amounts of AXs-1, -2 or -5. The respective AX was preincubated in separate series either with the substrate or with the enzyme. PLA_2 activity was determined by the release of fatty acids from a L-α-1-palmitoyl-2-^{14}C-arachidonyl-phosphatidyl-ethanolamine substrate. We found a partial inhibition of PLA_2 activity with all AXs tested when the respective AX was preincubated with the substrate (AX-5 > > AX-2 > AX-1). Inhibition occurred at substrate concentrations of 10 μM for AX-5 and 2μM for AX-1 and AX-2 at an AX concentration of 0,27 μM. When we preincubated the AX with the enzyme fraction instead of the substrate the inhibition was significantly lower (AX-1,2) or not detectable (AX-5) at identical substrate concentrations. That confirms the observation that AX binds to the substrate and not to the enzyme itself. Furthermore the inhibitory effect was completely abolished at higher substrate concentrations. There was no clear-cut difference in the inhibition profiles of the dermal and epidermal fractions.

This data demonstrates that the inhibition of human skin PLA_2 by AX occurres at unphysiologically low substrate concentrations, as it has been shown for PLA_2 of different origins as well. This observation makes it unlikely that AX is directly involved in the regulation of PLA_2 activity under physiological conditions.

P52

EFFECTS OF RETINOIDS ON THE LDL METABOLISM OF MACROPHAGES. Éva Remenyik, György Paragh, Gabriella Fóris, Péter Nyirkos, Dept. of Dermatology and 1st Dept. of Internal Medicine, Univ. Med. School, Debrecen, Hungary.

A well-known side effect of retinoid treatment is hyperlipidaemia. A possible explanation for this phenomenon might be the reduced consumption of lipids. Macrophages play an important scavenger role in lipid metabolism. The effect of retinoids on the lipid metabolism of macrophages has not been studied. 72 hour monocyte cultures provide means for studying both specific and scavenger LDL receptor pathways. In the case of the specific receptor 125I-LDL was used for binding and degradation assay. Cholesterol synthesis was also studied, as it inhibited by LDL. In the case of the scavenger receptor acetylated 125I-LDL (acLDL) was used for binding and degradation studies. ApoE synthesis was also studied, as it is enhanced by acLDL. The effect of pertussis toxin (PT), phorbol myristate acetate (PMA) and isotretinoin (RA) was studied on the lipid metabolism in monocyte cultures established from healthy volunteers. PT, PMA and RA did not effect the binding and degradation of LDL and acLDL. The cholesterol-synthesis inhibition effect of LDL was suspended by PMA, but was not effected by PT and RA. ApoE secretion induced by acLDL was slightly inhibited by PT, significantly reduced by RA and slightly increased by PMA. This suggests that the signal transduction is different for acLDL and LDL. The specific pathway was not effected by RA, while the apoE synthesis belonging to the effector part of the scavenger pathway was inhibited. RA, effecting the signal transduction, exerts its effect through the inhibition of protein kinase C. This may explain the lower levels of HDL-C observed during retinoid treatment.

P53

IMMUNOHISTOCHEMICAL DEMONSTRATION OF VITAMIN D RECEPTOR (VDR) EXPRESSION IN PSORIATIC SKIN AND BASAL CELL CARCINOMA BY LASER SCANNING MICROSCOPY. J.Reichrath[1], H.P.Baum[1], G.Unteregger[2], A.Theobald[1], G.Schock[1], F.A.Bahmer[1], E.W.Rauterberg[3]. [1]Department of Dermatology and [2]Institute of Human Genetics, University of the Saar, Homburg; [3]German Diagnostic Clinic, Wiesbaden; Germany.

The skin is a key tissue in both synthesis and function of vitamin D3. The most potent metabolite 1,25-dihydroxyvitamin D3 (1,25-D3) acts via binding to a nuclear high-affinity receptor (VDR). 1,25-D3 was shown to block proliferation and to promote differentiation in keratinocytes in vitro. Proliferation and differentiation of epidermal cells are autocrineously and paracrineously regulated by various growth factors, cytokines, hormones, and their corresponding receptors. We recently demonstrated a significant increase in VDR expression in epidermal keratinocytes and skin immune cells in lesional as compared to nonlesional psoriatic skin. VDR expression was furthermore very strong in the skin appendages i.e. hair follicles and sweat glands. We now analyzed the VDR expression in psoriatic skin and basal cell carcinoma (BCC) by confocal laser scanning microscopy (CLSM) applying an immunohistochemical method and the monoclonal antibody 9A7gamma to the VDR. Double staining procedures with monoclonal antibodies against PCNA and Ki-67 in conjunction with morphometric evaluation were performed to investigate the correlation between VDR expression and cell proliferation markers. Our observations indicate i) that BCC cells express VDR and ii) that VDR expression in BCC is correlated to the specific growth pattern of the tumour and iii) that CLSM represents a promising tool to analyze the coexpression of VDR and other growth or differentiation markers and iv) that VDR expression in psoriatic skin and BCC is not correlated to the expression of proliferation markers (Ki-67; PCNA). These findings point at a cell cycle independent VDR expression at least in these particular cells.

P54

1,25-DIHYDROXYVITAMIN D3 AND THE VITAMIN D ANALOGUE KH 1060 INDUCE HYPERPROLIFERATION IN NORMAL MOUSE EPIDERMIS

Claus Lützow-Holm*, Henrik Huitfeldt$, Per Brandtzaeg*, Ole P. F. Clausen* ,Inst. of Pathology, Univ.of Oslo*, The National Hospital, Rikshospitalet, and Natl. Inst. of Public Health$, Oslo, Norway.

1,25-dihydroxy vitamin D3 (calcitriol) affects differentiation and proliferation of epidermal keratinocytes in vitro and in vivo. We have studied the topical effects of 1,25 dihydroxyvitamin (0.08-2.0 ug/ml) and a new vitamin D analogue KH1060 (0.4.-2.0 µg/ml) on epidermal proliferation in hairless mice epidermis. Epidermis was examined at intervals from 4 h to 8 days after a single dose. The mitotic rate was assessed with the stathmokinetic method, and hyperplasia was scored in sections. Cell cycle parameters were measured by bivariate bromodeoxyuridine (BrdU)/DNA flow cytometry on isolated epidermal basal cells after pulse-labelling with BrdU.

Both calcitriol and HK1060 increased the mitotic activity from 8h, the latter drug being the most effective. Hyperplasia was evident from 20h. An altered cell cycle transit pattern with increased S-phase was observed. The results showed that a single dose of calcitriol and KH1060 induces increased epidermal proliferation in hairless mice, and influences the cell cycle in a way different from retinoic acid and other hyperplasiogens. This is also in contrast to the anti-proliferative effects of the same agents on the hyperproliferative disease psoriasis.

P55

DIFFERENTIAL EXPRESSION OF α6β4 INTEGRIN IN LESIONAL SKIN OF BULLOUS PEMPHIGOID AND EPIDERMOLYSIS BULLOSA ACQUISITA. Michalaki H., Staquet M.J., Cerri* A., Berti* E., Roche P., Machado P., Thivolet J., Nicolas J.F. INSERM U.346, Clinique Dermatologique, Pavillon R, Hôpital Edouard Herriot, 69437 Lyon Cedex 03, France and * First department of Dermatology, The University and IRCCS, Milan, Italy.

The integrin α6β4 complex is a protein of the membrane of basal keratinocytes, localized at the surface of cells in contact with the basement membrane zone in normal skin. The expression of α6β4 was investigated in several autoimmune blistering skin diseases including bullous pemphigoid (BP, n = 18), epidermolysis bullosa acquisita (EBA, n = 5), bullous systemic lupus erythematosus (BSLE, n = 2) and pemphigus vulgaris (PV, n = 7) using an indirect immunofluorescence technique. In lesional bullous skin of BP, α6β4 expression was either absent, or in some cases represented an unusual irregular patchy staining. In contrast, in lesional bullous skin from EBA, BSLE and PV, α6β4 expression was comparable to that observed in normal skin, i.e. a linear staining of the BMZ. Thus, analysis of the α6β4 integrin reactivity on lesional skin can allow a rapid and accurate diagnosis of BP. Furthermore, our findings support a potential role of α6β4 integrin in epidermal cell-BMZ adhesion.

P56

A STUDY TO DETERMINE THE SITE OF IgA ANTIBODY DEPOSITION IN LINEAR IgA BULLOUS DERMATOSES USING DIRECT IMMUNOFLUORESCENCE OF SODIUM CHLORIDE-SEPARATED SKIN. B.S.Bhogal, C.M.Stefanato, T.P. Chorzolski+, C. Kowalewski+, F. Wojnarowska*, and M.M. Black. Institute of Dermatology, UMDS,London.

+ Warsaw School of Medicine, Poland. *The Slade Hospital Oxford.

The Linear IgA bullous dermatoses (LABD) include LAD of adults and chronic bullous dermatosis of childhood (CBDC). Both are characterised by the linear deposition of IgA at the dermo-epidermal junction . The exact site of IgA antibody deposition remains an enigma.

Prost et al have demonstrated by direct immunoelectron microscopy that IgA is deposited in a mirror image pattern above and below the lamina densa (1). However, other workers have found the IgA deposits in a sublamina densa position alone or in a combination of sublamina densa and lamina lucida deposition (2,3).

We performed direct Immunofluorescence on sodium chloride separated- skin from biopsies obtained from 34 patients, 17 LAD and 15 CBDC to determine the site of IgA deposition.

Fourteen patients (8 LAD and 6 CBDC) showed linear BMZ staining with IgA only to the epidermal side of the split . Ten (8 LAD and 2 CBDC) showed binding only to the dermal side. . Four patients (all CBDC) were positive on both epidermal and dermal side of the split. In these four children the staining on the dermal side was always weaker than the epidermal side. Three patients (1 LAD and 2 CBDC) were positive on intact skin but became negative after sodium chloride separation.

The results of our study further emphasise the potential heterogeneity of the site of antibody deposition in LABD , thus suggesting that linear IgA antigen(s) may have both epidermal and dermal components.

1. Prost C., et al , Diagnosis of adult linear IgA dermatoses by immunoelectron microscopy in 16 patients with linear IgA deposits. J Invest Dermatol 1989;92.

2. Bhogal BS, et al , Linear IgA bullous dermatoses of adult and children: an immunoelectron microscopy study. Br. J Dermatol 1987;117.

3. Horiguchi Y. et al, Immunoelectron microscopy observation in a case of linear IgA bullous dermatoses of childhood. J Am Acad Dermatol 1981;14.

P57

THE ROLE OF α6 β4 INTEGRINS IN BLISTER DEVELOPMENT IN BULLOUS PEMPHIGOID. VA Venning, J Allen, G. Kirtschig, †J. Aplin, and F Wojnarowska. Dept of Dermatology, Oxford and † Dept of Obstetrics, University of Manchester, UK·

The α6β4 integrin is associated ultrastructurally with hemidesmosomes and the bullous pemphigoid (BP) antigen, a distribution suggesting an important role in adhesion of basal cells to the basement membrane zone (BMZ). Interference with this function is likely to be implicated in the pathogenesis of blistering. Using an immunofluorescent technique with monoclonal antibodies to the α and β subunits we investigated the tissue distribution of α6β4 in BP skin (uninvolved, perilesional and lesional) (n=9), and compared the findings with normal skin (n=3), and skin split by 1M NaCl incubation (n=2) and by suction (n=2), techniques known produce a split through the lamina lucida. The distribution of α6β4 was compared to the distribution of laminin and type 7 collagen.

In uninvolved and perilesional BP skin the distribution of the α6 and β4 subunits, laminin and type 7 collagen was identical to normal skin In early pre-blistered BP lesions, both integrin subunits were normally expressed. Within 5/6 BP blisters, both α6 and β4 integrin subunits were absent, in contrast to laminin and type 7 collagen which formed a continuous band along the base of the lesion. In only one BP blister was there retention of α6β4 integrin, both subunits being expressed along the blister roof. In chemically and suction split skin, α6β4 integrin was expressed along the epidermal aspect of the split, whereas laminin and type 7 collagen were expressed along the dermal aspect.

These findings rule out a widespread abnormality of α6β4 integrin expression affecting uninvolved BP skin and early non-blistered lesions. In the majority of developed BP blisters α6β4 integrin is undetectable either through loss of the molecule or through conformational changes preventing recognition of the epitopes by the monoclonal antibodies. The retention of α6β4 integrin in artificially split skin suggests that there are alternative mechanisms by which separation occurs through the lamina lucida.

P58

IMPROVED ASSESSMENT OF ANCHORING FIBRIL ABNORMALITIES IN DYSTROPHIC EPIDERMOLYSIS BULLOSA USING NOVEL PRE-EMBEDDING IMMUNOGOLD ELECTRON MICROSCOPY TECHNIQUES. J.A.McGrath, A.Ishida-Yamamoto, J.R.McMillan, A.O'Grady, R.A.J.Eady. Dept. of Cell Pathology, St. John's Institute of Dermatology, St. Thomas's Hospital, London SE1 7EH.

Dystrophic epidermolysis bullosa (DEB) encompasses a spectrum of inherited disorders characterized by trauma-induced mucocutaneous blistering and scarring. There are both autosomal dominant and recessive forms of the condition which range clinically from mild localized signs to more severe generalized manifestations. Laboratory diagnosis usually depends on demonstrating a reduced number or altered ultrastructure of anchoring fibrils associated with sub-lamina densa blistering, and on immunofluorescent staining of the dermo-epidermal junction using anti-type VII collagen antibodies. However, these techniques are not able to give any information about the precise localization of type VII collagen within anchoring fibril or fibril-like structures and cannot discriminate between certain dominant and milder recessive sub-types of DEB. In an attempt to define the protein abnormalities more clearly, we have examined DEB skin by immunoelectron microscopy. Skin samples from 19 DEB patients (4 dominant, 6 localized recessive, 9 generalized recessive) were incubated *en bloc* with either LH 7:2 or GDA-J/F3 (monoclonal antibodies labelling different anchoring fibril epitopes) and then with 1nm colloidal gold-labelled secondary antibodies. After fixation, the gold labels were silver enhanced before embedding in resin. Examination of ultrathin sections showed a range of immunolabelling patterns on anchoring fibrils and on the lamina densa, with dominant DEB cases showing only a slight but variable reduction in labelling density, in parts similar to normal human skin. In localized recessive DEB skin, some filamentous structures just below the lamina densa had specific immunogold labelling despite their lack of resemblance to definitive anchoring fibrils [1]. This labelling was most prominent beneath hemidesmosomes. The anti-type VII collagen labelling in some recessive DEB samples resembled that seen on developing anchoring fibrils in normal fetal skin, possibly indicating incomplete anchoring fibril formation. In generalized recessive DEB, occasional immunolabelling, even in the most severe phenotypes, was found within the lamina densa and on scanty filamentous structures at the site usually occupied by anchoring fibrils. In summary, this technique provides an improved means of assessing anchoring fibrils and type VII collagen expression at the dermo-epidermal junction in DEB and could be useful in clarifying disease subtypes and aiding genetic counselling. [1] J Invest Dermatol 1985:84:374-7

P59

MHC CLASS I AND II ANTIGENS IN LINEAR IGA DERMATOSIS. P M Collier and F Wojnarowska. Dept of Dermatology, Slade Hospital, Oxford, UK.

Linear IgA dermatosis (Lad) comprises two clinical entities which are part of the same disease, Chronic bullous disease of childhood (CBDC) and linear IgA disease of adults. The aetiology of the disease is unknown but an increased association with HLA B8 has been shown. The aim was to extend this study to the class II region.

The diagnosis of LAD was based on typical clinical features and positive direct immunofluorescence, 31 adults and 24 children were included in the study. HLA typing was performed using a lymphocyte microcytotoxicity assay with a large battery of antisera against defined HLA class I and II antigens. The results were compared to standardised gene frequencies for the population studied, and analysed using Chi square tests with Yates's correction.

A significant increase in the incidence of HLA B8, DR3 and DQw2 were found. The differences were most marked in the children, for DR3 this was 71% CBDC and 45% of adults (control 23% P=0.0001 & 0.003, and for DQw2 was 75% (p=0.0001) and 55% (p=0.003) respectively. As in SLE, patients not DR3 tended to be DR2 and HLA Cw4 was reduced (0/24 CBDC and 1/31 adults p=0.013 & 0.007). There was a total absence of DR1 in the CBDC group (p=0.02), and it was found in only 2 of the adults (p=0.093) (control 24%), DR4 was also reduced (2/24 & 8/31 p=0.005 & 0.232) suggesting that the 'rheumatoid susceptibility motif' present on DR1 and most DR4 subtypes may be protective for linear IgA dermatosis.

These results provide new insight into the pathogenesis of this disease, and further studies are in progress to study this linkage more closely.

P60

REDUCTION OF DAPSONE TOXICITY IN DERMATITIS HERPETIFORMIS (DH) BY THE CONCURRENT ADMINISTRATION OF CIMETIDINE L.E.Rhodes,[1] M.D.Coleman, A.K.Scott, J.L.Verbov,[1] P.S.Friedmann,[1] A.M.Breckenridge, and B.K.Park. Dept. of Dermatology[1], Royal Liverpool University Hospital & Dept. of Pharmacology & Therapeutics, University of Liverpool, UK.

Haemolysis and methaemoglobinaemia during therapy with dapsone are caused by the hydroxylamine derivative of dapsone. Since cimetidine reduces hepatic N-hydroxylation of the drug but does not inhibit acetylation, we wished to see if concurrent administration of cimetidine would reduce dapsone-induced haematological toxicity.

Six patients with DH on stable doses of dapsone ranging from 50-350mg daily were studied at six weekly visits. Cimetidine 400mg t.d.s. was taken during weeks 3 and 4. Trough and peak levels of methaemoglobin relative to haemoglobin were assayed by spectrophotometry, and of dapsone and monacetyl dapsone by HPLC. Dapsone and its metabolites were measured in 12 hr urine samples.

	Peak MetHb%	Trough Dapsone %dose/ml	Urinary Dapsone Hydroxylamine%dose
mean weeks 1&2	7.65(SD3.1)	0.018(SD.007)	17.63(SD8.9)
week 4*	5.61(SD2.12)**	0.025(SD.008)***	13.5(SD8.7)**
week 6	7.1(SD2.2)	0.019(SD.001)	14.7(SD8.5)

*2nd week of cimetidine therapy, ** p<0.05, ***p<0.01

Daily dapsone dosage and methaemoglobinaemia were linearly correlated, r=0.91(SD.04). A sustained fall of 27.3%(SD6.7) in methaemoglobin formation during cimetidine administration was accompanied by reduction of symptoms in all four of the patients who had previously complained of headaches and lethargy. There was a marked improvement in the usually cyanotic appearance of the patient taking the highest dose of dapsone. No patient experienced any deterioration in their DH.

Thus cimetidine produces improved patient tolerance of dapsone in DH.

P61

A REFLECTANCE SPECTROSCOPIC AND LASER DOPPLER FLOWMETRIC STUDY OF THE CUTANEOUS CORTICOSTEROID VASOCONSTRICTION ASSAY. Andersen P, Millioni K, Kubota K, Maibach H. Department of Dermatology, University of California, School of Medicine, UCSF, San Francisco

Visual cutaneous vasoconstriction induced by topical corticosteroids was investigated using noninvasive bioengineering techniques. *Methods*: In alcoholic solution different potency corticosteroids were applied topically under occlusion for 16 h and a special betamethasone-17-valerate 1% (w/v) 50 μ m thick patch (BIO-PSA®) was used to study time dynamic changes. Cutaneous blanching was investigated using visual scoring, reflectance spectroscopy (RS) and laser Doppler velosimetry (LDV). The applied RS technique allows separation of cutaneous hemoglobin content into oxygenized (Ox Hem) and deoxygenized hemoglobin (Deox Hem) components. *Results*: Betamethasone-17-valerate was the most potent vasoconstrictor inducing significant visual blanching, decreased Deox Hem (30%), Ox hem (33%) and blood flow (BF) (18%) ($p<0.01$). Only the most potent corticosteroid caused any significant decrease in blood flow although less potent steroids caused significant visual blanching and decreased Deox Hem. The time dynamic changes induced by the betamethasone-17-valerate caused maximal skin blanching 12 h post application at the 12 h exposure site. The highest decrease in Deox Hem at exposure times $\leq$ 8 h was also found 8 h - 12 h post application. All exposure loci except 6 h and 12 h sites still showed some venous vaso-constriction 24 h post application, but for time intervals $\geq$ 32 h a paradox increase in Deox Hem was found at all exposure sites $\geq$ 3 h. Both cutaneous vaso-constriction and post constriction hyperemia were caused by changes in Deox Hem. *Conclusion*: Measured reflectance spectroscopic the corticosteroid induced blanching was predominantly veno constriction and only the most potent steroid caused significant decrease in Ox Hem and BF. Our results may explain why previous attempts to improve the cutaneous vasoconstriction assay using laser Doppler flowmetry have been unsuccessful. Corticosteroid induced changes in vascular tonus are predominantly at venous vessels.

P62

DuP 654 IS A POTENT 12(S)-HYDROXYEICOSATETRAENOIC ACID RECEPTOR ANTAGONIST IN HUMAN EPIDERMAL CELLS. Thomas Ruzicka, Petr Arenberger, Lajos Kemény, Achim Raap* and Benjamin Armah*, Department of Dermatology, University of Munich, Beiersdorf AG, Hamburg, FRG*.

Substance DuP 654 (2-phenylmethyl-1-naphtol), a well studied 5-lipoxygenase inhibitor, has been reported to exert antiinflammatory effects in a murine skin inflammation model. Since recently we have described high affinity binding sites for 12(S)-HETE on epidermal cells, we were interested whether this substance may also influence 12(S)-HETE receptors in the human epidermal cell line SCL-II.

Standard radioligand binding assays with 12(S)-(3H)-HETE have been used to determine the antagonist potency of DuP 654. The binding data were analysed with a nonlinear computer fitting program MxN-FIT.

DuP 654 antagonized 12(S)-HETE binding in a dose-dependent manner with a Ki of 3.41±0.23 nM. The antagonistic effect was reversible. After 1 and 24h preincubation, the substance had no more significant inhibitory effect at concentrations between 0.1 nM and 10 µM on 12(S)-HETE binding sites (Bmax of 215,000±21,000 receptors per cell) or on receptor affinity (Kd of 3.25±0.42 nM).

Our results show that DuP 654 is a potent 12-HETE receptor antagonist, and therefore may be of benefit in skin diseases with elevated 12-HETE levels.

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INTERLEUKIN-1 INCREASES HYDROXY-LINOLEIC ACID ISOMERS FORMATION BY HUMAN DERMAL FIBROBLASTS IN CULTURE. Nuria Godessart and Luis Vila, Department of Inflammation and Cell Proliferation Mediators, FISP, Hospital de la Santa Creu i Sant Pau. Barcelona, Spain.

Interleukin-1 (IL-1) is present in human skin in normal and pathophysiological situations. IL-1 increases the production of prostaglandins by human dermal fibroblast. However, there is no information concerning its effect on the linoleic acid (LA) metabolism. LA metabolites specially 13-HODE exhibit an intriguing biological role apparently related with anti-adhesive and anti-inflammatory activities. The aim of this work was to investigate the ability of fibroblasts to metabolize linoleic acid and its modulation by IL-1. Cultured fibroblasts were incubated with several concentrations of human recombinant IL-1ß for different periods of time. Then IL-treated and untreated cells were incubated in the presence of exogenous labelled LA. The products formed were analyzed by HPLC. LA was metabolized by fibroblasts mainly in 13-HODE and 9-HODE. After 24 hours of treatment with 10 U/ml of IL-1ß LA metabolism increased a maximum of 8-10 times, in a time- and dose-dependent fashion. This effect was completely abolished by 3µg/ml cycloheximide. 1 mM aspirin or 50 µM indomethacin completely inhibited 13- and 9-HODE formation in controls and IL-1ß treated fibroblasts. Results from fibroblasts were consistent with those obtained with isolated ram seminal vesicles cyclooxygenase. These results indicate that IL-1 increases LA metabolism in human dermal fibroblasts by enhancing cyclooxygenase expression. Further investigations are needed to find out the biological relevance of the effect of pro-inflammatory cytokines such as IL-1 that can promote LA metabolism on skin.

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EFFECT OF INTERLEUKIN-1 ON THE BIOSYNTHESIS OF 15-HYDROXY-EICOSATETRAENOIC ACID IN HUMAN DERMAL FIBROBLASTS. Luis Vila, Nuria Godessart, Luis Puig* and Josep Mª de Moragas*. Department of Inflammation an Cell Proliferation Mediators, FISP and Department of Dermatology*, Hospital de la Santa Creu i Sant Pau. Barcelona, Spain.

Biological actions of Interleukin-1 (IL-1) are at least in part mediated through the induction of prostaglandins in several cell types including human dermal fibroblasts. No information about the effect of IL-1 on the biosynthesis of 15-HETE is at present available. Reduced levels of 15-HETE has been described in psoriatic dermis. Moreover, beneficial effects of application of 15-HETE on psoriatic lesions has been reported. The aim of this study was to investigate the effect of IL-1 on the biosynthesis of 15-HETE by fibroblasts. Cells were incubated with different concentrations of human recombinant IL-1α or β for several periods of time, prior addition of 25µM of ^{14}C-arachidonic acid. Eicosanoids were analyzed by HPLC. IL-1 increased significantly the production of 15-HETE (3-4 fold), but also HHT, 11-HETE and prostanoids, reaching a maximum at a concentration of 10 U/ml after 9-24 hours of treatment. This effect was completely abolished by 1µM actinomycin D or 3 µg/ml cycloheximide. Whereas 50 µM indomethacin strongly inhibited 15-HETE (>97%), aspirin (100-1000µM) was unable to significantly inhibit 15-HETE in both untreated and IL-treated fibroblasts. When isolated ram seminal vesicles cyclooxygenase was incubated with arachidonic acid, 15- and 11-HETE were also formed, and aspirin failed to completely inhibit 15-HETE. These results provide evidence that IL-1 increases 15-HETE formation mainly through cyclooxygenase expression. Since the regulation and activity of IL-1 on skin are not completely elucidated, more research is needed to asses the pathophysiological relevance of these findings.

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BIOSYNTHESIS AND ESTERIFICATION OF 13-HYDROXY-OCTADECADIENOIC ACID INTO PHOSPHOLIPIDS BY HUMAN EPIDERMAL CELLS IN SUSPENSION. Mercedes Camacho and Luis Vila, Department of Inflammation and cell proliferation mediators, FISP, Hospital de la Santa Creu i Sant Pau. Barcelona, Spain.

The presence of high amounts of free and esterified 13-HODE has been described in psoriatic skin. 13-HODE exhibit anti-adhesive and anti-inflammatory properties. Cellular and enzymatic origin of 13-HODE present in the skin lesions remains at present unclear. It was the aim of this study to investigate the biosynthesis and esterification of 13-HODE in epidermal cell (EC). EC suspensions ($2x10^7$cel/ml) were incubated with 100 µM ^{14}C-LA for 10 min and products were analyzed by HPLC. EC produced 13-HODE and 9-HODE as a major products (range 3.9-6.1 and 1.9-18.6 pmol/10^6-cel for 13-HODE and 9-HODE respectively). Both 13-HODE and 9-HODE were strongly inhibited by 100 µM ETYA (>97%) indicating their enzymatic origin, and by 1mM aspirin (>80%) that suggested a predominant cyclooxygenase common origin. Linoleic acid incubated in the presence of isolated ram seminal vesicles cyclooxygenase also yielded 13-HODE and 9-HODE as major products which were both 13-HODE and 9-HODE inhibited by aspirin. Labelled 13-HpODE and 13-HODE obtained from ^{14}C-LA using soybean lipoxygenase-1 and afterwards purified by HPLC, were used to perform esterification experiments. EC suspensions incubated with 5µM of ^{14}C-13-HpODE or ^{14}C-13-HODE for 2 hrs were extracted and analyzed by TLC. 13-HpODE was totally transformed, mainly in 13-HODE, after incubation with EC. 67-87% of the radioactivity incorporated into phospholipid fraction (≈1% total added radioactivity) was associated to phosphatidyl choline. These results provide evidence that 13-HODE is formed in normal epidermal cells mainly through cyclooxygenase activity, being part of it esterified in the glycero-phospholipids mainly in PC. Further research will be necessary to clarify the biological role of both free and esterified 13-HODE in normal and pathological circumstances.

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KERATINOCYTE-INDUCED TRANSFORMATION OF NEUTROPHIL DERIVED LTA_4 INTO LTB_4: EVIDENCE FOR EXISTENSE OF A PUTATIVE KERATINOCYTE LEUKOTRIENE A_4 HYDROLASE. Lars Iversen, Karsten Fogh, [1]Vincent Ziboh, Peter Kristensen, Anne Schmedes and Knud Kragballe. Departments of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark and [1]University of California, Davis, USA.

Although a previous study from our laboratory has indicated that the co-incubation of human keratinocytes (KC) with polymorphonuclear cells (PMN) resulted in the generation of leukotriene B_4 (LTB_4) the mechanism of this transcellular cooperativity has remained unclear. We have extended the above observation to test the hypothesis that PMN-derived leukotriene A_4 (LTA_4) is contributed for transformation into LTB_4 by a putative KC epoxy hydrolase. Specifically incubation of KC with LTA_4 (10 µM) resulted in KC-induced transformation of LTA_4 into LTB_4 as determined by cochromatography with authentic LTB_4 on HPLC, by UV-scan and by RIA. No LTA_4 was transformed into LTB_4 spontaneously. Furthermore it was shown that pre-incubating the KC for 15 min. or 60 min. with bestatin (70 µM), an inhibitor of LTA_4 hydrolase, resulted in a time-dependent decrease in KC-induced transformation of LTA_4 into LTB_4. These findings indicate that while KC apparently cannot directly metabolize AA via the 5-lipoxygenase enzyme it can transform PMN-derived LTA_4 into LTB_4 suggesting the possible existence of a KC-LTA_4 hydrolase.

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C5A ANAPHYLATOXIN - A POTENT ACTIVATOR OF HUMAN EOSINOPHILS

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The complement system probably represents an important amplification system for the propagation of allergic as well as pseudoallergic inflammatory reactions. In the present study, the effect of the major anaphylatoxin C5a, in comparison to PAF, on highly purified eosinophils (≥95%) (EO) was evaluated using functional (lucigenin-dependent chemiluminescence/-CL, release of eosinophil peroxidase/EPO) as well as morphological criteria (scanning and transmission electron microscopy, ultrastructural detection of H_2O_2). In parallel to PAF, C5a induced double-peaked CL responses in EO lasting for 30 min with a maximum within 3 to 7 min after addition of the stimulus. Maximal effects, comparable to IL-5 (100 U/ml), were observed at a concentration of 10^{-5} to 10^{-6}M and 10^{-7} to 10^{-8}M for PAF and C5a resp. Typical morphological changes were induced in EO by both stimuli. Upon stimulation with C5a EO maintained their spheric structure developing short pseudopodia-like protrusions, H_2O_2 production was detected only in the small cytoplasmic vesicles. In contrast, PAF induced the generation of a number of digitating pseudopodia-like protrusions, significant H_2O_2 production was observed on the outer surface of the plasma membrane in the contact zones in between adjacent cells. Morphological signs of degranulation induced by C5a and PAF were accompanied by the significantly increased release of EPO. To evaluate the modulating effect of cytokines on CL response, EO were preincubated with IL-2, IL-3, IL-5, IL-8, IFNγ, GM-CSF and TNFα for 60 min at 37°C and subsequently stimulated with C5a (10^{-7}M) or PAF (10^{-8}M). However, no significant differences between EO preincubated with cytokines and EO preincubated with medium alone could be detected. The data clearly indicate that C5a induces effects in human EO comparable to PAF in the assay system tested. Therefore, it is tempting to speculate that C5a generated during infections may be an additional inflammatory mediator, involved in the activation of EO in atopic diseases.

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EFFECTS OF SELECTIVE PROTEIN KINASE C-INHIBITORS ON THE EXOCYTOSIS FROM HUMAN BASOPHILS. U. Amon, F. Bauer[1] Dept. Dermatology, Medical University of Lübeck, FRG; [1]Dept. Exp. Dermatology, Hoffmann-La Roche Ltd, Switzerland

Skin mast cells and basophils play an important role in acute and chronic inflammatory skin diseases. It has been demonstrated recently that Fc_ϵRI-mediated activation of both cell populations involve activation of PKC. We have previously shown that mediator release from isolated human skin mast cells and basophils was strongly suppressed by the potent but unselective PKC-inhibitor staurosporine (STA) (1). In the present study we investigated the effects of four strong and selective PKC-inhibitors (STA-derivatives) on the exocytosis from human basophils in comparison to STA. Basophils were obtained by venipuncture from non-allergic blood donors and isolated by dextran sedimentation. Cells were preincubated with the inhibitors (1nM-10µM) for 15 min (37°C) before being challenged with an optimal concentration of anti-IgE for another 30 min (37°C). Histamine release (HR) was measured by an automated procedure. STA, which inhibits the PKC and other protein kinases in the nM-range, showed a dose-related highly significant inhibition of the HR with an IC_{50} value of 0.05µM. In contrast, the other compounds (Ro 31-8830, 31-7549, 31-8220, 31-8425) with selective *in vitro*-activity for the PKC (2) did not inhibit the IgE-mediated exocytosis. At concentrations of 10µM these substances did potentiate the HR up to 50 %. To exclude cytotoxic effects LDH measurements were performed, which, however, were not different from controls (buffer). Therefore, it is questionable, whether the inhibitory effects of STA are solely due to PKC-inhibition. To evaluate the role of the PKC as possible target for anti-inflammatory therapy further investigations with selective PKC-inhibitors of different chemical origin are necessary. (1) Amon U et al, Ann Allergy 1991; 66: 80; (2) Davis PD et al, FEBS Let 1989; 259: 61

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EFFECTS OF TRADITIONAL CHINESE HERBAL THERAPY EXTRACTS ON CON-A INDUCED CELL PROLIFERATION AND IL-4 INDUCED CD23 EXPRESSION ON MONOCYTES. Y. L. Latchman, M.H.A. Rustin, M.P. Sheehan, D.J. Atherton, G.A. Bungy Poor Fard, J. Brostoff, Departments of Dermatology, The Royal Free Hospital and School of Medicine and The Hospital for Sick Children and Department of Immunology, University College and Middlesex School of Medicine, London.

Traditional Chinese Herbal therapy has been shown to be an effective treatment of refractory atopic dermatitis in adult patients. In order to investigate the mode of action of this treatment, the effect of an extract of Traditional Chinese Herbal Therapy (TCHTE) on various cells of the immune system was studied.

Firstly the effect of TCHTE on the mitogenic activity of Con A on lymphocytes collected from non-atopic healthy individuals was studied. With a sub-optimal dose of Con A and using a range of concentrations of TCHTE there was no significant change in Con A induced T cell proliferation and no alteration of cell proliferation with TCHTE alone was observed. There was no effect on cell viability.

Secondly the effect of TCHTE on IL-4 stimulated monocyte CD23 (low affininty IgE receptor) expression was studied. Buffy coat cells were incubated with varying doses of IL-4 and the effect of simultaneous incubation with either TCHTE or a placebo extract was studied. Using double FACS analysis with monoclonal antibodies to monocytes and CD23 there was a greater than 60% inhibition of CD23 expression with the TCHTE compared to the placebo ($p < 0.0001$).

The mode of action of Traditional Chinese Herbal Therapy in atopic dermatitis is not yet known but modulation of CD23 expression may be an important effect.

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LOCALISATION AND SYNTHESIS OF GLYCOSAMINOGLYCANS IN ISOLATED HUMAN HAIR FOLLICLES. Watson, L.P., Blount, M.A., Westgate, G.E., Gibson, W.T. Personal Products Research Division, Unilever Research, Colworth House, Sharnbrook, Bedford, UK.

Previous biochemical and immunohistochemical studies on skin biopsies have demonstrated that proteoglycans (PG's) have an important role in the hair growth cycle. The levels of chondroitin PG's vary according to the stage of the cycle, being maximal during the growing phase and diminishing during the regression and resting phases. With the development of the human hair follicle (HHF) model, it became necessary to investigate whether the distribution and turnover of PG's in vitro resembled that found in whole skin. HHF were isolated from the skin of female patients. They were grown in Williams E medium containing insulin, hydrocortisone and antibiotics for 9 days. Follicles were taken on days 1,3,5,7,9 for immunohistochemical and biochemical analysis. For immuno-histochemistry, follicles were fixed in paraformaldehyde, frozen and cryosectioned. After chondroitinase ABC digestion, sections were stained with monoclonal antibodies for chondroitin and chondroitin-6-sulphate and visualised with immunoperoxidase. Biochemical analysis was by radiolabelling the glycosaminoglycans (GAG's) with ^{35}S sulphate for 24 hrs at each time point. The GAG profile was resolved using anion exchange chromatography (Mono Q) on FPLC. During culture, hair growth measurements showed that the HHF continued to grow at approximately 0.3mm/day. Sections showed that although the epithelial component of the HHF grew, the connective tissue sheath (CTS) did not extend, but nonetheless it maintained its integrity. Chondroitin and chondroitin-6-sulphate expression was observed throughout the 9 days in culture, being present in the dermal papilla and CTS. The biochemical analysis showed that the isolated follicles synthesise heparan sulphate and chondroitin sulphate throughout the time course, although the relative amount of chondroitin sulphate increased and heparan sulphate decreased by 8% over this time. The techniques used show that isolated HHF are able to maintain and synthesise GAG's in culture and are comparable to those seen previously in whole skin sections.

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INFLUENCE OF THE EXTRACELLULAR MATRIX AND GROWTH FACTORS ON THE REGULATION OF COLLAGENASE ACTIVITY USING *IN VITRO* WOUND HEALING MODELS. Edward J. Wood, Paul G. Genever and William J. Cunliffe*, *Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, and *Department of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, UK.*

Some of the factors affecting active and latent collagenase production by human dermal fibroblasts were investigated using the dermal equivalent system as an *in vitro* wound healing model. Dermal equivalents (DEs) are collagen gels containing fibroblasts. They may remain attached to the culture vessel or may be detached and allowed to float, each of these providing a different environment to the growing cells. Collagenase activity in the culture medium was assayed by measuring its ability to digest tritiated collagen gels, a brief preincubation with trypsin being used to activate any latent collagenase. In attached DEs, fibroblasts showed a bipolar morphology and underwent a 30-fold proliferation over 28 days whereas in detached DEs the extent of proliferation was about one-tenth of this and the cells showed a stellate morphology.The effect of growth factors on fibroblast collagenase production was dependent on the type of DE in which they grew (attached or detached) and the length of time spent in culture before the growth factors were added. After 2 days in culture, fibroblasts in attached DEs were significantly stimulated by 5ng/ml PDGF to produce a 3-fold increase in latent but not active collagenase levels, yet were unresponsive to EGF or TGF-β. However, in detached DEs, EGF stimulated a 3-6-fold increase in collagenase production whereas PDGF and TGF-β had little effect. Fibroblasts in both attached and detached DEs were sensitive to PDGF and TGF-β when they were added after 6 days in culture (causing up to a 2.5-fold increase and 40% decrease respectively in latent collagenase levels), but not EGF. These data confirm that fibroblasts are highly sensitive to their environment and that this modulates their response to growth factors. The attached and detached DE systems may offer models for the behavior of fibroblasts at different phases of the wound healing process.

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EFFECT OF GROWTH FACTORS ON COLLAGEN PRODUCTION AND GENE EXPRESSION IN FIBROBLASTS CULTURED IN DERMAL EQUIVALENTS. Jaswant K. Jutley, William J. Cunliffe*, Edward J.Wood. *Dept..of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, and *Dept .of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, UK.*

Growth factors are essential in the wound healing process. However, in the study of this process it has become increasingly evident that the behaviour of fibroblasts in monolayer cultures does not correspond well with the situation *in vivo*. Therefore the effects of growth factors on collagen production and gene expression are increasingly being studied in 'dermal equivalent' models (DEs). We studied attached DEs made by seeding fibroblasts into collagen gels which remained attached to the plastic wells. The effect of growth factors on collagen production was determined by measuring the incorporation of ^{35}S-methionine into protein in the presence of TGF-β (2-10ng/ml), EGF (2-20ng/ml), or PDGF (2-10ng/ml) for 18h. DEs were harvested, treated with collagenase to release cells, sonicated and samples analysed by 5% SDS-PAGE followed by autoradiography. *In situ* hybridisations were performed on cryosections of DEs grown in medium containing the growth factors. The sections were probed with biotin-labelled prepro α I(I), α 2(I) collagen (kindly provided by Dr.G.Tromp, Philadelphia, USA), and procollagen α I(III) (kindly provided by Dr.Ala-Kokko, Philadelphia, USA) . Autoradiographs showed increased levels of α I(I), α 2(I) and α I(III) collagen in DEs treated with TGF-β compared with controls. DEs treated with 2-10ng/ml EGF showed reduced levels of α I(I) and α I(III) collagen. However, EGF at 20ng/ml resulted in an increase to untreated control levels of α I(I) and α I(III) collagen. There was no significant effect on the levels of α 2(I) collagen. *In situ* hybridisation was scored as follows: 1-5 positively stained cells per field of view (over several areas) = +, 5-11 = ++, 11 > = +++. DEs treated with TGF-β showed increased mRNA expression for α I(I) (++), α 2(I) (+++) and α I(III) (+++) collagen. There was little collagen mRNA expression in DEs treated with PDGF or EGF, α I(I) (+), α 2(I) (+), αI(III) (+), compared with controls. Thus collagen gene expression by dermal fibroblasts in attached DEs is modulated by growth factors. This model has attractive characteristics for studying the process of ECM formation and its remodelling in wound repair.

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COLLAGEN PRODUCTION AND COLLAGEN GENE EXPRESSION BY ACNE KELOID FIBROBLASTS. Jaswant K Jutley, William J.Cunliffe*, Alison Layton* and Edward J. Wood. *Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, and *Department of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, UK.*

A keloid is a scar that grows beyond the confines of the original wound. The aetiology of keloids is unknown but both local and constitutional factors appear to be involved. The dermis of keloid scars is characterised by increased cellularity with the production of excessive extra cellular matrix (ECM) arranged in a loose and disorganised way. We investigated collagen composition and collagen gene expression in acne keloid fibroblasts.

Acne keloid samples were obtained from patients and diagnosed as early keloid (EK), late keloid (LK) and compared with unaffected samples adjacent to EK (nEK) and late keloid (nLK). Tissue was cultured for 24h at an air/liquid interface with either ^{3}H-Proline or ^{35}S-Methionine in organ culture. It was then washed, homogenised, centrifuged and samples run on 5% SDS-PAGE. The amounts of type I and type III collagen were quantified by densitometry. *In situ* hybridisation was performed on cryostat sections using biotin-labelled cDNA probes for prepro collagen α I(I), α 2(I) (kindly provided by Dr.G.Tromp, Philadelphia, USA) and procollagen α I(III) (kindly supplied by Dr.Ala-Kokko, Philadelphia).

EK tissue contained 11.4% α I(I) collagen as compared with 5.8% in nEK or 7.2% in nLK but the content in LK was much reduced (3.2%). Similarly EK (14.3%) and nLK (15.9%) contained much more α 2(I) collagen compared with LK (5%) or nEK (7.2%). In contrast, nLK contained 7.7% α I(III) collagen compared with LK (3%), EK (3.9%) or nEK (5.2%). The expression of α I(I) and α I(III) collagen mRNA in acne keloid dermis was demonstrated by *in situ* hybridisation. Moderate levels of α I(III) collagen mRNA were detected in the dermis. Thus acne keloid tissue in early stages has an increased percentage of type I collagen compared with adjacent apparently unaffected tissued. However, unaffected regions adjacent to late keloids contained more type III collagen compared with the keloids themselves.

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EHLERS-DANLOS TYPE VII C IS THE HUMAN COUNTERPART OF DERMATOSPARAXIS IN ANIMALS. B. V. Nusgens*, Ch. Verellen+, T. Le-Hermanns§, A. De Paepe*, G. E. Pièrard§ and Ch. M. Lapière*. * CHU University of Liège, Dermatology and § Dermatopathology, + Saint-Luc, Catholic University of Louvain, * Genetics, University of Ghent.

ED VII A and B are related to a defective processing of, respectively the pN-alpha1 and the pN-alpha2 collagen chain. Both forms are related to mutations of the genes coding for the collagen polypeptides resulting in the suppression of the cleavage site for procollagen I-N-peptidase. A third form, ED VII C depending on the lack of the enzyme, has also been hypothesized. We present here the first evidence of its existence. The patient is a two year old girl suffering of easy bruising, skin fragility and facial dysmorphy. By electronmicroscopy, the collagen fibrils in skin display a hieroglyphic pattern in cross section similar to that observed in animal dermatosparaxis. The collagen extracted from skin is made predominately of pN-alpha1 I and pN-alpha2 I polypeptides in a 2 to 1 ratio that can be converted to mature alpha chains by purified procollagen I-N-peptidase. pN-I collagen polypeptides also accumulate in skin cell cultures.

There are more analogies (clinical, morphological and biochemical) between ED VII C and dermatosparaxis in various animal species than between ED VII C and ED VII A and B in the human.

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T GAMMA DELTA RECEPTOR BEARING LYMPHOCYTES IN SYSTEMIC SCLEROSIS (SSc): A CLUE TO THE DISEASE PATHOGENESIS ? M. Matucci-Cerinic[1], L. Ghersetich[2], L. Sacerdoti[1], M. Dahm[2] and T. Lotti[3]. Dept. of Clinical Medicine IV [1] and Dermatology I [2], University of Florence; Dept. of Dermatology,[3] University of Siena.

T gamma delta cells are involved in mucosal immunity and in responses to heat shock antigens and may play a role in autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis (Klin. Woche 1990; **68:**489). SSc is actually considered as a disease mediated by T lymphocytes and autoimmune perturbation leading to endothelium derangement and fibroblast activation. An association has been found between SSc and an 11.3-kb Pvu II fragment in the T cell receptor gamma delta gene (Arthritis Rheum 1990; **33:**569). This evidence prompted us to investigate the presence of T gamma delta (Tgd) cells in the affected (5th finger of the left hand) and unaffected skin (buttock) of 6 patients affected with limited cutaneous SSc and in 6 controls (5th finger left hand) matched for sex and age. The skin was processed as described previously (J. Immunol. 1989; **143:**2480). Immunohistologic staining was performed using CD4 and 2 antibodies (BB3, A13) directed against different epitopes of the human gamma delta heterodimer. From each patient peripheral blood lymphocytes were also obtained and the percentage of gamma delta cells was derived. In normal human skin Tgd were infrequently seen and a very slight difference was appreciable between controls and SSc unaffected skin. In SSc skin the largest amounts of Tgd was found. The patients with the edematous early phase of the disease showed an increased percentage of Tgd when compared to the patients in the advanced atrophic stage. In the circulation, no difference was found between controls and SSc and among SSc phases of the disease. These data clearly demonstrate for the first time that SSc presents an increased accumulation of lymphocytes bearing Tgd receptor in the affected skin, in particular in the early phases of the disease. This suggests that Tgd cells might be involved in the pathogenesis of the disease.

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EXPRESSION OF ALPHA$_2$BETA$_1$ INTEGRIN IN SCLERODERMA FIBROBLASTS *E.Koslowska, *C.Mauch, *B.Eckes, #D.Dressel, #E.Klein, *Th.Krieg, Departments of Dermatology Universities of *Cologne and #Ulm, FRG

Systemic scleroderma (SSc) is characterized by excessive deposition of extracellular matrix (ECM) proteins. Cultured fibroblasts grown from fibrotic lesions exhibit increased levels of collagen. The mechanisms responsible for upregulated collagen gene expression are not well understood. However there are indications that an altered regulation of collagen metabolism by the ECM might play a crucial role. One group of specific cell surface receptors mediating the interactions between cells and the ECM are integrins. We therefore investigated the expression of collagen specific alpha$_2$beta$_1$ integrin at the protein and at the mRNA levels comparing normal and SSc fibroblasts.

Normal fibroblasts reveal a low expression of alpha$_2$beta$_1$ integrin mRNA. In SSc fibroblasts grown as monolayers the expression of alpha$_2$beta$_1$ is lower as compared to control fibroblasts both in Northern blot and in quantitative mRNA analysis. As substantiated by immunoprecipitation of ^{35}S-methionine labelled fibroblasts using monoclonal antibodies the decreased mRNA levels are also found at the protein level. In some patients it is reduced to about 20% as compared with the controls.

Our data indicate an altered control of alpha$_2$beta$_1$ integrin expression in scleroderma fibroblasts. Further experiments have to be carried out to clarify whether altered recognition of ECM proteins by SSc fibroblasts is involved in the mechanism leading to high collagen synthesis found in those cells.

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THE WOUNDED DERMAL EQUIVALENT: A NOVEL *IN VITRO* WOUND HEALING MODEL. Paul G. Genever, Edward J. Wood and William J. Cunliffe*, *Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, and *Department of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, UK.*

Dermal Equivalents (DE), fabricated by seeding fibroblasts into a collagen lattice, may be used as *in vitro* models for studying *in vivo* remodelling processes. We investigated fibroblast behavior in "wounded" DEs by measuring fibroblast migration, proliferation and collagenase production, in the presence and absence of peptide growth factors. DEs were fabricated by growing human foreskin fibroblasts in hydrated type I rat tail collagen lattices. Wounds (~6mm in length) were made with a scalpel blade in the centre of the collagen lattice after 3 days in culture. Collagenase activity in the culture medium was assayed by measuring its ability to digest gels containing tritiated collagen, with or without a brief preincubation with trypsin to activate any latent procollagenase.

Following wounding, fibroblasts migrated towards and into the wound defect and appeared to initiate the closure of the wound by bringing together the cut collagen surfaces over a period of 5-11 days. The fibroblast migratory response was significantly stimulated by the presense of EGF and PDGF (2.8- and 3.5-fold respectively) but not TGF-β, whereas type I collagen inhibited cell migration. Cell proliferation in wounded DEs was up to 21% greater than in non-wounded DEs and cell numbers were stimulated further by the addition of TGF-β, EGF and PDGF (1.4-, 1.6- and 1.9-fold respectively). Wounded DEs also displayed a 2.1-fold increase in latent collagenase production followed by a 1.7-fold increase in active collagenase levels compared to non-wounded DEs. Clearly, simply wounding the collagen matrix in which fibroblasts grow is sufficient to stimulate those fibroblasts to migrate towards and proliferate within the wound defect, to increase the amount of collagenase they produce and to initiate the closure of the wound. Thus the wounded DE system may provide a valuable model for studying wound healing *in vitro*.

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HUMAN FIBROBLASTS, MYOFIBROBLASTS AND VASCULAR SMOOTH MUSCLE CELLS DISPLAY DIFFERENT PHENOTYPES IN CULTURE. P. Lefebvre, B. Nusgens and Ch. M. Lapière, Lab. Exper. Dermatology, University of Liège, Belgium

The aim of our work was to compare the phenotype of the skin cells (F) with that of granulation tissue cells (MYO) and blood vessel wall cells (smooth muscle cells, SMC) derived from human tissues. The SMC retracted a collagen lattice more strongly than MYO, themselves more active than F (respectively at day 4: 80%, 60% and 40%). Heparin stimulated the lattice retraction by F and SMC. As measured by strain gauges, the mechanical force developed by SMC, much greater that in the other types of cells, was further enhanced by serotonin (5HT) and inhibited by ketanserine (K). These two agents had no effect on F. The biosynthetic phenotype evaluated by the steady-state level of various mRNAs, showed little difference in terms of procollagen I and III. The tissue procollagenase was 10 times more expressed in SMC than in MYO and F. As compared to F and SMC, MYO was characterized by a 30 times higher level of mRNA for TIMP, 5 times higher for 72 kd collagenase IV while stromelysin was 10 times lower. 5HT did not affect the phenotype of F, increased collagen type I and III in MYO and had the reverse effect on SMC while the collagenase is inversely regulated. The effect of 5HT was abolished by K. The significant differences in the phenotype support the contention that the three types of cells retain some of their identity in vitro and that cultured fibroblasts are different of myofibroblasts.

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IN VITRO EVALUATION OF COLLAGEN LATTICES, SERVING AS CARRIERS FOR DERMAL AND EPIDERMAL CELLS, A TREATMENT MODALITY FOR FULL THICKNESS WOUNDS. de Vries Henry JC#, Westerhof Wiete#, Wildevuur Charles HR§ # Dept. of Dermatology, Academic Medical Center, Univ. of Amsterdam, Amsterdam, The Netherlands. § Research Division Thorax surgery, Thorax Center, University Hospital Groningen, Groningen, The Netherlands.

Skin substitutes, comprising of a biodegradable carrier with seeded fibroblasts and keratinocytes, are able to close full thickness wounds in less time, with less wound contraction and scar tissue formation than, for example a conventional split skin graft method or cultured keratinocyte sheets. One of the problems still unsolved, is the composition of an ideal cell carrier.

To evaluate different cell carriers, we developed a model consisting of; the material to be tested, seeded with fibroblasts, on top of which a collagen gel with suspended keratinocytes is placed. Six different collagen lattices were tested: 1) Glutaraldehyde cross-linked lattice, 2) Aluminum alginate cross-linked lattice 3) Large fibre lattice, 4) and 5) Two non cross-linked lattices, and 6) Collagen/elastin lattice. Parameters were: a) cell toxicity, b) disintegration speed of the carrier, c) cell attachment and cell guidance, d) architecture of dermal and e) epidermal component. Evaluation times were day 0, 3, 7, 10 and 14.

The Glutaraldehyde and Aluminum alginate fixated lattices appeared to be cytotoxic. In the Large fibre carrier, fibroblasts were not able to attach. The two non cross-linked lattices were non-toxic, cells attached and proliferated but both materials disintegrated quickly and their fibers started to fall apart from day 3 on. The keratinocyte layer on top of the two non cross-linked latices was 4 to 5 cell layers thick and showed signs of cornification. The Collagen/elastin lattice gave the best results, the carrier kept its original fibrillar structure, fibroblasts proliferated and were able to spread out through the whole matrix. The keratinocyte layer resembled normal epidermis. Further testing of this cell carrier in a porcine skin model will have to prove its possible use as skin substitute for the treatment of full thickness wounds.

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EXPRESSION OF COLLAGEN VII AND FORMATION OF ANCHORING FIBRILS DURING HUMAN WOUND HEALING. G. Zambruno*, A. Cavani*, A. Giannetti*, and L. Bruckner-Tuderman**, Departments of Dermatology, *University of Modena, Italy, and **University Hospital, Zürich, Switzerland.

Expression of collagen VII and formation of anchoring fibrils is essential for effective dermo-epidermal cohesion during wound healing. The sequence of these events was investigated in both a model of normal human skin transplanted onto nude mice and directly in human volunteers. In the first case, split-thickness xenografts of normal human skin were transplanted onto nude mice and 50 days later a 3-mm wound was made in the centre of the graft (day 0). At serial times from day 0 to 28, wound sites were surgically removed. In 12 volunteers, a split-thickness 3-mm punch biopsy was performed and the site of biopsy was removed at intervals from day 1 to 28. Immunofluorescence (IF) was carried out on frozen sections using a polyclonal antibody to collagen VII. In addition, part of the human biopsies was processed at day 14, 21, and 28 for transmission electron microscopy (TEM). Similar results were obtained with both models. A faint labelling of the proximal part of the newly forming dermal-epidermal junction (DEJ) was evident from day 3/4. CVII expression extended to the median portion over the next days, and was present along the entire newly-formed DEJ when re-epithelialization was complete, appearing similar to that adjacent unwounded skin by day 28. In some specimens, a granular staining pattern was noticed in the cytoplasm of basal keratinocytes. Up to day 28, a granular labelling was also observed in the upper dermis, suggesting fibroblasts are involved in cVII synthesis during wound healing. TEM examination showed extremely sparse and immature anchoring fibrils at day 14, when collagen VII was well detectable by IF. At days 21 and 28, size and density of the anchoring fibrils had increased but were still not comparable to those of normal skin. Our results indicate that during human wound healing cVII synthesis occurs long before the formation of distinct anchoring fibrils.

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REGULATION OF EXPRESSION OF THE CD34 ANTIGEN IN HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS. Helen A Bull, *Linda S Wilkinson, C B Bunker, *J C W Edwards, Pauline M Dowd, Depts of Dermatology & *Rheumatology, UCMSM, The Middlesex Hospital, London. UK.

CD34 is a proteoglycan-like antigen expressed on vascular endothelium in a variety of tissues including skin. Its function and mechanisms regulating its expression are unclear. We have now examined expression of CD34 in normal skin and regulation of expression in human dermal microvascular endothelial cells (HDMEC) by immunocytochemistry and Western blotting.

4mm punch biopsies were taken from the forearm of 5 healthy male volunteers, snap frozen in hexane and 5μm sections cut. Confluent monolayers of HDMEC were incubated either alone in serum free medium, with 3 U/ml hrIL-1α or 0.5 μM PMA for upto 18 hrs and then cytospun onto glass slides. Skin sections and HDMEC were stained with an antibody directed against CD34 (QBEND 10), using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. In HDMEC, QBEND 10 staining was compared with that of Ki67 (proliferating cell nuclei) and an anti-CD44 antibody (the proteoglycan receptor for hyaluronic acid). Protein extracts were also prepared from HDMEC , size fractionated by SDS-PAGE, electroblotted onto nitrocellulose and probed with QBEND 10.

In normal skin, CD34 staining was present around the endothelium of all blood vessels throughout the dermis, predominantly on the abluminal side. CD34 staining was also present around sweat glands in the dermis and along the fine processes of dermal fibroblasts. In cytospin preparations, CD34 immunoreactivity was evident in 50% of unstimulated HDMEC. The staining was discrete and evenly distributed through the cytoplasm. Incubation with hrIL-1α or TPA did not increase the number of cells that expressed immunoreactive CD34. However, immunoreactivity was then much more closely associated with the cell membrane and in culture, HDMEC changed from being round to spindle-shaped cells. Ki67 and CD44 staining was confined to <5% of individual HDMEC and did not correspond with that of CD34. Western blot analysis of HDMEC identified a band at ≈ 98kD, expression of which was not upregulated in cells incubated with hrIL-1α or PMA for 8 hours.

These data indicate that the CD34 antigen is constitutively expressed by endothelium in normal skin and by a proportion of non-proliferating endothelial cells in culture. The distribution of immunoreactive CD34 in normal skin and change in its pattern of cellular distribution in stimulated HDMEC indicate a possible role as an extracellular matrix adhesion molecule.

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IMMORTALIZATION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS BY EPSTEIN-BARR VIRUS. Rudolf Berger, Karin Macfelda, Heidemarie Korschan, Klaus Wolff, and Georg Stingl, Div. Cut. Immunobiology, Dept. Dermatol. I, Univ. Vienna Medical School, Vienna, Austria.

Epstein-Barr virus (EBV), a member of the human herpesvirus group, has two target cell types in vivo : epithelial cells and B-lymphocytes. The virus enters its targets via an interaction between the viral envelope protein (gp 350/220) and its cellular receptor, the CR2/CD21 molecule. Following attachment and penetration of the virus, the majority of infected cells are activated to leave the G_0 phase of the cell cycle and to enter G_1. As a result, EBV infection causes indefinite cell proliferation. Since we recently found CD21 to be expressed on the surface of human umbilical vein endothelial cells (HUVEC), we addressed the question, as to whether HUVEC can be infected with EBV and whether this event might lead to growth factor-independent, long-term proliferation of HUVEC in vitro.

Primary HUVEC were propagated on fibronectin-coated culture plates in the presence of optimal concentrations of endothelial cell growth factor (ECGF) and heparin. HUVEC were infected using cell free supernatants of a EBV-producing Burkitt lymphoma cell line (BL 74). As a selection criterium, cells were kept in medium (MCDB 104 plus 10% FCS) devoid of ECGF, heparin or fibronectin. While non-infected HUVEC died within 7 days, EBV-infected HUVEC started to proliferate after a time interval of 6 weeks. Interestingly, cells were not adherent as the initial cell population, but rather grew in suspension. EBV-transformed HUVEC are now being cultured for more than ten months without the need of exogenous growth factors. The presence of EBV within cellular DNA of HUVEC was detected by Southern blotting using the EBV Bam HI-W-fragment. Immunocytochemical analysis of the EBV-transformed HUVEC reveiled the expression of certain endothelial cell-specific markers like collagen type IV, EN-4, EN-2/3, EN-7/4 and BMA 120. Surprisingly,these cells expressed certain B-cell markers (CD19, CD20) together with CD23 and CD45, cell surface moieties which cannot be detected on primary HUVEC.

The establishment of the permanent HUVEC line described here may be useful for the identification of new endothelial cell-specific growth factors induced by EBV. In addition, this cell line might also allow to investigate the mechanisms responsible for extinction or reexpression of lineage specific genes.

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ESTABLISHMENT AND CHARACTERIZATION OF SOMATIC CELL HYBRIDS BETWEEN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AND B LYMPHOCYTES. Karin Macfelda, Georg Stingl, Klaus Wolff, and Rudolf Berger, Div. Cut. Immunobiology, Dept. Dermatol. I, Univ. Vienna Medical School, Vienna, Austria.

Successful culture of primary human umbilical vein endothelial cells (HUVEC) requires the presence of exogenous growth factors (i.e. endothelial cell growth factor, ECGF) and extracellular matrix proteins (ECMP). However, despite the addition of growth factor(s) and ECMP, HUVEC cultures have a limited life span (< 25 passages). To analyze whether continuous proliferation can be achieved after somatic cell hybridization of HUVEC with an exogenous growth factor-independent cell line, we fused Raji cells (EBV-positive Burkitt lymphoma cells, TK$^-$) with primary HUVEC. After selection in HAT-containing culture medium, hybrid cells were propagated either in culture medium (MCDB 104 plus 10% FCS) alone, in the presence of fibronectin or fibronectin + ECGF + heparin. Immunocytochemical analysis of hybrid cells revealed a mixed phenotype between the two parental cell types. Hybrid cells continued to express HUVEC-specific antigens (vWF, BMA 120, EN 2/3, and urokinase), but additionally expressed markers found to be exclusively expressed on the parental B cell line (HLA-DR, CD22, CD23, CD45). This mixed phenotype was neither influenced by the composition of the culture medium, nor dependent on the growth characteristics of the hybrid cells (suspensions vs. adherent). In order to test whether cytokine or intracellular adhesion molecule type 1 (ICAM-1) secretion patterns of the hybrid cells are identical to one of the parental cell types, we screened culture supernatants for the presence of certain cytokines (sCD 23, IL-8) or ICAM-1 by ELISA. While IL-8 is highly expressed in HUVEC, but not in Raji cells, soluble CD23 (sCD23) is typically secreted by Raji cells, but not by HUVEC. All hybrid cell lines tested so far did expressed sCD 23 and ICAM-1. Expression of these molecules was downregulated by fibronectin/ECGF. Conversely, IL-8 which is not expressed in hybrid cells cultured in medium devoid of ECGF and without fibronectin, could be detected in high amounts in supernatants from hybrid cells cultured on fibronectin and in the presence of ECGF. This suggests that secretion of sCD 23, ICAM-1 and IL-8 can be modulated by ECGF and fibronectin. These cell lines should prove useful to search for growth factor(s) required for long-term HUVEC cultures. Additionally, these somatic cell hybrids might represent a proper model system for analyzing the regulation of endothelial cell-specific genes by cellular trans-acting factors.

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INDUCTION OF ICAM-1 EXPRESSION BY EPIDERMAL KERATINOCYTES VIA A PARACRINE PATHWAY INVOLVING DERMAL DENDRITIC CELLS. I. Bruynzeel[1], B.J. Nickoloff[2], E.M.H. van der Raaij[1], D.M. Boorsma[1], T.J. Stoof[1], and R. Willemze[1], [1]Department of Dermatology, Free University Hospital, Amsterdam, The Netherlands; [2]Department of Dermatology and Pathology, University of Michigan Medical School, Ann Arbor, MI, USA.

Recent studies have attributed a central role to epidermal keratinocytes (KC) in the initiation of cutaneous inflammation. This concept was based on the observation that contact with various stimuli causing skin inflammation (e.g. contact allergens, toxic chemicals, UV-light), can directly induce epidermal KC to produce specific cytokines and to express adhesion molecules. Recently, in our laboratory, the capacity of a number of cytokines and other inflammatory mediators (IL-1, IL-2, IL-4, IL-6, IL-8, IFN-α, IFN-γ, TNF-α, TGF-α, PMA and LPS) to induce ICAM-1 expression in epidermal KC was investigated, both in monolayers of cultured KC and in skin biopsy specimens kept in short-term organ culture. KC monolayers expressed ICAM-1 following incubation with IFN-γ, TNF-α and PMA, but not with LPS or any of the other cytokines tested. In the short-term organ culture ICAM-1 was induced on epidermal KC not only by IFN-γ, TNF-α and PMA, but also by LPS. Using antibodies against TNF-α and IL-8, dermal dendritic cells in the papillary dermis of the LPS incubated biopsies were found to express TNF-α and IL-8. Cryostat sections from skin biopsies not stimulated by LPS did not show TNF-α or IL-8 staining in the dermis. These findings support our hypothesis that LPS induces ICAM-1 expression by epidermal KC indirectly via induction of TNF-α production by dermal dendritic cells and demonstrates that not only epidermal cells, but also dermal dendritic cells may act as initiators of cutaneous inflammation.

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INTRACELLULAR pH CHANGES IN NORMAL HUMAN KERATINOCYTES
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In many cells a transient rise in intracellular calcium ($[Ca]_i$), with growth factors, preceeds a rise in intracellular pH (pH_i). In human keratinocytes (HKs), EGF elicits a transient $[Ca]_i$ rise, whilst differentiation, induced by a high extracellular calcium concentration ($[Ca]_o$) or phorbol ester TPA, is associated with a sustained rise in $[Ca]_i$. We therefore studied the effects of $[Ca]_o$, TPA and EGF on HK pH_i. HKs were grown in MCDB153 ($[Ca]_o$ 70μM, EGF 10ng/ml). pH_i and $[Ca]_i$ were measured in single cells using the dyes BCECF and FURA-2 with microspectro-fluorimetric techniques.

Control cells had pH_i 7.01±0.19 (±SEM, n=210) and $[Ca]_i$ 104±3 nM (n=40). Increasing $[Ca]_o$ to 1mM gave no immediate pH_i change, however a rise was seen at 30min (pH 7.33±0.02, n=30), 2 hrs (pH 7.41±0.04, n=30) and sustained at 6 days (pH 7.65±0.09, n=90). A proportion of cells responded immediately to 500nM TPA with an increase in pH_i (pH 7.20, 6/9) and $[Ca]_i$ (200-500nM, 8/12). 20nM TPA caused both a $[Ca]_i$ increase (132±2nM at 6hr, n=50) and a gradual pH_i rise (pH 7.75±0.06 at 6hr, n=60). Initial studies with HKs deprived of EGF for 5-10 days gave an immediate but transient $[Ca]_i$ rise (100-250nM, 4/7) and pH_i increase (pH 7.66 at 10min, 2/2) with EGF (10ng/ml). We have shown both pH_i and $[Ca]_i$ increases occur in NHK differentiation with different stimuli and this is likely to be distinct from the transient $[Ca]_i$ and pH_i rise observed with EGF.

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MODIFIED LIPID SYNTHESIS AND INCREASED BrdU INCORPORATION IN VITRO BY EPIDERMAL KERATINOCYTES OF NON-BULLOUS CONGENITAL ICHTHYOTIC ERYTHRODERMA (NBCIE). M. Haftek, C. Amsellem, C. Berlioz, A. Réano, MC. Martini*, J. Thivolet, INSERM U.346/CNRS and *ISBP, Lyon, France.

The NBCIE has been individualized from the group of lamellar ichthyosis on the basis of particular clinical and histological pictures as well as the characteristic keratinocyte hyperproliferation associated with a modified epidermal lipid synthesis.

We used lesional keratinocytes from two patients with NBCIE and normal epidermal cells from four control subjects for studies of a reconstructed epidermis in the emerged cultures on dead de-epidermized dermis. The cultures were maintained for 14 and 25 days. Keratinocyte proliferation was estimated through the rate of BrdU incorporation during the last 4 hours of culture. Qualitative and quantitative biochemical analysis of the epidermal lipid composition was performed. Histological and immunohistochemical study using various epidermal differentiation markers completed the study. No major macroscopic differences nor any marked variations in epidermal immunoreactivity were observed between the cultures of normal and pathological origin. By contrast, a clearcut increase in BrdU incorporation rate characterized the 14-day (but not the 25-day) NBCIE keratinocytes. Also, a significant hyperproduction of epidermal n-alkanes could be demonstrated in the ichthyotic cultures. Our findings demonstrate that the major markers of the ichthyotic epidermis, i.e. keratinocyte hyperproliferation and n-alkane hyperexpression, are maintained in vitro in the emerged culture conditions. They also indicate that the characteristic n-alkane increase in NBCIE is indeed endogenous and not merely related to the possible contaminations resulting from topical treatments.

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A RAPID AND SIMPLE GROWTH ASSAY FOR KERATINOCYTES BY PHOTOMETRIC MEASUREMENT OF HEXOSAMINIDASE ACTIVITY.
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In order to examine proliferative activities of various growth factors on keratinocytes (KC) in vitro, a rapid and reliable method to cell proliferation is needed. N-acetyl-D-Hexosaminidase is a lysosomal enzyme widely distributed in many cells and tissues. P-nitrophenol-N-acetyl-ß-D-glucoseminide, as substrate for the hexosaminidase (hex), can be employed for spectrophotometric quantification of the enzyme's activity. After removal of media, 50 µl of a 7.5 mM substrate solution were added to attached KC in flat bottom microtiter wells. All plates were incubated at 37°C for 4 hours. Color reaction was developed by addition of 0.1 M NaOH-Glycine buffer, pH 10.4, 100 µl per well. Absorbances were measured at 405 nm.

Cell numbers obtained by counting in a Neubauer chamber and fluorescence units established by a fluorometric assay correlated with the absorbances obtained by the hex assay (r=0.99 and 0.90, respectively). Performance of this assay in proliferating KC revealed continuous increase of absorbances after 2,4,6 and 8 days of culture, depending on initial cell density. Measurement of hex activity in cultures of rapidly frozen keratinocytes revealed only background levels. Thus, we confirmed the growth-promoting effect of Insulin-like growth factor (IGF) II on KC (control 0.142, 50 ng/ml IGF II 0.201, 100 ng/ml IGF II 0.223). The hex assay is rapid, easy to perform and inexpensive. Therefore, trouble-some handling of isotopes, time-consuming cell counting or expensive tools for fluorometric assays can be omitted.

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EXPRESSION PATTERNS OF LORICRIN IN DERMATOLOGICAL DISORDERS Daniel Hohl,[1] Marcel Huber,[1] Scott Thacher,[2] and Edgar Frenk[1]; Dermatology, University Hospital of Lausanne[1]; Medical Biochemistry, Texas A&M University, College Station, Texas[2]

Loricrin is a glycine-, serine- and cysteine-rich protein expressed very late in epidermal differentiation in the granular layers of normal human epidermis. Subsequently, loricrin becomes cross-linked by the activity of type I/III transglutaminase as a major component of the cornified cell envelope by γ-glutamyl-lysyl-isopeptide bonds. In this study, 110 biopsies from a wide range of inflammatory, inherited and tumoral skin lesions, characterized by morphologically altered epidermal differentiation, were analyzed using immunohistology with monospecific, polyclonal antibodies to a unique carboxy terminal peptide of loricrin and to involucrin. In addition, antibodies to filaggrin and to type I (keratinocyte) transglutaminase were used for ichthyotic lesions. In contrast to involucrin, loricrin expression was consistently downregulated in parakeratotic keratinization as observed in psoriasis, eczema, pityriasis lichenoides, porokeratosis or precancerous and malignant squamous lesions. High levels of loricrin were found in hypergranulotic and hyperorthokeratotic epidermis as observed in lichen planus, benign papillomas and pseudocarcinomatous hyperplasia. 10 biopsies from patients with ichthyosis vulgaris showed a normal peripheral staining in the granular layers. However, in 3 cases of lamellar ichthyosis a high expression of loricrin as well as involucrin with a peculiar and abnormal cytoplasmic staining was observed and correlated with a diminished cytoplasmic staining of type I transglutaminase as assessed by antibodies B.C.1 and K.D.3. Most interestingly, this pattern was absent in a collodion baby at birth but present 3 weeks later when clinically and histologically a phenotype of lamellar ichthyosis appeared. Our results indicate (i) that loricrin expression is closely linked to an orthokeratotic phenotype of human epidermal keratinization and (ii) that in lamellar ichthyosis disturbed membrane ancorage of type I transglutaminase might alter loricrin and involucrin expression and the formation of the cornified cell envelope.

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EXPRESSION OF CALPROTECTIN (LEUCOCYTE L1 PROTEIN) BY HUMAN KERATINOCYTES IN VITRO. Tor-Øivind Gabrielsen and Per Brandtzaeg. Dept of Dermatology and Lab for Immunohistochem and Immunopathol, Inst of Pathol, Univ of Oslo, Rikshospitalet, Oslo, Norway.

Calprotectin (or L1 antigen) is a calcium-binding myelomonocytic protein first isolated from human granulocytes, in which it constitutes 60% of the cytosol proteins. L1 antigen is also found in monocytes and certain reactive tissue macrophages and is normally present in squamous epithelium of mucous membranes. While absent in normal epidermal cells, L1 antigen is expressed suprabasally in a wide variety of inflammatory and tumorous skin conditions. L1 is also seen in well differentiated areas of squamous cell carcinomas. The biological function of L1 remains obscure but both antimicrobial and antiproliferative effects have been demonstrated *in vitro*. We have investigated the induction of L1 in human keratinocytes *in vitro* and found that this epidermal protein is expressed in skin biopsy specimens within 24 hours. Single cell suspensions of keratinocytes in culture medium also express L1 within the same period. In confluent stratified keratinocytes in culture, grown on mitomycin treated 3T3 fibroblasts as feeder layer, L1 is expressed suprabasally. These findings indicate that keratinocyte expression of L1 is strictly linked to cell differentiation.

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EXPRESSION OF RIBONUCLEOTIDE REDUCTASE IN HUMAN KERATINOCYTES:A NEW MARKER OF PROLIFERATION. A. Cerri, E. Roscetti, L. Restano, L. Crosti, G. Cattoretti*, E. Berti, R. Caputo 1st Department of Dermatology, University of Milan and *National Tumours Institute; Milan, Italy.

Ribonucleotide reductase (RR) is an enzyme present in all dividing cells, it is essential in DNA synthesis because it catalyzes the conversion of ribonucleotides to deoxyribonucleotides.While the activity of RR seems to be related with cellular DNA synthetic rate, the kinetic features of RR expressing cells are still poorly understood. It is well known that epidermis is a self renewal compartment and a useful model to study cellular proliferation and differentiation.In an attempt to investigate the relationships between RR expression and keratinocytes(KC) proliferation and differentiation we analyzed, using a specific anti-RR monoclonal antibody (Mab AD 203) and an APAAP method, the RR distribution on frozen sections of normal skin, psoriasis and on KC cultured in different Ca++ concentrations.Finally to correlate the RR expression with proliferation, we performed a double labelling with Mab anti BrdU on cytospins of cultured KC.In normal epidermis the Mab AD203 showed a cytoplasmatic reactivity that was primarily localized on basal KC, the suprabasal cells stained weakly and rarely.In contrast in psoriasis, a paradigmatic hyperplastic skin conditions, we detected an increased RR expression in basal and suprabasal cell layers. The cytoplasm of KC cultured at low Ca++ concentration was strongly labelled with Mab AD203, while in the culture grown in a high Ca++ medium the cells were unevenly stained. The double staining study showed that the BrdU+ KC were also AD203+ but the RR expression is not phase S restricted(AD203+/ BrdU- KC).These data indicate that the RR expression correlates with the proliferative state of human KC and suggest that the AD203 Mab may be an useful marker to distinguish proliferating and quiescent cells, as an alternative to Ki-67 Mab that shows cross-reactivity to basal KC cytoskeleton.

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LOCALIZATION OF ACTIN AND SPECTRIN IN KERATINOCYTES IS CALCIUM DEPENDENT. Hans W. Kaiser, Ed J. O'Keefe, Dorothee Beichelt, Winfried Ness, Welisar Petrow and Hans W. Kreysel, Department of Dermatology, University of Bonn, Germany; Department of Dermatology, University of North Carolina, Chapel Hill, N.C.

Actin and spectrin, major components of the cytoskeleton of keratinocytes, are presumed to associate with transmembrane proteins mediating cell-cell contact. A calcium shift assay was used to study the presence and distribution of actin and spectrin before and after induction of cell-cell contacts in cultured human keratinocytes. immunoblots revealed equal amounts of spectrin and actin at low (0.1 mM) and high (1.0 mM) Ca^{++}. Actin filaments were randomly oriented in low Ca^{++} but became oriented and concentrated at sites of cell-cell contact at high Ca^{++}. Although distribution of spectrin by immunofluorescence did not change with calcium shift, extraction of soluble proteins with nonionic detergent before fixation showed that cytoplasmic spectrin was extractable at both high and low Ca^{++} but was insoluble in high Ca^{++} exclusively at sites of cell-cell contact. Induction of cell-cell contact in keratinocytes may lead to stabilization of the actin filament system by association of actin into an insoluble spectrin-actin complex.

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MICRODIALYSIS MEASUREMENT OF TRANSCUTANEOUS ETHANOL ABSORPTION Thomas Andersson and Chris Anderson, Department of Dermatology, University Hospital, Linköping, Sweden.

Microdialysis, a bioanalytical sampling technique enables measurement of substances in the extracellular space. The technique's usefulness in the field of percutaneous absorption of solvents has been studied, using ethanol as test substance. 70 mm microdialysis probes, equipped at the tip with a 10 mm semi-permeable polycarbonate membrane were inserted into the skin of the ventral forearm in volunteers, at a depth of around 1 mm. Probe depth was measured by ultrasound technique. 99.5% ethanol was applied to the skin in excess in a glass reservoir over the tip of the probe.The probe was perfused with a physiological Ringer's solution. Analysis of ethanol was performed by gas chromatography. In-vitro recovery for ethanol is good. Ethanol does not damage the microdialysis membrane.

In initial experiments, with a flow rate of 1 ul/min, ethanol absorption could be demonstrated in all 9 patients during a 50 minute collection period. Values obtained ranged from 10 ug/ml to 800 ug/ml. With improved analysis technique, the flow rate could be increased to 3 ul/min and samples collected at 10 minute intervals. Results from a small number of patients indicate that peak ethanol levels occur at about 100 minutes, that probe depth is an important determinant of absorption level, and that "stripping" of the skin increases early absorption.

The method opens new possibilities in the investigation of skin barrier function in man.

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EPIDERMAL TRANSGLUTAMINASE GENE EXPRESSION BY A TUMOR PROMOTER 12-*O*-TETRADECANOYLPHORBOL-13-ACETATE (TPA) IN CULTURED NORMAL HUMAN KERATINOCYTES.

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Epidermal transglutaminase (ETG) catalyzes formation of ε-(γ-glutamyl) lysine isopeptide bond among substrate proteins such as loricrin and involucrin to form cell envelope in the late stage of keratinization. Recently, we have cloned a cDNA of the human ETG and characterize the primary structure of the enzyme. In this study, we investigated the effect of the tumor promoter TPA, a protein kinase C (PKC) activator, on the expression of ETG gene in cultured normal human keratinocytes. TPA induced expression of ETG mRNA in a dose dependent manner. The induction was completely inhibited by the PKC inhibitors H-7 and staurosporine, as well as retinoic acid. Our results suggest that the expression of ETG gene in keratinocytes is up-and down- regulated by the PKC and retinoic acid receptor systems, respectively.

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CODISTRIBUTION OF HYALURONAN and CD44 IN THE EPITHELIA OF HUMAN SKIN APPENDAGES. Chao Wang, Markku Tammi, and Raija Tammi, Department of Anatomy, University of Kuopio, Kuopio, Finland.

Biotinylated hyaluronan (HA) binding region (HABR) from bovine articular cartilage was used as a histological probe to study the localization of HA in human skin. The distribution of HA was correlated with the distribution of its presumptive receptor, CD44, using monoclonal antibodies. In the epidermis HA and CD44 were stained in the basal and spinous cell layers, whereas the stratum granulosum and stratum corneum were negative. In the keratinizing parts of hair follicle, i.e. the outer and inner epidermal root sheath, pilosebaceous duct and the actual hair, HA and CD44 were also found between the vital but not the terminally differentiated cells. In the sebaceous glands HA and CD44 stainings were restricted to the basal cell layer. However, only a part of the basal cells in the bottom of the sebaceous acini were stained. The secretory acini of the sweat glands showed intense staining with the anti-CD44 antibodies but only a weak staining with HABR. In this gland CD44 was localized on the basal and lateral surfaces of the clear cells, whereas the dark cells and the myoepithelial cells were negative. Both the lower and the upper layers of the sweat gland ducts showed a faint but constant staining with for CD44 but contained only minor amounts of HA. HA and its presumptive receptor were thus particularly abundant around the multilayered, keratinizating epithelial cells, suggesting an important role in their differentiation.

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INOSITOL PHOSPHATE FORMATION AND CALCIUM RELEASE IN HACAT KERATINOCYTES. T. Rosenbach, C. Liesegang, S. Binting, B.M. Czarnetzki, Dept. of Dermatology UKRV, FU Berlin, Berlin, FRG.

The signal transduction via inositol phosphates is a major regulatory component of cell growth and differentiation. Specific receptors for growth factors, hormones, and neurotransmitters may exert their function via activation of phospholipase C (PLC) which in turn cleaves phosphatidylinositolbisphosphate into the second messengers inositoltrisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+} from intracellular storage sites and DAG activates protein kinase C. In the present study we characterized this pathway in the human keratinocyte cell line HaCaT. Inositol phosphates were measured by means of anion exchange chromatography and intracellular free Ca^{2+} was determined with the fluoresence dye FURA-2AM. After stimulation of cells over a broad range of concentration of EGF, TGF_α, $Il1_\alpha$, $Il1_\beta$, Il6, Il8, retinoic acid, carbachol, and prolactin for up to 5 min, all these substances exerted no effect on intracellular Ca^{2+}. The only ligand which caused a rapid and transient rise of IP_3 and Ca^{2+} was bradykinin. The maximal response was observed 20 sec after addition of bradykinin. IP_3 in turn was rapidly dephosphorylated to IP_2 and IP. These results suggest that HaCaT cells possess a receptor for bradykinin and that this receptor is coupled to the inositol phosphate signal transduction pathway.

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PHORBOLESTER PREVENTS FORMATION OF NORMAL INTERCELLULAR JUNCTIONS IN CULTURED HUMAN KERATINOCYTES. Friedhelm Achenbach, Hans W. Kaiser, Andrea Balcerkiewicz, Ulrich Issberner and Hans W. Kreysel, University of Bonn, Germany.

Cell-cell junctions are dynamic structures with a constant turnover of their components. In this study we investigated the effects of the tumorpromotor phorbolester on the development and maintenance of cell-cell contacts like desmosomes and adherens junctions. Normal human keratinocytes were incubated with nanomolar amounts of phorbolester (TPA) before and after induction of cell-cell contact by raising the calcium concentration in the culture medium. Immunohistochemistry revealed an irregular staining pattern for antibodies against desmoplakin to localize desmosomes and monoclonal antibodies against vinculin to detect adherens type junctions at cell-cell contact sites. In addition an increase of cytoplasmic staining was noted in phorbolester-treated cells. Westernblot analysis of desmoplakin and vinculin in TPA-treated cells showed no degradation of proteins. The tumorpromotor TPA prevents the formation of normal intercellular junctions and might indicate one possible mechanism for the loss of contact-inhibition in the development of malignant diseases.

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INTERMEDIATE FILAMENT ARRANGEMENT OF COEXPRESSED KERATIN AND VIMENTIN IN NORMAL HUMAN KERATINOCYTES. A. Wevers, B. Bonnekoh, and G. Mahrle, Department of Dermatology, University of Köln, Germany

Coexpression of keratin and vimentin has been shown to occur in epithelial cell lines, transformed keratinocytes (Differentiation 14:35, 1979), and under less optimal culture conditions, such as serum-free medium (Arch Dermatol Res 282:512, 1990), lack of feeder cells (Cancer Cell 1:217, 1984), and seeding at low density (J Cell Biol 97:858, 1983). In the present study we demonstrate the coexpression of keratin and vimentin in cultured non-transformed human keratinocytes grown on 3T3 mouse feeder cells in serum containing medium and their distribution pattern within the cell after double-immunostaining.

At the end of the second subculture of normal human keratinocytes (10 weeks after onset of cell culturing), intermediate filaments were extracted, separated in 12 % SDS-PAGE, and immunoblotted with different monoclonal antibodies. Double staining with anti-keratin and anti-vimentin antibodies was performed for immunocytological and electron microscopical investigations. For electron microscopy second antibodies labeled with 6 nm and 15 nm gold particles were applied.

Coexpression of keratin and vimentin was observed in the keratinocyte cytoplasm by immunocytochemistry. Immunoblots with different monoclonal antibodies to vimentin showed a specific reaction in the molecular range of vimentin. There was no cross-reaction to keratin polypeptides and mouse vimentin. Immunoelectron microscopy revealed, that the antibodies to keratin labeled distinct tonofilament bundles, whereas the label for vimentin was more arranged in spots of higher electron density intermingled with the tonofilaments or enriched in areas with electron denser and slightly thicker filaments than tonofilaments.

The results show, that overlapping keratin and vimentin cytoskeletons are formed in long-term expansion cultures of normal keratinocytes.

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LIPID CHARACTERIZATION OF HUMAN SKIN AFTER GRAFTING ONTO ATHYMIC NUDE MICE. Isabelle Higouneng[1], Ferry Spies[2], Michel Demarchez[1], Braham Shroot[1], Maria Ponec[3]. Centre International de Recherches Dermatologiques Galderma (CIRD Galderma), Sophia Antipolis, 06565 Valbonne, France[1], Department of Electron Microscopy[2] and Dermatology[3], University Hospital Leiden, The Netherlands.

Human skin grafted onto nude athymic mice provides an attractive in vivo model for various pharmacological investigations since human skin retains its inherent histological functions for months after grafting. It can thus be used for the evaluation of percutaneous absorption and cytotoxicity studies of new drugs, cosmetics and consumer products.

In the present study we characterize the barrier properties of human epidermis grafted for two months onto nude mice and compare it with normal human skin. Since the barrier function and the transepidermal waterloss (TEWL) of the skin are closely related to its lipid composition, we analysed the lipids by high performance thin layer chromatography (HPTLC), measured the TEWL and evaluated the ultrastructure of the intercorneocyte lipid arrangement by freeze fracture electron microscopy (FFEM).

The TEWL of human skin exhibits no significant variation before and after grafting onto nude mice. FFEM analysis showed that grafted epidermis has the same morphological pattern as normal human epidermis. Furthermore, regular desmosomes and lamellar lipid structures were observed. Grafting did not qualitatively affect lipid composition of human epidermis. In particular, ceramides which contribute largely to the barrier function exhibit the same distribution profile. Beside human lipids, we detected the presence of mouse-specific lipid fractions in grafted human skin. We could however show, that these lipids are contamination of the graft by mouse superficial lipids. In summary, these results indicate that human skin grafted onto athymic mice maintains its original lipid composition and barrier properties and provides us a valuable in vivo model for penetration and cytotoxicity studies.

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ANTIBODIES TO MYCOBACTERIAL 65-KDa HEAT SHOCK PROTEIN AND RELEVANT IMMUNOCROSSREACTIVE ANTIGENIC COMPONENTS IN PSORIASIS.

A. Rambukkana, S. Yong, L. Witkamp, M.M.H.M. Meinardi, J.D. Bos, P.K. Das. Departments of Dermatology and Pathology, Academisch Medisch Centrum, Universiteit van Amsterdam, The Netherlands.

In view of the fact that IgG antibody levels to mycobacterial hsp in patients with rheumatoid arthritis were found significantly higher even than in tuberculosis, the possibility that mycobacteria may trigger other automimmune diseases has been raised. Among other factors, autoimmunity and the involvement of microbial immunity have been suggested for the pathogenesis of psoriasis. Against this background we performed immunoblotting analysis (ImBA) using sonicated preparations of different species of mycobacteria to explore the possible connection between mycobacteria immune response and psoriasis. ImBA showed that 50% of psoriasis patients had significantly elevated IgGAb to 65 KDa singlet, 45/48 KDa doublet, whereas only 20% showed IgG Ab to 16 KDa and 80 KDa mycobacterial ImmunoCrossReactive Antigenic Components (ImCRAC). In the light of Ab activity to mycobacterial ImCRAC 65 KDa singlet, we further carried out an ELISA as well as ImBA using both recombinant mycobacterial hsp 65 KDa and the sodium dodecyl poliacrylamide gel electrophoretically (SDS-PAGE) purified 65 KDa protein(s). 50% of Ab positive patients sera by ImBA showed strong IgG Ab activity in ELISA to hsp 65 KDa but not to SDS-PAGE purified 65 KDa protein. Interestingly, in these patients high Ab to hsp 65 KDa was positively correlated with disease activity. Since immune response to hsp 65 KDa may be explained on the basis of so called antigenic mimicry as in autoimmune disease, our present results are suggestive that the underlying pathomechanism of psoriasis may be associated with previous bacterial sensitisation. The present data also indicate that an ELISA serology of IgG Ab to hsp 65 KDa may be useful for the management of patients during therapy.

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SKIN-DERIVED ANTILEUKOPROTEINASE: CELLULAR LOCALISATION IN PSORIATIC AND TAPE STRIPPED SKIN. **H. Alkemade, I. v. Vlijmen-Willems, G. de Jongh, J. Schalkwijk. Dept. of Dermatology, University Hospital Nijmegen, The Netherlands.**

Recently we described a novel elastase inhibitor (skin-derived antileukoproteinase, SKALP) that inhibits human leukocyte elastase and porcine pancreatic elastase. It is expressed in inflammatory skin disorders e.g. psoriasis and in cultured keratinocytes, but not in normal epidermis.

We have now prepared a polyclonal rabbit antiserum against SKALP and used it to investigate SKALP expression immunohistologically and on Western blotting, both in psoriatic lesion and in epidermis following tape stripping, a model in which inflammation and hyperproliferation are induced by surface trauma.

1. Immunohistology showed that SKALP expression was restricted to the differentiating keratinocytes of the upper layers of the epidermis. Basal keratinocytes were completely negative. This was found in the psoriatic lesion and in tape stripped skin.

2. Western blotting showed an increase in SKALP expression which peaked at 48 hours after tape stripping; at 0, 8, 24, 96, 192 and 384 hours after tape stripping faint bands were stained. These findings are in accordance with functional measurements of the induction of SKALP activity following sellotape stripping. Only one band was stained on Western blots of the tape stripping experiments, while Western blots of psoriatic lesions showed two distinct bands, most likely as a result of cleavage of the inhibitor.

We conclude that SKALP can be considered as an acute phase protein, which is locally produced by keratinocytes and which might act as an off-switch mechanism of inflammation.

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DISTRIBUTION OF INTERLEUKIN 1 RECEPTOR ANTAGONIST PROTEIN (IRAP), INTERLEUKIN 1 RECEPTOR (IL-1R), AND INTERLEUKIN 1α (IL-1α) IN NORMAL AND PSORIATIC SKIN. M. Kristensen, B. Deleuran,* D.J. Eedy,** M. Feldmann, S. M. Breathnach** and F. M. Brennan, Charing Cross Sunley Research Centre, London, U.K., *Kennedy Institute of Rheumatology, London, U.K. and **St. John's Institute of Dermatology, London, U.K.

The keratinocyte-derived cytokine interleukin 1 (IL-1) is thought to play an important role in a variety of inflammatory skin diseases. However, in psoriasis, epidermal IL-1 bioactivity has been reported to be reduced. An IL-1 receptor (IL-1R) binding protein with IL-1 inhibitory activity, termed IL-1 receptor antagonist protein (IRAP), has recently been defined. We therefore sought to determine the presence and distribution of IL-1α, its receptor, and its naturally occurring inhibitor IRAP, in normal and psoriatic skin. Immunohistochemical staining was carried out, using the APAAP technique and specific monoclonal antibodies, on cryostat sections of skin from 10 normal subjects, and paired skin biopsies from uninvolved and involved sites from 7 patients with stable plaque psoriasis.

We document for the first time that in normal skin IRAP is distributed throughout the viable layers of the epidermis, and is also associated with sebaceous glands and eccrine sweat glands. We also report the novel finding that IL-1R in normal skin is localised to the viable layers of the epidermis, sebaceous and eccrine glands, upper dermal blood vessels, and to a prominent network of dermal dendritic cells. IL-1α was localized predominantly to the basal layer in normal epidermis,and was also present in eccrine sweat glands, sebaceous glands, and upper dermal blood vessels. The overall distribution of staining with antibodies to IL-1α and to IL-1R in both uninvolved and lesional psoriasis skin was broadly qualitatively the same as in normal skin, except that in lesional skin staining with anti-IL-1α was seen throughout the epidermis, rather than being restricted to the basal layer, and there was prominent staining of tortuous papillary blood vessels with this antibody. In 6 of 7 lesional psoriatic skin biopsies there was a consistent reduction, compared with normal and uninvolved psoriasis skin, in the intensity of epidermal staining with antibodies to IRAP. Our findings that the inhibitor protein IRAP is present in areas where the pro-inflammatory cytokine IL-1α is distributed provides strong evidence for the existence of a cytokine regulatory system in normal skin. Modulation of expression of the keratinocyte IL-1R does not seem to occur in psoriasis. By contrast, decreased keratinocyte expression of IRAP in psoriatic lesional skin indicates that alterations in the level of this inhibitor protein may be of importance in the pathogenesis of inflammatory skin conditions.

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LOCALIZATION OF TUMOUR NECROSIS FACTOR ALPHA (TNFα) AND ITS RECEPTORS IN NORMAL AND PSORIATIC SKIN. M. Kristensen, C.Q. Chu,* D.J. Eedy,** M. Feldmann, S.M. Breathnach** and F.M. Brennan. Charing Cross Sunley Research Centre, London, U.K., Kennedy Institute of Rheumatology, London, U.K., and *St. John's Institute of Dermatology, London, U.K.

The immunoregulatory cytokine tumour necrosis factor alpha (TNFα) has been implicated in the pathogenesis of inflammatory skin diseases. Two distinct cell surface receptors for TNFα, the 55 kD (TNFR-55) and the 75 kD (TNFR-75) TNF receptors, have recently been described, but their localisation in normal and diseased skin has not been characterized. We have therefore investigated the distribution of TNFα, TNFR-55 and TNFR-75 in normal skin and skin from patients with psoriasis. Immunohistochemical staining was carried out, using the APAAP technique, on cryostat sections of skin from 8 normal subjects, and paired skin biopsies from uninvolved and involved sites from 7 patients with stable plaque psoriasis. Antibodies used included a polyclonal rabbit anti-TNFα, and monoclonal murine antibodies to TNFR-55 (htr-9) and to TNFR-75 (utr-1).

We report here the novel finding that TNFR-55 in normal skin is distributed throughout the viable layers of the epidermis in a cytoplasmic and peri-nuclear manner, and is also associated with a network of upper dermal dendritic cells. A similar pattern of staining was seen in uninvolved and lesional skin from patients with psoriasis, except that in lesional psoriasis skin there was staining of the parakeratotic stratum corneum, and there was upregulation of expression of TNFR-55 in upper dermal blood vessels. Staining for TNFR-75 in normal skin was restricted to eccrine sweat ducts and dermal dendritic cells, and was absent from the epidermis. There was prominent staining for TNFR-75 in association with upper dermal blood vessels and perivascular infiltrating cells in lesional skin from psoriasis patients. Our finding that keratinocytes in normal skin express the TNFR-55 suggests that the actions of TNFα on epidermal cells in vivo are mediated by binding to this receptor. TNFα was predominantly localized to the basal cell layers of the epidermis in normal skin, and was seen in association with eccrine ducts and sebaceous glands; in lesional psoriasis skin, and to a lesser extent in uninvolved psoriasis skin, TNFα was more evenly distributed throughout the epidermis, and was also localized to upper dermal blood vessels. While marked alterations in the expression of epidermal TNFα and TNFR-55 do not seem to be a feature of psoriasis, upregulation of TNFα, TNFR-55 and TNFR-75 on dermal blood vessels may play an important role in the pathogenesis of this condition by promoting recruitment of inflammatory cells into the skin.

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EXPRESSION OF THE COMPLEMENT REGULATORY MOLECULES CD46, CD55 AND CD59 IS REDUCED IN PSORIATIC LESIONS. Gerard T. Venneker, Pranab K. Das, Marcus M.H.M. Meinardi, Jan D. Bos and Shafi S. Asghar, Department of Dermatology, University of Amsterdam, Academisch Medisch Centrum, Amsterdam, The Netherlands.

CD46 (membrane cofactor protein; MCP), CD55 (decay accelerating factor; DAF) and CD59 (homologous restriction factor; HRF20) are inhibitors of autologous/homologous complement activation. These proteins occur in cell membrane bound forms on various cell types as well as in soluble forms in various body fluids. They protect tissues and cells from complement mediated injury associated with inflammation. In the present immunohistochemical studies, we have shown that monoclonal antibodies specific for CD46, CD55 and CD59 recognize a series of normal human skin structures, namely hair follicles, sebaceous glands, sweat glands and ducts, endothelium and intercellular spaces between keratinocytes. In addition, CD55 and CD59 were present on elastic fibres. In psoriasis patients, staining of CD46 was found to be decreased specifically in the intercellular spaces between keratinocytes of the basal layer. Staining of CD55 and CD59 around the basal keratinocytes and in the endothelium was decreased moderately in non-lesional skin and drastically (virtually abolished) in psoriatic lesions. Our findings indicate a misregulation of the expression of CD55 and CD59 in psoriatic lesions. This might be due to a possible defect in the phosphatidylinositol mediated signal transduction involving phospholipase C, protein kinase C and phospholipase D. The decreased expression of CD46 can be explained by tissue specific regulatory mechanisms of certain cytokines.

P104

PLATELET ACTIVATING FACTOR (PAF) RELEASE IN SKIN IN PSORIASIS AND DELAYED PRESSURE URTICARIA (DPU). M.R. Judge, R.M. Barr, P.F. Courtney, A.I. Mallet, A.Kobza Black & M.W. Greaves. St. John's Institute of Dermatology, London, UK.

PAF injected intradermally causes inflammation, but the extent of endogenous PAF release and its contribution to inflammation in skin disorders is unclear. We have measured PAF and lysoPAF in skin of patients with psoriasis or DPU.

Suction blister exudates were obtained from patients with DPU and from normal subjects. Exudates from abraded sites on lesional and uninvolved psoriatic skin and skin of normal subjects were collected in 1ml PBS in chambers. The exudates were analysed for PAF and lysoPAF by GC-MS.

There was no significant difference between PAF levels in exudates from lesional and uninvolved psoriatic skin and that of normal subjects, 0.86±0.27, 0.77±0.31 and 0.42±0.11 pmole/abrasion (mean±sem, n=8) respectively. There was less lysoPAF in uninvolved than in involved psoriatic skin (n=4) and skin of normal subjects (n=8), 3.44±0.57, 14.33±2.38 & 12.03±2.9 pmole/abrasion respectively. Suction blister exudates from control DPU skin, and from skin immediately and at 6h after pressure stimulation, contained 1.02±0.28, 1.71±0.59 & 1.73±0.39 nM PAF (n=5) respectively, compared with 1.85±0.92 nM in exudates from normal subjects (n=7).

We conclude that the cutaneous inflammation in DPU and psoriasis is unlikely to be related to increased PAF and lysoPAF in the skin.

P105

DITHRANOL INDUCES DOWN-REGULATION OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTORS. Lajos Kemény*+, Petr Arenberger*, Günter Michel* and Thomas Ruzicka*, + Department of Dermatology, University of Szeged, Hungary, * Department of Dermatology, Univ. of Munich,FRG

Dithranol is highly effective in the treatment of psoriasis, but its mechanism of action is still not fully understood. Since altered expression of epidermal growth factor receptors (EGF-R) in psoriatic epidermis is supposed to have pathogenetic significance, we were interested whether the effects of dithranol may be mediated by an influence on epidermal EGF-R.

Human squamous carcinoma cells (SCL-II) were incubated for 1 h with increasing concentrations (0.1-1.0 µg/ml) of dithranol or its inactive oxidation product, danthrone. After treatment, culture medium was replaced, and standard radioligand binding assays were performed at varying time intervals thereafter with 125J-EGF.

Dithranol, in contrast to danthrone, induced a dose-dependent decrease in EGF binding amounting to 75% at 1.0 µg/ml. The inhibition of binding was also time-dependent with a maximum at 24 h after dithranol treatment. The analysis of saturation curves showed that the inhibition of EGF binding was due to a decrease in receptor number (Bmax: 48000 receptors/cell for control; 18000 receptors/cell for dithranol), while the Kd values remained unchanged (Kd:0.21 for control; 0.19 for dithranol). Due to the postulated role of EGF-R in psoriasis, the modulation of EGF-R by dithranol may contribute to its antipsoriatic action.

P106

SERUM-SOLUBLE IL-2RECEPTOR AND SOLUBLE CD8 ANTIGEN CORRELATE WITH SEVERITY OF DISEASE PROCESS IN PSORIASIS AND REMAIN ELEVATED AFTER CLINICAL HEALING. Franz G. Dunkel, Janine Bock, Volker Bielefeld*, Michael Ständer*, Albert A. Hartmann, Department of Dermatology, Univ. School of Medicine, Würzburg; *Fachklinik for Psoriasis, Bad Bentheim, Germany.

An unexpected low incidence of infectious diseases in 2800 psoriatic patients (among 40,000 pat.; Henseler et al., J. Invest. Dermatol. in press) prompted us to look for parameters indicating an abnormal permanent immunological activation in psoriasis.

Serum levels of soluble Interleukin2-receptor (sIL-2R), soluble CD4 (sCD4) and soluble CD8 antigen (sCD8) were measured by ELISA techniques, samples were taken on admission to hospital (n=67 patients) and three days after last anthralin therapy when all skin lesions had been cleared (n=27). The severity of each patient's psoriasis was measured by the Psoriasis area and severity index (PASI). Erythrocyte sedimentation rate (ESR) was performed in the routine way. Serum sCD4 levels of psoriatic patients were in the normal range, but significant higher levels of sIL-2-R and sCD8 were observed in the psoriatic group [sIL-2-R 748 U/ml (control group,n=26: 359 U/ml); sCD8 505 U/ml (311 U/ml)]. SIL-2R and sCD8 levels correlated with the PASI, but not with the ESR. Both sIL-2R and sCD8 remained elevated after complete clinical healing (before/after therapy: sIL2-R 689/650 U/ml, sCD8 568/575 U/ml). Our results confirm similar findings of a smaller group treated by coal tar. Furthermore, in addition to sIL-2R sCD8 rather than sCD4 appears to be an indicator of chronic immunological activation in psoriasis.

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MACROPHAGES LINING THE EPIDERMO-DERMAL BORDER ARE A CHARACTERISTIC MORPHOLOGICAL FEATURE IN PSORIASIS. Wolfram Sterry, Silke Bruhn, Wolf-Henning Boehncke, Department of Dermatology, Univ. of Ulm, Germany.

We have recently proposed a classification discriminating three subpopulations of dermal macrophages according to their morphology, distribution and immunophenotype. Here, we report the characterization of a new macrophage subpopulation most prominent in psoriasis.

Biopsies from patients with psoriasis (n=4), atopic dermatitis (n=4) and contact dermatitis (n=4) were immunohistochemically analyzed using a panel of monoclonal antibodies directed against macrophage markers.

In all dermatoses, peri- and intervascular macrophages were readily detectable staining positive for CD11c (LeuM5, Ki-M1), CD68 (Ki-M6) and Ki-M8. Additionally, macrophages lining the epidermo-dermal border were detected. These cells also expressed CD11c and, although to a lesser degree, CD68 and the antigen seen by Ki-M8. This subpopulation was most prominent in psoriasis, where a median of 34 macrophages neighboured 100 basal keratinocytes, thus covering about 80% of the border area. In contrast, the numbers for atopic and contact dermatitis were 11 and 13, respectively, accounting for about 20% of the border area.

This is the first report on macrophages lining the epidermo-dermal border, a subpopulation absent in normal skin and most prominently exhibited in psoriatic lesions.

P108

THE CYTOKINE-INDUCED EXPRESSION OF ICAM-1 ON HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS AND PSORIATIC KERATINOCYTES IN VITRO IS NOT INHIBITED BY ANTIPSORIATIC DRUGS. M. Detmar, S. Tenorio, U. Hettmannsperger, C.E. Orfanos. Dept. of Dermatology, Medical Center Steglitz, The Free University of Berlin, Germany.

It has been previously reported that treatment with retinoids or cyclosporin A might suppress the increased expression of ICAM-1 on epidermal keratinocytes (KC) or dermal microvascular endothelial cells (HDMEC) in inflammatory skin diseases. In order to proove whether this effect is mediated by direct suppression of ICAM-1, we investigated the effect of several antipsoriatic drugs on the cytokine-induced ICAM-1 expression of HDMEC and psoriatic KC in vitro.

HDMEC were isolated from neonatal foreskins by trypsin treatment and density gradient centrifugation, and were cultured in supplemented EGM (Clonetics). KC were isolated from involved skin of chronic plaque-type psoriasis (n=8), and were cultured in serum-free KGM. Second passage HDMEC or KC were treated with interleukin 1-beta (IL 1b), IFN-gamma or TNF-alpha (0.1-1000 U/ml) either alone or in combination with acitretin (10^{-6}M), cyclosporin A (10^{-6}M), or calcitriol (10^{-7}M) for 1 day. The expression of ICAM-1 was assessed by APAAP immunocytochemistry of cytospin preparations or by FACS analysis.

IL 1b, IFN-gamma, and TNF dose-dependently induced the expression of ICAM-1 on HDMEC, while only TNF and IFN-gamma induced ICAM-1 expression on psoriatic KC in a dose-dependent manner. Coincubation with acitretin, cyclosporin A, or calcitriol did not inhibit the cytokine-induced ICAM-1 expression on HDMEC or KC. Instead, treatment with acitretin led to a slight increase of ICAM-1 expression on cytokine-stimulated KC.

These data suggest that the therapeutic effect of antipsoriatic drugs is not mediated by direct down-regulation of ICAM-1 on dermal endothelial cells or epidermal KC. The diminished ICAM-1 expression after treatment in vitro might rather be caused by effects on infiltrating leukocytes and/or on the production of inflammatory cytokines.

P109

EXPRESSION OF CYCLOPHILIN ISOFORMS IN HUMAN EPIDERMAL CELLS. Dominique Chatellard-Gruaz, Georges Siegenthaler, Jean-Hilaire Saurat, Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Geneve 4, Switzerland.

Cyclosporine A (CsA) is effective in the treatment of severe psoriasis. CsA has an effect on both keratinocytes and chronic inflammatory cells. It has been suggested that cyclophilin (Cyp) a cytosolic receptor of CsA is involved in CsA's mechanism of action, but the role of Cyp particularly in keratinocytes is unknown. We have developed a PAGE radiobinding assay for analysis Cyp isoforms expression in cytosolic extracts of human normal skin (HNS), psoriatic plaques (PP), non-differentiated (NDCK) and differentiating (DCK) cultured keratinocytes. Two specific Cyp isoforms were detected in HNS (major and minor forms), both having a Mr of 17 kDa. The major form was expressed at similar level (values in pmol[14C]CsA bound/mg protein) in HNS (54.96±18.18) in PP (56.30±24.54) in NDCK (37.55±16.16) and in DCK (40.47±11.25). In contrast the minor isoform content varied significantly; *in vivo* it was decreased in PP as compared to HNS (4.78±9.06; 27.51±17.25 respectively $p<0.01$); and *in vitro* it was increased in DCK as compared to NDCK (1.94±1.42; 11.32±6.96 $p<0.02$). These observations show that (i) our PAGE assay is able to identify isoforms of Cyp in keratinocytes and (ii) there are variations in the relative amount of these isoforms as related to the differentiation of keratinocytes which might be relevant to the response of PP to CsA.

P110

DISTRIBUTON OF RETIONOIC ACID RECEPTORS ALPHA1 AND GAMMA1 IN HUMAN NORMAL EPIDERMIS, ORAL MUCOSA AND PSORIASIS. I. Masouyé[1], M.P. Gaub[2], C. Rochette-Egly[2], L.Didierjean[1], P.Chambon[2], J.H. Saurat[1]. [1]Clinique de Dermatologie, Hôpital Cantonal Universitaire,1211 Genève 4, Switzerland - [2]Unité 184 de Biologie Moléculaire de l'INSERM, Faculté de Médecine, 67085 Strasbourg Cedex-France

A panel of mouse monoclonal antibodies (MoAb) raised against synthetic peptides specific to either RAR-α1 or RAR-γ1 has been screened for immunoreactivity on frozen sections of normal human epidermis. A specific immunostaining was obtained with two MoAb produced respectively against the F region (aminoacid residues 420-462) of the RAR-α1 and to the A region (aminoacid residues 39-50) of the RAR-γ1, indicating that these epitopes were accessible in vivo.

In normal human epidermis and non-keratinizing oral mucosa, a strong RAR-α1 nuclear immunoreactivity was demonstrated in about two third of the keratinocytes, the staining being predominant in the stratum spinulosum; psoriatic epidermis exhibited a significant increase of RAR-α1 expression. In all samples, we observed a diffuse RAR-γ1 immunoreactivity in all epidermal layers except the stratum corneum and a shift from a predominent cytoplasmic pattern in the basal layers to a predominent nuclear labelling in the uppermost layers. This study shows that both RAR-α1 and RAR-γ1 proteins are expressed in human epidermis and oral mucosa which is concordant to the previous detection of mRNA for RAR-α and -γ ; Their expression appears to vary with both the stage and type of keratinocytes differentiation.

P111

IMMUNOHISTOCHEMICAL ANALYSIS OF NEUROPEPTIDES IN PSORIATIC SKIN DURING THE KOEBNER RESPONSE INDUCED BY TAPE-STRIPPING. Anita Naukkarinen[a], Ilkka T. Harvima, Kari Paukkonen, and Maija Horsmanheimo, Departments of Pathology[a] and Dermatology, University of Kuopio, Kuopio, Finland.

The distribution of neuropeptides Substance P (SP), vasoactive intestinal polypeptide (VIP) and calcitonin gene related peptide (CGRP) was studied immunohistochemically in psoriatic skin during the Koebner response (6h, 2d, 7d, 14d, 21d), and in mature psoriatic plaques of 37 psoriatic patients. The morphological association of SP and VIP with papillary mast cells was also monitored.

The nerves containing SP, VIP or CGRP were very scanty in control skin, nonlesional and Koebner-negative psoriatic skin. The first psoriatic lesions were seen 7 days after tape-stripping the symptomless psoriatic skin. Both SP and VIP containing nerves were increased in Koebner-positive specimens, their appearance being most prominent in dermal papillae of mature psoriatic plaques. In the plaques, only SP-positive fibers were detected in epidermis and in contact with papillary mast cells. VIP was mainly located around capillaries where SP was also found. No marked change was noticed in the distribution of CGRP between nonlesional specimens and psoriatic lesions.

Both SP and VIP obviously function as vasodilators in the psoriatic lesion, and they seem to participate in maintaining the inflammatory reaction rather than initiating it. Morphological contacts between mast cells and SP containing nerves give further evidence to the view that SP is capable of amplifying the inflammatory reaction also through the axon-reflex mechanism.

P112

ELEVATED EXPRESSION OF INTERLEUKIN-4 and TNF-α RECEPTORS ON PSORIATIC KERATINOCYTES. E. Prens, J. Hegmans, K. Benne, R. Debets, Th. van Joost and R. Benner. Departments of Dermatology and Immunology, University Hospital Dijkzigt and Erasmus University Rotterdam.

An elevated expression of cytokines such as IL-6, IL-8, INF-γ and TNF-α and the growth factor TGF-α have been observed in psoriatic lesions. These cytokines may *trigger* keratinocyte hyperproliferation either directly by serving as a growth factor or via activation of the keratinocyte. Proliferation of the keratinocyte may be *maintained* by e.g. autocrine growth stimulation by cytokines or growth factors (TGF-α) produced by the activated psoriatic keratinocyte. The regulation of keratinocyte activation and autocrine growth is a complex proces involving interactions between cytokines, growth factors and their receptors. Little is known about the expression of cytokine receptors in healthy or diseased skin. We therefore studied the expression of IL-1α, IL-1β, IL-4, IL-6 and TNF-α receptors in epidermal cell suspensions (ECS) from healthy individuals and psoriasis patients. The cells were labeled using biotinylated recombinant human cytokines and streptavidin-FITC or Phycoerythrin as the second step, followed by FACscan analysis. Psoriatic ECS expressed significantly elevated levels of IL-4R and TNF-αR compared to control ECS. Normal ECS rapidly upregulated their IL-4R after stimulation with LPS and PMA, whereas TNF-αR was not, in contrast to psoriatic ECS. The latter together with the occurrence of elevated levels of IL-4R on carcinoma cells, may reflect a state of proliferation. The exact role of these receptors in psoriasis needs further clarification.

P113

STREPTOCOCCAL ANTIGEN-SPECIFIC T LYMPHOCYTES IN SKIN LESIONS OF GUTTATE PSORIASIS. Barbara S.Baker, S.Bokth, A.V.Powles, *H.Valdimarsson and Lionel Fry. Department of Dermatology, St Mary's Hospital, Paddington, London and *Department of Immunology, Landspitalinn, Reykjavik, Iceland.

To demonstrate the presence of streptococcal-specific T cells in the skin, T cell lines were established from biopsies of lesions from 5 patients with guttate psoriasis; all 5 T cell lines responded in a proliferation assay to heat-killed isolates of group A streptococci.

One of the T cell lines was cloned in the presence of type 5 streptococcal M protein. The 9 clones obtained were all CD3+, CD4+, CD45RO+, α,β+, γ,δ-. However, they were all unreactive with antibodies to TCR Vβ 5,6,8 or 12. Eight of the 9 clones reacted to one or more of 3 preparations of group A streptococci expressing different M proteins. The responses of 4 clones which remained stable were HLA-DR8-restricted and inhibited by anti-HLA-DR antibody in a dose-dependent manner. On stimulation these clones secreted high levels of gamma-interferon and detectable levels of IL-2, IL-10 and GM-CSF depending upon the nature of the stimulus. However no production of IL-4 or TNF-α was detected.

This study has shown, for the first time, that T lymphocytes specific for group A streptococcal antigens can be isolated from guttate psoriatic lesions.

P114

NEUROGENIC CHANGES IN PSORIASIS: AN IMMUNOHISTOCHEMICAL STUDY. M.S.K. Al'Abadie, H.J. Senior, S.S. Bleehen, D.J. Gawkrodger, University Department of Dermatology, Royal Hallamshire Hospital, Sheffield S10 2JF, UK.

To test the hypothesis of neuronal involvement in psoriasis, we examined the distribution of nerves and neuropeptides in psoriatic skin. Immunohistochemistry using antibodies to general neuronal marker PGP 9.5, substance P (SP), vasoactive intestinal polypeptide (VIP), calcitonin gene related peptide (CGRP) and neuropeptide Y (NPY), was used to study neuronal activity in skin biopsies (taken from arm or leg) from lesional psoriatic skin (11), non-lesional psoriatic skin (1) and non-psoriatic controls (7). Semiquantitative evaluation was made of the dermis, epidermis, dermo-epidermal junction and appendages. VIP showed the most striking change: reactivity in lesional psoriatic skin was increased around sweat glands, in the dermis and epidermis and most prominently at the dermo-epidermal junction where the fibres ran perpendicularly through the junction (9/11). Neuronal marker PGP 9.5 was increased in psoriatic skin, mainly in the papillary dermis (8/11 cases). SP was increased in psoriatic skin but only at the dermo-epidermal junction where the fibres ran perpendicularly (6/11). CGRP and NPY reactivity did not deviate from the norm in the 3 groups. Non-lesional psoriatic skin (1 case) showed slight changes similar to the psoriatic pattern for SP and VIP. Non-psoriatic control skin (7 cases) showed normal reactivity for all antibodies. These findings support the hypothesis of a neurogenic inflammatory background in the pathogenesis of psoriasis.

P115

DEVELOPMENT OF A NEW METHOD FOR PLASMA-MEMBRANE ISOLATION FROM KERATINOCYTES FOR THE SENSITIVE DETECTION OF MEMBRANE ANTIGENS IN NORMAL AND PSORIATIC SKIN.

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The signal function of altered membrane proteins is discussed for many pathologic processes such as tumor growth and metastasis or homing of leukocytes. The Alteration of distinct membrane proteins is also discussed for the pathogenesis of psoriasis. The method described in the following is enzymefree and allows a selective and more detailed research of membrane protein alterations within a short time. As a result, membrane proteins of normal and psoriatic cultivated cells can be distinguished. For the plasma-membrane isolation the cells were homogenized in EGTA-medium, centrifugated at 500 x g, then 100 000 x g, and finally layered on a succrose gradient. Trypsin or other enzymes were avoided. Cell cultures of normal, foreskin, transformed and psoriatic cells were used after one passage. SDS-solubilized homogenate and membrane portions of equal protein content were electrophoretically separated and subsequently blotted on nitrocellulose. Unspecific protein-staining and specific carbohydrate-stainings with lectins (Con-A, DBA, UEA I, PNA, WGA) were performed. As a result, a membrane fraction of high purity was obtained with enriched activity of membrane marker-enzymes, and endoplasmatic reticulum as the only contamination. In the lectin staining, the glycoproteins were commonly slightly enriched in the membrane fractions, as expected. Up to 75 glycoproteins could be distinguished in the membrane fraction. The following differences appeared between normal and psoriatic keratinocyte membranes: 5 proteins after Con A-staining (98, 84, 63, 45 and 44 kDa), 6 proteins after UEA I-staining (2 x 125, 2 x 68, 64, 53 kDa) two 18 kDA-proteins after PNA-staining, one 34 kDa-protein after WGA-staining, and no differences after DBA-staining. We conclude from our experiments that the method demonstrated for plasma-membrane isolation allows an advanced selective and sensitive research of membrane proteins from normal and pathologically altered keratinocytes. The differing psoriatic membrane glycoproteins observed correspond to known histological findings. They may lead to a better understanding of the pathogenesis, to a differenciated possibility of therapy control, and to new therapeutic concepts for this disease.

P116

mRNA LEVELS FOR INTERLEUKIN 1 ALPHA AND THE 80 KD INTERLEUKIN 1 RECEPTOR IN COCULTURES OF EPIDERMAL AND DERMAL CELLS

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Interleukin 1 (Il 1) alpha and beta are potent mediators of inflammation with pleiotropic effects on virtually all cells. While keratinocytes synthesize biologically active IL 1 alpha in vivo as well as in vitro little is known about transcriptional regulation of the 80 kd IL 1 receptor (IL 1 R I) in IL 1 responsive cells i.e. fibroblasts. Recently, the 80 kd IL 1 receptor expressed on T cells and fibroblasts was cloned. Total RNA preparations of normal human skin and cultured cells (keratinocytes, fibroblasts and microvascular endothelial cells (HDMEC)) were analyzed in northern hybridization experiments for expression of IL 1 alpha and Il 1 R I mRNA. In normal human skin of six different individuals IL 1 alpha as well as IL 1 R I were almost undetectable. In addition, we found neither IL 1 alpha nor IL 1 R I transcripts in keratinocyte cultures (in the presence of hydrocortisone 0,4 ug/ml). In contrast, some fibroblast and HDMEC cell strains expressed variable IL 1 R I mRNA levels whereas in some strains IL 1 R I mRNA was undetectable. IL 1 alpha signals were absent in all dermal cell populations. Low IL 1 R I mRNA levels in fibroblasts and HDMEC could be substantially stimulated by coculture with keratinocytes in monolayer cultures. In addition, IL 1 alpha and IL 1 R I mRNA expression in three dimensional organotypic cocultures consisting of keratinocytes cultured on fibroblast populated collagen (type I) gels at the air medium interface (organotypic cocultures) were analyzed. In keratinocytes IL 1 R I was absent throughout whereas IL 1 alpha mRNA peaks were detected early i.e. 1 and 2 days after air exposure slowly decreasing with time in culture. Fibroblasts regulated IL 1 R I mRNA reciprocally, first visible after 4 days in culture increasing steadily while IL 1 alpha was not expressed. Future work will have to investigate the mechanisms for coordinate regulation of IL 1 alpha and its receptor in epidermal and dermal cell populations *in vivo*.

P117

DEMONSTRATION OF INTERLEUKIN-8 RECEPTOR RESPONSIBLE FOR CHEMOTAXIS OF NORMAL HUMAN EPIDERMAL CELLS. Günter Michel, Lajos Kemény, Christine Ried, Axel Beetz, Thomas Ruzicka, Department of Dermatology, University of Munich, FRG

Since recently chemotactic effects of interleukin-8 (IL-8) on epidermal cell lines had been described, we were interested whether this proinflammatory cytokine could influence the chemotaxis of normal human keratinocytes acting via specific receptors.

Epidermal cells were separated from healthy individuals. The chemotactic response was studied in modified Boyden chambers with freshly isolated epidermal cells. To study the putative IL-8 receptors, radioligand binding assays with 125J-rhIL-8 (72 amino acid form) were performed on cultured normal human keratinocytes.

Epidermal cells showed significant chemotactic response towards IL-8 with a maximum at concentrations of 1-10 nM. A rather unusual IL-8 binding characteristics of cultured normal human keratinocytes suggested IL-8 dimer binding with a Kd in the range of 1 nM and Bmax of 15,000 receptors/cell. Dimer formation was demonstrated by SDS-PAGE and Western blot techniques.

Thus, we could show for the first time that normal human keratinocytes possess IL-8 receptors, which are likely to be involved in IL-8-induced chemotaxis of epidermal cells. These data suggest that IL-8 could play a role in the activation of epidermal cells in skin conditions with increased IL-8 levels, such as psoriasis.

P118

THE INTERLEUKIN-2 RECEPTOR IN LESIONS AND SERUM OF BULLOUS PEMPHIGOID. D. Zillikens[1], C. Zieglmeyer[1], R. Dummer[2], M. Burger[1], A.A. Hartmann[1], and G. Burg[2], [1]Department of Dermatology, University of Würzburg, FRG, and [2]Department of Dermatology, University of Zürich, Switzerland.

The interleukin-2 receptor (IL-2R) is mainly expressed on activated lymphocytes. According to its rate of synthesis, a part is released from the cell surface as soluble IL-2R (sIL-2R). Since the role of lymphocytes in the pathology of bullous pemphigoid (BP) is not well understood, we determined the sIL-2R by ELISA in both blister fluid and serum of 15 BP patients with generalized disease before initiating a systemic treatment. In addition, we obtained both lesional and perilesional skin biopsies from all patients and examined the mononuclear infiltrate with a panel of monoclonal antibodies.

In BP blisters, sIL-2R levels were significantly increased (2070±350 U/ml) (±SEM) compared to serum samples taken at the time of blister puncture (1340±290 U/ml) ($p<0.001$). Serum levels in 25 healthy controls were 410±70 U/ml. In patients with 2° burns (n=4), friction-induced bullae after sports (n=3) and in suction blister volunteers (n=5), sIL-2R levels were normal in both blister fluids and serum. In BP, elevated serum levels decreased to normal during therapy, correlating with disease activity. The immunohistology of BP lesions showed that 70% of mononuclear cells expressed CD3, 75% were positive for CD4, and 30% expressed the IL-2R, whereas only 15% were positive for the IL-2R in perilesional skin. IL-2R+ cells of the dermal infiltrate are the most likely source of the shed receptor in BP blisters. Our results indicate the presence of activated lymphocytes in lesions and peripheral blood of BP and thus underline the importance of cell-mediated immune mechanisms in the pathology of this disease.

P119

TNF-α IN BLISTER FLUIDS OF BULLOUS PEMPHIGOID IS NOT BIOLOGICALLY ACTIVE

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In order to further elucidate the role of TNF-α in blister formation in Bullous Pemphigoid (BP) the levels of bioactive and immunochemical TNF-α were determined in blister fluids of BP and compared to controls.

Ten patient samples were obtained by blister puncture. Five patients were untreated with clinical, histological and immunofluorescent (IF) findings of BP. Two patients (untreated) had clinical and histological findings of BP with negative IF. One patient (untreated) had clinical and IF findings suggestive of BP with histology indicative of Herpes Gestationis. One patient was diagnosed BP but on prednisolone therapy and the last patient had Pemphigus vulgaris. Four control samples were obtained from normal volunteers using a suction blister apparatus.

Immunochemical TNF-α was determined by ELISA (British Biotechnology). Bioactive TNF-α was measured using the L929 cytotoxicity assay in the presence of actinomycin-D to improve sensitivity. The bioassay was validated for use with trauma blister fluid. Bioactive human recombinant TNF-α was fully recovered from blister fluid and stable upon storage in blister fluid for one month at -70°C. Thus, there was no evidence that blister fluid contained inhibitors of TNF-α. Immunochemical TNF-α was detected in 3 of the 5 untreated BP blister fluids (90, 260 & 1120 pg/ml). The 2 blister fluids from patients with negative IF contained low levels of immunochemical TNF-α (12 & 13 pg/ml). The remaining 3 patient samples were negative. Two of the 4 control suction blister fluids contained immunochemical TNF-α (8 & 150 pg/ml). There was no significant difference between the mean levels of immunochemical TNF-α in blister fluids from the 5 untreated BP patients and control suction blister fluids (analysis of variance).

Bioactive TNF-α was not detected in any of the 10 patient samples or controls thus although immunochemical TNF-α may be present in blister fluids from some BP patients, it is not bioactive. Moreover immunochemical TNF-α may be present in suction blister fluids from normal individuals. It is unlikely that TNF-α plays a major role in blister formation in BP.

P120

INTERFERON-GAMMA (IFN-γ) INDUCES KERATINOCYTE PROLIFERATION IN VIVO.

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Studies indicate a pro-inflammatory role for the activated T-cell derived cytokine, IFN-γ, in cutaneous immunopathology. To determine whether IFN-γ also contributes to keratinocyte hyperproliferation in vivo, normal human volunteers received intradermal injections of recombinant human IFN-γ (3-30μg), or tumour necrosis factor-α (TNF-α) and keratinocyte proliferation assessed on whole skin biopsies using 3 independent histochemical parameters; (a) silver-nucleolar organiser region (AgNOR) counts, (b) proliferating cell nuclear antigen (PCNA), and (c) hyperproliferative keratin 16 (K16) expression.

Results obtained from biopsies taken on day 6 following first IFN-γ injection (n=9) revealed significantly increased scores for each parameter. (a) AgNOR (counts per nucleus): IFN-γ; mean=3.99 (sem=0.2), control =2.48 (sem=0.42) ($p<0.01$), (b) PCNA (per hpf); IFN-γ=40.9 (sem=3.9), control =16.6 (sem=3.5) ($p<0.01$) and (c) K16; IFN-γ=1.7 (sem=0.36), control =0.14 (sem=0.14) ($p<0.01$). Proliferation indices were not elevated at 48 hours following IFN-γ injection nor following TNF-α (100U) intradermally at day 6 despite the presence of a similar inflammatory infiltrate. As expected proliferation indices were upregulated in psoriatic epidermis (n=5).

These studies demonstrate that, in contrast to its in vitro growth inhibitory effects, IFN-γ induces keratinocyte hyperproliferation in vivo, indicating both a pro-inflammatory and growth regulatory role for this cytokine. Since IFN-γ production is detectable in psoriatic epidermis, these findings may be of particular importance to the pathophysiology of this disease.

P121

A FIBROBLAST GROWTH FACTOR-LIKE ACTIVITY SECRETED BY A DENDRITIC B-CELL LINE,

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We have recently established an Epstein-Barr virus (EBV)-positive B-cell line (R594-4) with a high stimulatory capacity for T-lymphocytes in allogeneic mixed leukocyte reactions, comparable to dendritic cells (i.e. epidermal Langerhans cells). Screening for cytokine mRNA's revealed abundant expression of K-fgf, a member of the fibroblast growth factor (FGF) family. To test as to whether functional K-fgf is secreted by R594-4, cell lines of mesenchymal origin were cultured in the presence of conditioned medium (CM) derived from R594-4. Cell cultures, kept in serum-free medium, proliferated in a dose-dependent fashion as judged by ^{3}H-Thymdine incorporation or staining with a monoclonal antibody against a proliferation-associated antigen (Ki-67). Interestingly, R594-4-derived CM also supported long-term growth of cell lines derived from Kaposi's Sarcoma (KS) lesions.

After incubation of CM with heparin-sepharose, the growth promoting activity was abolished indicating a close relationship to heparin-binding growth factors. Neutralizing antibodies to IL-1α, IL-1β, TNF-α, aFGF and bFGF did not block proliferation suggesting that the factor(s) in R594-4CM differs from these cytokines. Analysis by gel filtration showed that the active component in R594-4CM had a molecular size of about 52 kD. Further functional and biochemical studies are currently under way to delineate the nature of this factor(s).

P122

THE LYMPHOCYTE ATTRACTANT EFFECTS OF INTERLEUKINS 1α AND 8 ARE NOT DUE TO THE RELEASE OF A SECONDARY CHEMOATTRACTANT FACTOR.

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The in vitro peripheral blood lymphocyte (PBL) attractant effects of cytokines, including interleukin (IL)-1α and IL-8 which have been proposed to play a role in skin disease, may require further characterisation. We have therefore determined whether these effects of IL-1α and IL-8 are direct or due to in vitro production by PBL of secondary chemoattractant substance(s) during the migration assay. In preliminary experiments the concentration of specific neutralising antibody (NAb) needed to neutralise maximal responses to IL-1α and IL-8 in the PBL migration assay were determined. Subsequently, PBL ($2x10^7$ in 0.1ml) were incubated with a range of concentrations of IL-1α or IL-8 for 30min at 37°C. Neutralising concentrations of corresponding NAb were then added after 30min, supernatants harvested and tested in the PBL migration assay after serial dilution[1]. Responses were compared with those due to the corresponding IL alone[2], and NAb-free supernatant from PBL incubated in the absence of IL (background, Bg[3]), in the same assay. Results were as follows:

IL-8[2]	IL-8 NAb[1]	Bg[3]	IL-1α[2]	IL-1α NAb[1]	Bg[3]
2.07±0.09	1.42±0.12	1.37±0.03	1.97±0.03	1.31±0.12	1.36±0.09

[results expressed as a mean maximal migration index ± S.E.M., n=3]

The lack of attractant activity above Bg in supernatants to which NAb had been added following incubation of PBL with IL-1α and IL-8 for 30min, indicates that the in vitro PBL attractant effect of these cytokines is direct and not due to the release of a secondary chemoattractant factor.

P123

NORMAL HUMAN EOSINOPHILS ARE ACTIVATED BY TUMOR NECROSIS FACTORS (TNFα and β) AND EXPRESS THE 55 kD AND 75 kD TNF-RECEPTOR.

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Eosinophils play a major role in allergic inflammatory diseases. Cytokines, released from activated immune cells may be relevant stimuli for these effector cells. In the present study, it is investigated whether TNFα which is released by keratinocytes and monocytes in the skin during inflammatory reactions may be a significant activator of eosinophils (EO). TNFα induced a dose-dependent long-lasting lucigenin-dependent chemiluminescence (CL) response in highly purified normal human EO. Based on its effect in cytotoxicity assays TNFβ was significantly less active. Both stimuli, however, did not induce significant degranulation as measured by the release of eosinophil peroxidase. These functional findings were paralleled by morphological changes induced by TNFα. As estimated by transmission and scanning electron microscopy almost all TNFα-stimulated EO appeared to be strictly adherent to the plastic layer, exhibiting a typical "polarized hemispheric" shape. Hydrogen peroxide production, detected by an ultrastructural technique, was observed on the outer surface of the plasma membrane in the contact zones in between adjacent cells. TNF-induced activation of the EO is apparently mediated by binding to the 55 kD and the 75 kD TNF-receptor which could be detected both by FACS analysis and immune-electron microscopy using receptor-specific monoclonal antibodies. Based on FACS analysis the 75 kD TNF-receptor is expressed to a significantly lesser extent. These results indicate that TNFα represents an additional important eosinophil-activating cytokine which may be of relevance in atopic diseases.

P124

INTERLEUKIN 6 ACTIVITY IN POLYMORPHIC LIGHT ERUPTION AND CHRONIC ACTINIC DERMATITIS.

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Biologically active or immunoreactive interleukin (IL)-6-like material has been identified in skin samples from certain dermatoses, including mycosis fungoides, deep pressure urticaria and psoriasis. IL-6 has also been found to induce peripheral blood lymphocyte (PBL) migration in vitro (Bacon et al, Cytokine 2:100-105,1990). As polymorphic light eruption (PMLE) and chronic actinic dermatitis (CAD) are characterized by a lymphocyte-rich inflammatory infiltrate, we have studied IL-6 activity in suction blister fluid (SBF) at different time points following irradiation of susceptible patients with a solar simulator. Centricon 3 ultrafiltration retentates were assayed for IL-6 in a B9 proliferation assay:

	Control*	1-3 hours*	24 hours*
PMLE (n=5)	0	82 ± 21	284 ± 137
CAD (n=4)	0	972 ± 550	145 ± 89

[*results expressed as mean pg IL-6 activity/ml SBF ± SEM]

Multiple dilutions of SBF were also assayed for PBL attractant activity in an in vitro microchemotaxis assay, and effects of IL-6 antiserum determined. In 24 hour PMLE SBF samples, maximal PBL migration indices were reduced from 2.51 ± 0.19 to 1.25 ± 0.06 (means ± SEM, n=4, p=0.003, paired T test) by IL-6 antiserum. IL-6-like material is therefore produced in a range of skin diseases including these photodermatoses. It may have multiple effects, but at least in PMLE is produced in amounts sufficient to induce PBL migration.

P125

INTERLEUKIN 8 INDUCES T LYMPHOBLAST ADHESION TO FIBRONECTIN. Perry J. Hartfield and Richard D.R. Camp, St. John's Institute of Dermatology, St. Thomas's Hospital, United Medical and Dental Schools, London.

Interleukin 8 (IL-8) may play an important role in the pathology of psoriasis. A number of studies have shown IL-8 to be chemotactic for both neutrophils and lymphocytes, and recent observations have implicated IL-8 in the events mediating adhesion of neutrophils to matrix proteins and endothelial monolayers *in vitro*. In the present study we have investigated the effects of recombinant IL-8 (monocyte-derived and endothelial-derived proteins) on T lymphoblast adhesion to the extracellular matrix (ECM) protein, fibronectin (FN), in an *in vitro* 96-well microtitre plate assay. Lymphoblasts (1 x 10^6/ml) were incubated for 1 hr at 37° C in the absence or the presence of IL-8 (10^{-9} M, 10^{-8} M and 10^{-7} M). Adhesion was measured by staining of adherent cells with Rose Bengal, followed by lysis and determination of the optical densities spectrophotometrically. Both monocyte-derived and endothelial-derived IL-8 increased lymphoblast adhesion to FN-coated plates, as follows:

% Increased Adhesion vs. Control (mean ± S.D., n=3)

	10^{-9} M	10^{-8} M	10^{-7} M
monocyte-derived IL-8	240 ± 53	251 ± 68	245 ± 39
endothelial-derived IL-8	258 ± 50	275 ± 65	271 ± 39

Activated lymphoblasts (pretreated with phorbol ester, PMA, 20 ng/ml, 30 min, 37° C) also adhere to FN-coated plates with similar levels of increased avidity (% increase vs. control = 208 ± 39, n = 4). These results suggest that IL-8 may activate T lymphoblasts by increasing surface ligand avidity and that IL-8 may play a role in adhesive pathways regulating cell-ECM interactions, and possibly, cell-cell interactions.

P126

NCOI POLYMORPHISM OF THE HUMAN TNF-ß GENE IS ASSOCIATED WITH IMMUNOGENETIC AND CLINICAL SUBTYPES OF SYSTEMIC SCLERODERMA. Gerald Messer, Sabine Franz, Elisabeth H. Weiss[2] and Michael Meurer. Dept. of Dermatology, Ludwig-Maximilians-University of Munich, Frauenlobstr. 9-11, W-8000 München 2; [2] Institute of Immunology, L.-M.-University of Munich, Goethestr. 31, W-8000 München 2; F.R.G.

Autoimmune disorders in humans are often associated with particular alleles of genes of the major histocompatibility complex (MHC). The so far among cytokines unique genomic localization of the tumor necrosis factor locus, between the gene clusters of MHC class I and class II genes, has prompted the idea that a polymorphism of the TNF genes might play a role in autoimmune diseases, including scleroderma. We have isolated genomic DNA of 63 patients with systemic scleroderma (SSc) and studied the allelic distribution of an TNF-ß NcoI RFLP by amplification of part of the TNF-ß gene by polymerase chain reaction and endonuclease digestion thereafter. A first screening of all 63 patients indicated no difference in the genetic distribution of the TNF-ß alleles TNFB*1 and TNFB*2. However, an association to the RFLP of the TNF-ß gene was seen when the different clinical subtypes of SSc were investigated. In patients with diffuse SSc (N=26) the allele frequency of the TNFB*1 allele is markedly reduced (0.21) compared to the controls (0.33) and so far no cases of homozygosity for TNFB*1 could be detected. In limited SSc (N=29), the allele TNFB*1 is more frequent (0.38) and presents with 20.7% of patients homozygous for TNFB*1. The most apparent difference occured in the allelic distribution of the NcoI RFLP in patients with sclerodermatomyositis (N=8), who were all positive for the PM-Scl antibody and the HLA-A1, -B8, -DR3 haplotype. No homozygous patients for TNFB*2 are found and the allele TNFB*1 is present homozygously in 3 of 8 cases. This indicates the same close linkage as previously observed for HLA-DR3 in sclerodermatomyositis (Genth et al. 1991, Arth Rheumatism). The TNF-ß RFLP serves as an informative marker in the search of susceptibility genes in SSc and in addition, shows the strongest linkage between HLA-DR and TNF-ß.

P127

QUANTITATIVE DETERMINATION OF EPIDERMAL CYTOKINE mRNA LEVELS BY THE POLYMERASE CHAIN REACTION. Kirsten Paludan and Kristian Thestrup-Pedersen. Department of Dermatology, Marselisborg Hospital, Århus, Denmark.

To obtain detailed information of relative levels of cytokine gene expression in different skin compartments in various disease types we developed a protocol for quantitative analysis of cytokine mRNAs in large numbers of small samples. We used a combination and modification of methods for quantification by polymerase chain reaction (PCR) cDNA amplification demanding only a few analyses per sample and only picogram amounts of RNA per analysis. We present our protocol and show the results of analysis of more than a hundred epidermal samples for relative levels of interleukin (IL) 8, and representative samples among these for IL-1α, IL-6, and tumor necrosis factor-α (TNF-α) mRNA.

Epidermal samples were obtained by gentle curettage from normal test persons and patients with various skin diseases or induced skin reactions. Cytokine mRNA levels were determined relative to glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA under quantitative PCR conditions. IL-8/GAPDH signal ratios were high (15 - 400%) in diseases showing a high degree of inflammation and in induced allergic reactions, low (0 - 10%) in patients in a chronic stage of eczema and in irritant patch test reactions, low in steroid treated patients, and zero in normal control persons. The ratios for IL-1α and IL-6 were low (0 - 5%) in all cases tested (34 and 32, respectively), with no clear distribution pattern. No TNF-α signal was seen in any of 29 representative samples analyzed.

The results point out high IL-8 gene expression as an epidermal marker for certain inflammatory conditions. The lower epidermal levels of IL-1α, IL-6, and TNF-α mRNA make analysis of other skin compartments important. Our method should be useful for this as well as for any analysis of rare mRNAs in large numbers of small samples.

P128

IFN-GAMMA INDUCED MHC CLASS II EXPRESSION ON HUMAN KERATINOCYTES IS SPECIFICALLY SUPPRESSED BY TGF-BETA. S. Kukel, U. Reinhold, H. Abken, H.-W. Kaiser, and H.-W. Kreysel. Department of Dermatology and Institute for Genetics, University of Bonn, Bonn, Germany.

IFN-γ is secreted by T cells in response to stimulation by specific antigen or mitogens and is a potent regulator of class II MHC antigen expression on the surface of different cell types including human keratinocytes. In certain pathologic states such as inflammatory skin diseases class II antigen may be expressed on keratinocytes in vivo and might be the result of local IFN-γ secretion by skin infiltrating T cells. In contrast to expanding results on cytokine-mediated upregulation of activation antigens little is known about downregulatory mechanisms. In the present study the effect of TGF-β_1 on IFN-γ induced MHC class II expression was tested. Normal human keratinocytes were cultured in different concentrations of human TGF-β_1 and/or IFN -γ in vitro for different periods of time. Surface expression of MHC class I and II antigens and ICAM-1 on these cells was analyzed by flow cytometry.TGF-β_1 mediated a dose dependent inhibition of IFN-γ induced MHC class II expression by an optimal dose of 1 ng/ml. The effect was enhanced when keratinocytes were preincubated with TGF-β_1 for 6 hours. Northern blot analysis revealed that downregulation of HLA-DR antigen expression in cultured keratinocytes was at the transcriptional level. The expression of MHC class I antigen and the IFN-γ induced ICAM-1 expression was not affected by TGF-β_1. Consistant with the finding that human keratinocytes as well as lymphocytes are able to produce TGF-β_1 our results implicate the possibility of a cytokine-mediated autoregulatory system leading to downregulation of activation antigens on epidermal cells in inflammatory skin reactions.

P129

DECREASED GAMMA-IFN AND IL-6 RELEASE OF PBMC FROM PATIENTS WITH ACTIVE ALOPECIA AREATA IN VITRO Harald Gollnick, Rainer Kirchhof, Martin Owsianowski, Birgit Bogdanoff, Constantin E. Orfanos, Dept. Dermatology, University Medical Center Steglitz The Free University of Berlin, Berlin, Germany.

In alopecia areata (AA) patients a decreased lymphocyte stimulation in vitro and decreased $CD8^+$-T-cells in the peripheral blood have been repeatedly reported. Recently reduced sensitivity of peripheral mononuclear blood cells (PBMC) to IL-2 in vitro was observed. The purpose of our study was to investigate the release of mediators involved in cell-cell interactions and inflammation, such as interferon gamma (g IFN) and Interleukin-6 (IL-6).

PBMC from healthy volunteers (n=5) and AA patients (n=11) were isolated and 10^5 cells/200 l incubated in RPMI 1640 with autologous plasma for 48h. Stimulation was performed with PHA (2,5 g/ml), Con A (10 g/ml), g IFN (125 u/ml) and IL-4 (125 u/ml). Supernatants were stored at -20°C and IL-6 and IFN were measured with higly sensitive and specific in house ELISA's. Unstimulated cells served for spontaneous release as additional controls.

The results are shown in the following table:

stimulus	spontaneous		PHA		Con A		g IFN		IL-4	
cytokines	IL-6	g IFN	IL-6	g IFN	IL-6	g IFN	IL-6	g IFN	IL-6	g IFN
AA	0	0	17.3	0	0.6	0	0.5	353.0	0	0
controls	5.2	0	93.9	9.2	37.8	43.8	3.7	510.7	1.1	0

datas given in ng/ml

Our results points toward either a profound defect in the IL-6 and g IFN production of PBMC to appropiate mitogen stimulation or the presence of suppressive circulating factors in the blood of AA patients with active disease.

P130

CYTOKINE-INDUCED INTERLEUKIN 6 PRODUCTION OF HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS IS INHIBITED BY CORTICOSTEROIDS AND CALCITRIOL. U. Hettmannsperger, M. Detmar, M. Owsianowski, S. Tenorio, C.E. Orfanos. Dept. of Dermatology, Steglitz Medical Center, The Free University of Berlin, Berlin, Germany

Interleukin 6 (IL 6) seems to play a major role in the pathogenesis of psoriasis. Previously, we have shown that cytokine-stimulated human dermal microvascular endothelial cells (HDMEC) were able to produce significant amounts of IL 6. In the present study we investigated whether antipsoriatic drugs were able to inhibit endothelial cell derived IL 6 production in vitro.

HDMEC were isolated from neonatal foreskins and cultured (EBM, Clonetics) supplemented with hydrocortisone, epidermal growth factor (EGF) and 10% fetal calf serum. Second passage HDMEC were treated with LPS (10 ug/ml), interleukin 1-beta (IL 1b), TNF-alpha or interferon-gamma (IFNg, 10-1000 U/ml) for 4h and 24h. IL 6 production was assayed by ELISA and by Northern Blot analysis. In addition, HDMEC were treated with IL 1b (1000 U/ml) or TNF (1000 U/ml) either alone or in combination with hydrocortisone, dexamethasone, acitretin, cyclosporine A (10^{-5}-10^{-7} M) or calcitriol (10^{-6}-10^{-8}M) and assayed for IL 6 production.

Treatment of HDMEC with LPS, IL 1b and TNF, but not IFNg induced the production of IL 6 in a dose dependent manner, as detected by ELISA and Northern blot analysis. Stimulation with IL 1b for 4h led to high levels of IL 6 in supernatants together with strong expression of IL 6-mRNA, while after 24h IL 6-mRNA was absent and supernatants showed only slightly increased IL 6 levels. Stimulation with LPS and TNF showed a less pronounced IL 6 production after 4h, but after 24h transcription of mRNA was still detectable and IL 6 levels were significantly increased. Combination of IL 1b as well as TNF with dexamethasone or hydrocortisone markedly inhibited the cytokine-induced IL 6 production. Also, calcitriol reduced the IL 6 production by HDMEC, in contrast to acitretin and cyclosporin A.

These results demonstrate that also HDMEC are a major source for IL 6. Furthermore, the beneficial effects of corticosteroids and of vitamin D_3-analoga in psoriasis may be partly explained by their ability to suppress HDMEC IL 6 production. In contrast, cyclosporin A and acitretin did not suppress IL 6 production by HDMEC, suggesting other mechanisms of action in psoriasis.

P131

DOWN-MODULATED EPIDERMAL KERATINOCYTE HLA-CLASS I EXPRESSION IN MELANOCYTIC TUMORS OF THE SKIN. Antonius Schwaaf, Franz Trautinger, Karl-Heinz Vehring, Elisabeth Schauer, Thomas A.Luger, Eva-B.Bröcker, Dept.Dermatol. Münster and LBI Münster and Vienna, Germany and Austria.

Previously we observed that keratinocytes overlying melanomas may lack detectable amounts of HLA-class I antigens (HLA-I). In order to investigate this phenomenon, we performed the following study:

57 primary melanomas and 146 nevi were stained with the anti-HLA-I antibody W6/32. Staining intensity of keratinocytes in the overlying epidermis was compared to perivascular cells. Normal skin served as control. In vitro, the epidermoid carcinoma cell line A431 and hemopoetic cell lines (U-937,HL-60) were incubated with different melanoma cell conditioned media (KRFM-CM, A375-CM), and HLA-I expression was determined by ELISA and FACS. Because αMSH was reported to down-modulate HLA-I in A-431 cells A375-CM was fractionated by gel filtration and RP-HPLC, and tested for αMSH by RIA.

While in normal skin HLA-I expression of epidermal keratinocytes was equal to perivascular cells, decreased HLA-I above 43% of melanomas and 66% of nevi was found. Incubation of hemopoetic cell lines with KRFM-CM down-modulated HLA-I. Though biologically relevant quantities of immunoreactive αMSH could be purified from A375-CM, it did not affect A431 cell HLA-I.

In the view of the presence of αMSH in A375-CM and its reported capacity to down-modulate A-431 HLA-I expression, we assume that either A-431 cells do not respond to the concentrations of αMSH in A375-CM, or that the immunoreactive A375-CM αMSH is biologically inactive. Thus, the in vivo phenomenon of decreased HLA-I in keratinocytes in the close vicinity to melanocytic tumors requires further analysis.

P132

IDENTIFICATION OF ELAFIN-LIKE ACTIVITY IN URINE OF PATIENTS WITH ACUTE INFLAMMATORY DISEASES. Volker Streit, Oliver Wiedow, and Enno Christophers. Dept. of Dermatology, University of Kiel, Germany.

Low molecular weight serine protease inhibitors have received increasing interest in the recent past due to their ability to regulate protease mediated tissue proteolysis in inflammatory conditions. In the urine of patients with acute inflammatory diseases (e.g. peritonitis, multi organ failure) from a surgical intensive care unit as well as dermatological patients with erysipelas we were able to demonstrate an acid stable inhibitor of human leukocyte elastase (range 28 - 77 nmol/l urine, control group: 1 - 5 nmol/l urine). The acid stable inhibitor was isolated from perchloric acid treated urine by cation exchange HPLC and was further purified by C_8-reversed phase - and Poly LC-HPLC. Neither cathepsin G nor trypsin were inhibited. Analytical TSK 2000 size exclusion HPLC showed an apparent molecular weight within 6 - 8 kD. Physicochemical and inhibitory properties of this acid stable inhibitor are identical with the properties of elafin, an elastase specific inhibitor, which has recently been demonstrated in human epidermis and bronchial mucus. This elafin-like activity in urine might point towards a pathophysiological role of elafin in inflammatory diseases and could well serve as a diagnostic marker of inflammation.

P133

SUBSTANCE P INHIBITS THE VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)-INDUCED PROLIFERATION OF CULTURED HUMAN KERATINOCYTES. Carlo Pincelli, Fabrizio Fantini, Cinzia Sevignani, Luisa Benassi and Alberto Giannetti. Institute of Dermatology, University of Modena, Modena, Italy.

Vasoactive intestinal polypeptide (VIP) levels are increased, whereas substance P (SP) levels are decreased in lesional psoriatic skin. Because this disease is characterized by an increased keratinocyte proliferation rate, in this study we investigated the effects of SP and VIP on the proliferation of cultured human keratinocytes. Keratinocytes from normal human skin were grown according to the Green's method. Keratinocytes were then subcultivated in serum-free medium containing albumin, EGF, SP (0.1μM) and different VIP fragments (0.1μM). Furthermore, SP and VIP (1-28) were co-added to the medium. VIP (1-28), VIP (10-28) and VIP (22-28) significantly stimulated keratinocyte proliferation as compared to EGF alone with a mean % increase of 44.08±6.81 ($p<0.01$), 49.24±13.06 ($p<0.02$) and 30.67±11.98 ($p<0.05$) respectively. On the other hand, SP and VIP (1-12) failed to exert any stimulatory effect on keratinocytes. The addition of SP significantly inhibited the VIP-induced proliferation of keratinocytes ($p<0.05$). This study confirms that the VIP carboxy-terminal fragments are effective in the regulation of keratinocyte growth and that SP and VIP exert disparate effects on the proliferation of cultured human keratinocytes, thus suggesting that these neuropeptides might intervene with different effects in the pathogenesis of psoriasis.

P134

DIFFERENTIAL REGULATION OF ICAM-1 AND LFA-3 BY INTERFERON-GAMMA AND TUMOR NECROSIS FACTOR ALPHA ON HUMAN DERMAL FIBROBLASTS. Hunzelmann N[1], Roux M[1], Goebels N[2], Majewski S[1], Krieg T[1]. [1]Dept. of Dermatology, University of Cologne; [2]Max Planck Institute of Biochemistry, Munich, FRG.

In the dermis direct cell-cell contacts between fibroblasts and resident or infiltrating mononuclear cells play an important role in the development of inflammatory reactions. The antigen independent contact between lymphocytes and their target cells depends at least partially on the adhesion receptor pair LFA-1/ICAM-1 and CD2/LFA-3. In previous studies we were able to demonstrate that in systemic sclerosis, a disease which is characterized by inflammatory dermal infiltrates, ICAM-1 but not LFA-3 expression is increased on fibroblasts adjacent to MNC infiltrates. Therefore we asked to which extent the expression of the adhesion molecules ICAM-1 and LFA-3 is regulated in vitro by two mediators known to be involved in inflammatory conditions, i.e. IFN-g and TNF-a.

Human dermal fibroblasts were incubated for 48 hours with increasing concentrations of IFN-g (1-100 ng/ml), TNF-a (0.1-10 ng/ml) or their combination. The expression of ICAM-1 and LFA-3 was then examined by immunohistochemistry, FACS analysis and northern blot analysis. Our results show that the expression of ICAM-1 was synergistically induced by a factor of 5 after the incubation with TNF-a and IFN-g both on the protein and mRNA level. The expression of LFA-3, however, remained unchanged compared to untreated cultures, when fibroblasts were incubated with IFN-g, TNF-a alone or in combination. These results indicate that the differential expression of ICAM-1 and LFA-3 in human dermal fibroblasts might be involved in the regulation of tissue specific cell interactions.

P135

INTEGRIN EXPRESSION DURING HUMAN WOUND HEALING

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Integrins are a family of heterodimeric (α/β) receptors and represent primary mediators of cell-extracellular matrix adhesion. In normal human epidermis, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ complexes are preferentially localized on the lateral-apical surface of basal keratinocytes (BK), whereas $\alpha_6\beta_4$ is strongly expressed on their basal pole. The α_v subunit is also faintly expressed on BK, most probably in association with β_5. The aim of the present study was to investigate integrin expression on keratinocytes during human wound healing. Split-thickness 3 mm ø punch biopsies were performed on 12 volunteers. At daily intervals from day 1 to 8 and subsequently on days 11, 14, 21, and 28, the wound site was surgically removed. Immunofluorescence was carried out on frozen sections using antibodies (Ab) anti-α_2, α_3, α_5, α_6, α_v, β_1, β_3, β_4, collagen IV, laminin, vitronectin, fibronectin and a bullous pemphigoid (BP) antiserum. During reepithelialization, linear α_6, β_4 and BP antigen staining was detected along the entire newly formed dermal-epidermal junction (DEJ). A similarly polarized expression of the α_v subunit at the advancing wound edge alone was regularly detected from day 3 onwards. α_3 and β_1 subunits were expressed not only on BK but also on several suprabasal layers of advancing epithelium. In contrast, α_2 staining of BK in the advancing epidermis was much fainter compared to adjacent unwounded epidermis. α_5 and β_3 could be detected neither in wounded nor unwounded epidermis. A diffuse staining of the wound bed was observed with Abs to both fibronectin and vitronectin, the latter confined to elastic fibers in normal skin. Upregulation and polarization of α_v subunit could be induced by vitronectin deposit in the wound bed and could play a key role in keratinocyte migration. The early and simultaneous $\alpha_6\beta_4$ integrin and BP antigen expression suggests these may be the first two components synthesized in the newly formed DEJ, thus serving as initial anchoring of BK.

P136

EVIDENCE THAT DNA IS THE MOLECULAR TARGET FOR THE SUPPRESSION OF CYTOKINE-INDUCED ICAM-1 UPREGULATION BY ULTRAVIOLET (UV) RADIATION. F Parlow, EG Jung+, E Bohnert+, A Kapp, E Schöpf, and J Krutmann, Depts. Dermatology, University of Freiburg and +Mannheim Medical School, University of Heidelberg, F.R.G.

Human keratinocyte (KC) ICAM-1 molecules are important for the generation of an inflammatory infiltrate by mediating leukocyte/KC binding. KC ICAM-1 expression may be markedly inhibited by UVB irradiation, and this effect may at least partially account for the therapeutic effectiveness of UV therapy in inflammatory dermatoses. Accordingly, immediately after UVB exposure, IFNγ-induced ICAM-1 surface and mRNA expression is inhibited. This suppressive effect is transient, since UVB-irradiated KC may be restimulated to express ICAM-1 after a 12 hr incubation period. At the molecular level, UVB radiation is known to induce photoproducts in cellular DNA. To study the role of DNA as a target molecule for UVB-induced modulation of ICAM-1 expression, cells were exposed in vitro to sublethal doses of UV-light from FS 20 sunlamps and subsequently examined for ICAM-1 mRNA expression by Northern blot analysis. UVB-induced suppression of IFNγ-induced ICAM-1 expression was not specific for KC, since an essentially identical inhibition could be observed, if primary human skin fibroblasts (FB) were used. In order to study the role of DNA damage as an intermediate in this inhibition, FB from a healthy donor (wildtype) were compared with cells obtained froma patient with xeroderma pigmentosum (XP-D; UDS 15%). XP cells can not remove DNA lesions and thus, lower UVB doses are required to retain equivalent numbers of DNA photoproducts at a given time point after UVB-irradiation in XP cells, as compared to wildtype cells. In XP cells, 25 J/m² UVB were found to completely inhibit IFNγ-induced ICAM-1 mRNA expression, while in wildtype cells, a 3-fold larger dose was required. Moreover, in marked contrast to wildtype cells, no restoration of IFNγ-responsiveness could be observed in irradiated XP cells. These data indicate that the inhibition of IFNγ-induced ICAM-1 upregulation by UVB light involves the induction of DNA photoproducts of a type, which may not be removed by XP cells.

P137

MATRIX-DEPENDENT MIGRATORY ACTIVITY OF HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS. Marc Heckmann*, Yves Saurret§, David Woodley§, Marvin Karasek§. Dermatologische Klinik und Poliklinik der Universität München, Germany* and Department of Dermatology, Stanford University Medical Center, Stanford, USA§.

Dermal microvascular endothelial cells may not only function as a cellular barrier between blood and tissue; they also seem to participate actively in cell-matrix-interaction which may play an important role in reconstructive dermal processes such as wound healing. Using cultured dermal microvascular endothelial cells in a phagokinesis-assay we studied cell migration in dependence of defined matrix proteins. Computerized image analysis of migratory cellular traces revealed maximal cell migration on collagen type IV coated surfaces which were 460% of migration on uncoated surfaces. Migration on laminin, another major basement membrane component, was 300%. The interstitial collagen type I yielded only an 230% increase in migratory activity. Stimulation of endothelial cells with interleukin-1 (IL-1) which has been shown to be a highly active component of wound fluids could further enhance cell migration on collagen I up to 350%, whereas migration on collagen IV remained unaffected. At the same time Northern blot analysis of steady-state mRNA-levels revealed no detectable message for collagen type I but only collagen type IV which was independent of IL-1 stimulation.

These results suggest that dermal endothelial cells are able to discriminate between defined extracellular matrices resulting in different migratory patterns. Migration on the interstitial collagen type I to which endothelial cells are exposed physiologically only after tissue injury can be enhanced by IL-1.

P138

ANALYSIS OF THE REGULATION OF INTERCELLULAR ADHESION MOLECULE EXPRESSION IN HUMAN ANTIGEN PRESENTING CELLS. J Krutmann, U Jonas*, F Parlow, U Trefzer, M Berger*, E Schöpf, and J Bauer*, Departments of Dermatology and *Psychiatry, University of Freiburg, Freiburg, F.R.G.

ICAM-1 molecules on the surface of human antigen presenting cells (APC) such as epidermal Langerhans cells and peripheral blood monocytes (MN) deliver an important costimulatory signal in antigen-induced human T cell activation. Although ICAM-1 expression is known to be highly regulated in various cell types, little is known about the mechanisms by which ICAM-1 may be induced in human APC. Studies with MN have been substantially hampered by the fact that adhesion of freshly isolated MN to plastic tissue dishes, in the absence of additional stimuli, markedly upregulated ICAM-1 surface expression. In the present study, ICAM-1 surface expression was examined by FACS analysis on MN, which were cultured on hydrophobic teflon foils in order to prevent adhesion-induced ICAM-1 upregulation. Accordingly, constitutive MN ICAM-1 expression was essentially unaltered, if cells were cultured in teflon bags. Moreover, a 24 hr stimulation of teflon bag cultured MN with recombinant human (rh) IFNγ, rh interleukin (IL) 1α, rh IL1ß and rh IL6 markedly induced ICAM-1 surface expression with IFNγ being the most potent stimulus. Cytokine-induced ICAM-1 expression was corroborated by Northern blot analysis, demonstrating increased ICAM-1 mRNA expression in IFNγ-stimulated MN. We have previously demonstrated that MN cultured for 8 days in teflon bags mature into terminally differentiated macrophages. Maturation of MN into macrophages was associated with a time dependent increase in ICAM-1 surface and mRNA expression. In contrast to ICAM-1, ICAM-2 mRNA and surface expression of freshly isolated MN decreased to background levels upon maturation of MN into macrophages. These data clearly demonstrate that ICAM-1 expression in human MN may be induced by cytokines via a transcriptionally regulated mechanism. In addition, our studies provide evidence that ICAM molecules may be differentially regulated in human APC depending on their state of differentiation.

P139

EXPRESSION OF INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1) ON NORMAL HUMAN EOSINOPHILS - REGULATION BY CYTOKINES.

Wolfgang Czech, Jean Krutmann, Anne Budnik, Roland Kurrle*, Erwin Schöpf, Alexander Kapp, Department of Dermatology, Univ. of Freiburg, Freiburg and *Research Laboratories, Behring Werke, Marburg, Germany

Adhesion molecules, such as ICAM-1, play a crucial role in mediating cell-cell interactions in inflammatory reactions. Eosinophils (EO) appear to be the major effector cells in late phase allergic responses. To obtain more information about the capacity of EO to interact with LFA-1 positive inflammatory leukocytes, in the present study the expression of ICAM-1 on highly purified EO (≥95%) of normal nonatopic controls was investigated in vitro. Using FACS analysis, it could be shown, that EO do not constitutively express ICAM-1. However, a significant dose-dependent induction of ICAM-1 expression could be observed upon stimulation with IFNγ or TNFα for 24h in vitro. Moreover, simultaneous addition of these two cytokines induced ICAM-1 expression in a synergistic fashion. This observation was corroborated by Northern blot analysis. Accordingly, in contrast to unstimulated cells, significant amounts of ICAM-1 specific mRNA could be detected in EO which had been stimulated with IFNγ and TNFα. Cytokine induced ICAM-1 expression was highly specific, since IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, GM-CSF, C5a and PAF did not significantly affect ICAM-1 surface expression at physiologically relevant concentrations. In summary, this study indicates, that EO may be activated to express ICAM-1 upon stimulation with selected cytokines via a transcriptionally regulated mechanism. This finding points to a novel mechanism by which cross-talk between EO and other inflammatory cells may occur in atopic diseases.

P140

E-CADHERIN: Ca^{++}-DEPENDENT ASSOCIATION WITH ADHERENS JUNCTIONS AND ACTIN FILAMENTS IN KERATINOCYTES. Friedhelm Achenbach, Hans W. Kaiser, Andrea Balcerkiewicz, Welisar Petrow, Ed J. O'Keefe, Hans W. Kreysel, Departement of Dermatology, University of Bonn, Germany, Departement of Dermatology, University of North Carolina, Chapel Hill, N.C.

E-cadherin is a 124 kDa transmembrane glycoprotein mediating Ca^{++}-dependent homophilic binding of epithelial cells. It has been suggested that cadherins may associate with microfilaments and stabilize cell-cell contacts. We investigated this possibility in human keratinocytes (NHK) using a calcium shift assay with immunofluorescence and immunoblot analysis and antibodies to E-cadherin. E-cadherin was present in equal amounts at both 0.1 and 1.0 M Ca^{++} on immunoblots; immunofluorescence revealed a diffuse distribution of E-cadherin at 0.1 mM Ca^{++}, but after shift to 1.0 mM calcium, E-cadherin became concentrated at regions of cell-cell contact. Actin filaments were diffusely distributed at low Ca^{++} but colocalized with E-cadherin at contact sites in high Ca^{++}. Vinculin, a marker for adherens junctions known to colocalize with actin filaments at cell-cell and cell-matrix contact sites, was compared with E-cadherin by double immunofluorescence; vinculin and E-cadherin colocalized at cell-cell contact sites, but unlike vinculin, E-cadherin was not present in focal adhesions in NHK or at the basal lamina in epidermis. These studies are consistent with the possibility that vinculin and actin may stabilize E-cadherin-mediated cell-cell contact sites in keratinocytes.

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ADHESION MOLECULE EXPRESSION AND THE CELLULAR INFILTRATE IN TIMED, INDUCED LESIONS OF DELAYED PRESSURE URTICARIA. RJ Barlow, E Ross, A Kobza Black, DM MacDonald*, MW Greaves. St John's Institute of Dermatology, St Thomas' Hospital and *Department of Dermatology, Guy's Hospital, London, UK.

Delayed pressure urticaria (DPU) is characterised by persistent, painful, erythematous swellings which develop 30 minutes to 9 hours after applying pressure to the skin. The pathogenesis is unknown and we aimed to study the expression of cytokine induced adhesion molecules and the composition of the cellular infiltrate in timed skin biopsies. Lesions were elicited using weighted steel rods applied to the thighs of 13 patients with DPU. Three punch biopsies were taken from each volunteer, from unchallenged skin or at 0, 2, 6, 24, 48 or 120 hours after pressure application. Immunohistochemical analysis was made with monoclonal antibodies detecting Endothelial Adhesion Molecule 1 (ELAM 1), Intercellular Adhesion Molecule 1 (ICAM 1) and leucocyte subsets. Using a visual grading score (0 to +3), marked upregulation of ELAM 1 was observed at 6 and 24 hours. Median cell counts per high power field are tabulated below.

	Neutrophils (neutrophil elastase)	Eosinophils (eosinophil cationic protein)	Monocytes/Macrophages (EBM 11)
Unchallenged skin (control)	0.00	0.00	20.29
6 h	18.54*	0.27	17.38
24 h	31.15*	3.09*	47.53*

*$p < 0.05$, 24 h vs control (Mann Whitney U) *$p < 0.05$, 6 h vs control (Mann Whitney U)

These results suggest that vascular endothelial activation is an early response to pressure challenge in DPU and it is likely that ELAM 1 is involved in cell recruitment.

P142

THE ALLERGEN NICKEL CHLORIDE DIRECTLY INDUCES DISTINCT EXPRESSION PATTERNS OF LEUKOCYTE ADHESION MOLECULES ON HUMAN ENDOTHELIAL CELLS. Georg Meinardus-Hager, Matthias Goebeler, Johannes Gutwald, and Clemens Sorg, Institute of Experimental Dermatology, University of Münster, Münster, F.R.G.

During contact hypersensitivity to nickel chloride progressive endothelial expression of the leukocyte adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule (ELAM-1) is observed. In order to evaluate whether nickel chloride directly leads to activation of endothelium, we studied its in vitro effects on cultured human umbilical vein endothelial cells (HUVEC).

The expression of adhesion molecules after incubation of HUVEC with nickel for either 6 or 24 h was subsequently analyzed by ELISA technique. The allergenic nickel ion has a direct effect on HUVEC. At subtoxic concentrations of 2mM it augments the expression of ICAM-1 and VCAM-1. Regarding its effect on the induction of ELAM-1 on HUVEC, we found different result with individual HUVEC lines: Out of 7 lines tested so far 3 showed a pronounced upregulation of ELAM-1 after nickel stimulation, 2 only a weak response and 2 failed to react.

The induction of adhesion molecules by nickel is not mediated by the autocrine effect of interleukin-1, but more likely by a mechanism that resembles the non-specific action of LPS. The involvement of prostaglandins is less probable since dexamethason did not modulate the nickel effect.

The induction of adhesion molecules by nickel may rather be responsible for its well-known irritant properties than for specific allergic reactions to nickel. However, nickel induced endothelial adhesion structures (e.g. ELAM-1) may facilitate the influx of skin homing memory T-cells supporting a challenge reaction in sensitized patients.

P143

ION AND WATER DISTRIBUTION PROFILES IN HUMAN SKIN

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ABSTRACT

In the recent decade data on elemental distributions over normal and pathologically changed skin has been available through the use of particle probes. These studies have been confided to freeze-dried specimens and have given the amounts of physiologically interesting elements such as Na, K, Cl, Ca, Mg S, and P in dry weight measures. Obviously, the need for wet weight data is at hand in order to understand the physiological role of said concentrations.

Using particle probes to analyze frozen hydrated specimens, which are subsequently freeze-dried and renew the analysis makes it possible to establish the water concentration profiles over a tissue cross section such as the epidermis. We have investigated the water profile and elemental distribution profiles in three healthy individuals each offering three biopsies. The analyses were perfomed using a Phillips 525 scanning electron microscope fitted with a Polaron E7400 cryopreparation chamber working at liquid nitrogen temperatures.

Our results show that the physiologically interesting elemental profiles correspond well to data previously published. Thus the concentration of monovalent ions decline towards the stratum corneum to be very low in this stratum. This may suggest a mechanism for recycling of electrolytes. There is an almost constant water concentration distribution over the skin from the stratum corneum to the dermis. Since the increasing amount of fibrous proteins can hold substantial amounts of water, the free water available for the ions may be low in the strata having high protein concentrations thus providing an effective concentration gradient towards the dermis.

P144

CHEMILUMINESCENCE FOR THE STUDY OF JUNCTIONAL AND CYTOSKELETAL PROTEINS: A RAPID AND SENSITIVE METHOD WITHOUT ENVIRONMENTAL HAZARD, YIELDING AN IMAGE ON X-RAY FILM. Winfried Ness, Hans W. Kaiser, Czesary Kowalewski and Hans W. Kreysel. Department of Dermatology, University of Bonn, Germany, Department of Dermatology, University of Warzawa, Poland.

Radioactive waiste is an increasing concern in todays environmental discussion. Enhanced chemiluminescence (ECL) is a non-radioactive method for detection of proteins in Westernblot analysis. Sensitivity of this method was analyzed for the detection of junctional proteins like desmoplakin, vinculin, E-cadherin and cytoskeletal proteins like spektrin and adducin in samples of split skin, normal human keratinocytes, MDCK cell line and A-431 cells. Proteins immobilized on nitrocellulose or PVDF membranes after SDS gel-electrophoresis were detected within seconds on x-ray film with the use of monoclonal or affinity-purified polyclonal antibodies. Sensitivity was comparable to 125 iodine detection. ECL also required less antibodies than other non-radioactive detection methods. ECL allows a rapid detection of proteins by Westernblot analysis techniques without environmental hazard caused by radioactivity.

P145

FAST AND QUANTITATIVE METHOD FOR DETECTION OF MESSENGER RNA BY USE OF POLYMERASE CHAIN REACTION. Peter Kristensen*, Brian F.C. Clark* and Knud Kragballe+, *Dept. of Chemistry, Division of Biostructural Chemistry, University of Aarhus, Aarhus, Denmark. +Dept. of Dermatology, Marselisborg Hospital, Aarhus, Denmark.

Previous studies have shown that the rate of protein synthesis decreases when cells are allowed to age in culture. Our previous studies have shown that this decrease is paralleled by a decrease in activity of peptide elongation factor 1 alfa (EF-1α). To progress in these studies, we have established a method for quantitation of the mRNA for EF-1α using the polymerase chain reaction (PCR).

The fact that PCR is not a quantitative amplification of templates, has been solved by introducing an external standard. This has been constructed in such a way, that it at its 5'end contains the promotor sequence for T7-RNA polymerase and after this a deleted cDNA for EF-1α. From this we can obtain deleted mRNA for EF-1α by transcription of the cDNA by T7-RNA polymerase. This standard is then added to a sample before RNA isolation, then the mRNA is made into cDNA by reverse transcription and amplified. The product is loaded onto a TSK DEAE-NPR HPLC column, which separates the deleted product from the non-deleted, at the same time the two products can be quantitated. The deleted standard gives us the amount of amplification, which also applies to the non-deleted template. From this we can quantitate the amount of mRNA for EF-1α in the sample.

P146

TECHNIQUES USED IN CELL TRANSFECTION AFFECT THE REGULATION OF THE ENDOGENOUS COLLAGENASE, C-FOS AND C-JUN GENES Charles A. Lambert, Pascal Y. Lefebvre, Betty V. Nusgens, and Charles M. Lapière, Laboratory of Experimental Dermatology, Liège University, Sart Tilman, Belgium.

In the course of experiments aiming at introducing various collagenase constructs in human dermal fibroblasts (HDF), a several-fold increase in the collagenase mRNA was observed in sham electroporated HDF as compared to the naive cells. This increase was directly voltage-dependent and persisted maximally at least for 72 hours. Similar results were obtained with human smooth muscle cells (HSMC), whereas no collagenase mRNA could be detected in HeLa cells, control or electroporated. A voltage-dependent up-regulation of both c-Jun and c-Fos genes, two oncoproteins triggering the regulation of collagenase in response to various agents was also observed in HDF, HSMC and HeLa cells harvested one hour after electroporation. Liposomes, DEAE-dextran, chloroquine and glycerol were also shown to induce a several-fold increase in the collagenase gene expression in HDF.

We emphasize that the modification of endogenous gene expression by the transfection techniques must be taken into consideration in studies on gene regulation and could lead to misinterpretation of the results.

P147

CELL MOTILITY CALCULATED BY COMPUTER SIMULATION IS A PROGNOSTIC PARAMETER IN MALIGNANT MELANOMA. Rainer Hofmann-Wellenhof, Regina Kofler, Hans-Peter Soyer, Helmut Kerl, Josef Smolle, Department of Dermatology, University of Graz, Graz, Austria.

Proliferation and motility are considered to be important for tumor cell invasion and metastasis. Up to now, tumor cell motility could only be observed and measured in artificial in vitro systems. We recently showed, that analytical comparison of morphological patterns of malignant melanoma (MM) and patterns of tumors simulated by a computer program facilitates estimates of tumor cell motility. In this study we used the computer simulation technic to investigate the relationship of cell motility estimates and the metastatic potential of MM.

184 specimens of patients with primary MM were consecutively sampled. The follow up time was at least 5 years. The prognostic significance of MAXMOT defined as maximal number of cell shifts (i.e. number of cell movements of single cell width per mm^2 while the mitotic cells divide) and mitotic counts (mitotic figures per mm^2) were evaluated by CART analysis using the log-rank test.

According to mitotic counts we separated 2 prognostic groups ($p<0.05$): mitosis $\geq 17/mm^2$: 5-year survival rate 52%; mitosis $\leq 17/mm^2$: 82%. Within the latter group with low proliferation, however, there was one subgroup with bad prognosis, characterized by a high degree of tumor cell motility (MAXMOT ≥ 126: 5-year survival rate 35%) and a second with a significant better prognosis (MAXMOT ≤ 126: 85%). We found similar results for a 5-year metastasis free interval.

In conclusion, estimates of cell motility calculated by computer simulation could be useful to separate primary MM into different prognostic groups. This study shows a correlation between melanoma cell motility in vitro and metastatic potential. The knowledge of essential biological parameters of tumor cells like cell proliferation and cell motility could be useful planing further treatment of patients presenting with MM.

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p53 PROTEIN EXPRESSION IN BENIGN AND MALIGNANT SQUAMOUS AND MELANOCYTIC SKIN TUMOURS: AN IMMUNOHISTOCHEMICAL STUDY. Y.S. Ro*, B. Vojtesek**, P.N. Cooper, J.A. Lee, D. Harrison, B. Angus, J. Rees*, C.H.W. Horne, D.P. Lane**, Departments of *Dermatology and Histopathology, University of Newcastle upon Tyne, and **Cancer Research Campaign Laboratories, Department of Biochemistry, University of Dundee.

There is considerable evidence that mutation of the p53 gene has a important role in human malignancy. Mutation results in stabilisation of the p53 protein which then accumulates in the cell to levels detectable by immunohistochemistry. Using a new monoclonal antibody, DO-7, we have studied p53 expression in a series of 133 benign, dysplastic and malignant squamous tumours, and benign and malignant melaocytic tumours.

None of 26 benign tumours showed significant p53 expression; 2 of 12 actinic keratoses and 3 of 10 cases of Bowen's disease were positive; 1 of 9 keratoacanthomas showed staining. Of the malignant tumours the following proportions showed positive staining: 14/26 squamous cell carcinomas; 8/25 basal cell carcinomas; 1/25 malignant melanomas.

p53 protein was thus only detectable in dysplastic or frankly malignant lesions. The proportion of squamous lesions which over-expressed p53 increased with malignant potential, with the highest proportion of positive cases seen among the squamous cell carcinomas (14 out of 26; 54%). The findings that 8 of 25 basal cell carcinomas, but only one of 25 cases of malignant melanoma were positive, indicates that p53 mutation may not be related to metastatic potential in non-squamous lesions.

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THE CULTURE OF HUMAN MELANOCYTES IN A SKIN EQUIVALENT SYSTEM.

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In vivo melanocytes grow on a basement membrane and are closely associated with keratinocytes. Since these two vital elements are lacking in normal melanocyte culture methods, we have examined the possibility of growing these cells in a skin equivalent system. Early passage melanocytes and keratinocytes (0,3,10,20% melanocytes) from asian or caucasian donors were established upon de-epidermised dermis and after 3 days raised to the air-liquid interface. On day 9 they were processed for H+E, dopa or immunoperoxidase (MEL5) staining. Dendritic cells being dopa/MEL5 positive and found within the basal keratinocyte layer were present in all samples, including those seeded with pure keratinocytes. Melanin was seen in keratinocytes adjacent to melanocytes. Seeding densities above 10% melanocytes did not result in higher proportions of these cells in the basal layer and this was true for melanocytes from either source. In a few experiments where we used asian melanocytes we examined the effect of irradiation (UVB total dose 4670J/m^2). Whilst the skin equivalent became very obviously more pigmented following irradiation, there was no obvious change in pigment quantity or distribution at the light microscope level. Neither was there an increase in the staining intensity or melanocyte numbers. These results show that melanocytes can be grown in a skin equivalent system and form functional relationships with keratinocytes. We are now using this system to investigate further melanocyte-keratinocyte interactions and the effects of UV irradiation.

P150

DEMONSTRATION OF SPECIFIC MELANOCYTE STIMULATING HORMONE RECEPTORS ON HUMAN MELANOCYTES GROWN IN A SYSTEM FREE OF ARTIFICIAL MITOGENS.

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While others have utilized cholera toxin (CT) and 12-O-tetradecanoyl phorbol 13-acetate (TPA) as mitogens in melanocyte culture, we are currently employing a novel method which, because it does not use these artificial mitogens, is probably closer to normal physiological conditions. Although specific MSH receptors have been demonstrated on human and murine melanoma cells, there is little data relating to MSH binding to cultured human melanocytes. We have investigated the binding of the MSH analog Nle^4DPhe^7-MSH to human melanocytes cultured in our system. Cells were incubated for 2h at room temperature with increasing concentrations of ^{125}I-labelled analog ± excess 'cold' peptide. Binding was specific and saturable with a KD of $2.9x10^{-11}M$ and ∿500 binding sites/cell. There was no specific binding to human keratinocytes or dermal fibroblasts. After 2 day culture in the presence of 1mM dibutyryl cAMP, there was a 62% increase in specifically bound peptide. $10^{-9}M$ CT, which activates cAMP-dependent mechanisms, caused a 25% increase in binding while 25nM TPA, one of the actions of which is to activate the protein kinase C system, caused a 24% decrease in binding. In conclusion, we have demonstrated specific MSH binding to cultured human melanocytes and have been able to estimate a KD value and the number of binding sites/cell. Our protocol, which avoids the use of those mitogens which induce changes in MSH binding, may be the culture system of choice for elucidating the role and mechanism of action of MSH in vitro.

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IDENTIFICATION OF FREE CIRCULATING INTERCELLULAR ADHESION MOLECULE-1 IN PATIENTS WITH METASTATIC MELANOMA. A.M. Manganoni (1), M. Temponi (2), D. Braga (1), A. Lonati (1), S. Ferrone (3) and G. De Panfilis (1). Department of (1) Dermatology, (2) Institute of Chemistry, School of Medicine, Brescia University Hospital, Brescia, Italy; and (3) Department of Microbiology and Immunology, New York Medical College, Valhalla, N.Y., U.S.A.

The intercellular adhesion molecule-1 (ICAM-1), which is a member of the immunoglobulin supergene family, is a ligand for the lymphocyte function associated antigen-1 (LFA-1). Interaction between LFA-1 and ICAM-1 is a crucial event in numerous inflammatory and neoplastic processes. ICAM-1 has been identified on melanoma cells and the expression increased in advanced melanomas. Moreover, the incidence of positivity for free circulating ICAM-1 antigen in malignant diseases was recently demonstrated to be higher than that in benign diseases or in healthy controls. This prompted us to investigate the presence of free ICAM-1 molecule in the sera of melanoma patients. Serum samples of 10 healthy controls and of 6 patients with advanced melanoma were subjected to a double-determinant-immunoassay (DDIA), using two monoclonal antibodies (moAb) against ICAM-1 namely moAb CL 203.4 (catcher) and moAb RR1/1 (tracer). The values of circulating ICAM-1 antigen detected in advanced melanoma patients (19665.8±6294.778) were significantly ($p<0.001$) higher in comparison to those detected in normal controls (78692.84±23532.86). This is the first study demonstrating a significantly high incidence of free ICAM-1 antigen in malignant melanoma. The evaluation of circulating ICAM-1 in melanoma may represent a useful parameter in the study of this disease. In fact, the increased presence of circulating ICAM-1 molecule in the serum from melanoma patients in advanced stages might correlate with an increased ICAM-1 expression by melanoma and/or stromal cells, as most recently demonstrated for other malignancies. Moreover, soluble ICAM-1 could block the LFA-1 molecule on the surface of cytotoxic cells, thus triggering the escape of melanoma cells from host immune control. However, further studies are needed to clarify whether this marker could have also a prognostic value, and even allow the monitoring of the therapy of melanoma.

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ATYPICAL ACTIVATION OF MELANOCYTIC NEVI BY RECOMBINANT HUMAN GROWTH HORMONE. G.E. Piérard, J. Arrese Estrada, C. Piérard-Franchimont, J.P. Bourguignon, C. Ernould. Depts of Dermatopathology and Pediatrics, Univ. of Liège, Belgium.

Children with Turner syndrome (TS) or hypopituitarism (HP) are currently treated with recombinant human growth hormone (GH : 0.06 - 0.15 U/kg/d). Computerized image analysis of clinical photographs taken at 6 month intervals revealed a 4 to 6 fold increase in the growth rate of melanocytic nevi (MN) in 12 TS and 3 HP compared to 21 sex and age matched normal children and 3 untreated TS.

We studied histologically MN larger than 60 mm^2 including 27 GH-TS, 8 GH-HP, 7 untreated TS and HP, and 49 normal children. We used immunohistochemistry to HMB45 and PCNA, computerized image analysis of nevocyte nuclei, ^3H-TdR autoradiography and flow cytometry.

We found some heterogeneity in the size of nevocyte nuclei in HG-TS and HG-HP (Coef. Variat. 49 % VS 23 % in controls). HMB45 immunoreactivity was often focal but was abnormally prominent and diffuse in 9 MN of GH-TS. In these latter specimens PCNA and ^3H-TdR revealed a cell proliferation increased over 20 times compared to controls. Flow cytometry failed to reveal any aneuploidy.

These findings suggest that high dosage of HG activates melanocytic nevi, at least in genetically predisposed children.

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DIFFERENCES IN MEMBRANE CALCIUM CHANNEL COMPOSITION OF TWO CLOSELY RELATED MELANOMA CELL SUBLINES WITH DIFFERENT METASTATIC CAPABILITIES. Elmar Innauer[1], Wolfgang Schreibmayer[1], Christine Helige[2], Josef Smolle[2], Helmut A.Tritthart[1]. [1]Department of Medical Physics and Biophysics, University of Graz, Austria. [2]Department of Dermatology, University of Graz, Austria.

The patch clamp technique was engaged to detect calcium channels at the single channel level in two closely related murine melanoma cell sublines, namely K1735-c116, a low metastatic cell clone and K1735-M2, a highly metastatic cell clone. Calcium channels are known to be the main link between the extracellular and intracellular calcium concentration. The K1735-c116 cell clone showed a calcium channel, whereas in the K1735-M2 cell subline this calcium permeating channel was not present. To characterize the calcium channel, 90 mM calcium, 90 mM barium, 90 mM calcium and 1 µM Bay K-8644 containing solutions were engaged in the pipette. With these solutions slope conductances and mean open times of the channels were evaluated, indicating that this channel is not a L- or N-type calcium channel. The present results demonstrate major differences in calcium channel composition of two closely related melanoma cell sublines with different biological properties, indicating a possible role of calcium channels in calcium homoestasis and calcium signal transduction pathways interrelated to the metastatic potency.

P154

LOSS OF MELANOCYTES IN VITILIGO LESIONS. J.C. Le Poole, R.M.J.G.J. van den Wijngaard, W. Westerhof, R.P. Dutrieux, P.K. Das. Depts. of dermatology and pathology, Academic Medical Center, Amsterdam University and Leiden Cytopathological Laboratory, The Netherlands.

In vitiligo, no melanin pigment is present in lesional skin. Whether pigment-forming melanocytes are still present in an inactive form in lesional skin or have disappeared through destruction is still a matter of debate. Husain et al (J Invest Dermatol 78: 243-252, 1982) have found residual tyrosinase activity in lesional epidermis, suggesting that at least some melanocytes are still present. We have investigated the absence or presence of melanocytes in vitiligo lesions with a panel of 18 antibodies prepared to detect melanoma or non-transformed melanocytes. Immunoperoxidase indirect stainings were performed on cytospins of melanoma cells and of cultured normal melanocytes and frozen skin sections from healthy control biopsies and nonlesional as well as lesional vitiligo biopsies. 7 monoclonal antibodies from the panel could be used to detect melanocytes in normal and nonlesional vitiligo skin, namely NKI-beteb, MEL-5, TMH-2, NKI-C3, HMB-45, G7E2 and K-1-2-58. Other antibodies either did not discriminate melanocytes from neighbouring cells, would not react with normal melanocytes in situ or only recognized transformed melanocytes. None of the antibodies from the panel detected lesional melanocytes in vitiligo skin frozen sections, supporting the hypothesis that melanocytes are lost in vitiligo lesional skin. To investigate the eventual presence of residual melanocytes within lesional epidermis, a different technique was employed. Using epidermal split skin preparations stained with NKI-beteb, an approximately 500-fold increase in the number of detectable melanocytes can be obtained as compared to frozen skin sections. By immunoperoxidase staining combined with conventional light microscopy, an occasional melanocyte can be found in lesional skin. Abberant staining patterns in expanding lesions indicate the presence of melanocyte debris. Using confocal laser scanning microscopy after fluorescent staining, offering a three dimensional view of reacting particles, the presence of fewer, highly dendritic cells was apparent in perilesional skin taken from the inside of the lesion. Hereby depigmentation was shown to precede melanocyte disappearance. In conclusion, melanocytes are destroyed in vitiligo lesions. Therapies for vitiligo should thus aim at induction of melanocyte proliferation from hair follicles and from the perilesional area, rather than at reactivation of melanocytes present.

P155

SPECIFIC RELEASE OF TNF-α AND IFN-γ BY HUMAN TUMOR INFILTRATING LYMPHOCYTE (TIL) CLONES AFTER AUTOLOGOUS TUMOR STIMULATION.

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The means by which TIL exert their antitumor effects in vivo are unknown. The possible mechanisms include cell-mediated cytotoxicity and secretion of cytokines that destroy the tumor or recruit other elements of the immune system for this task. The current study investigates cytokine release by TIL clones after stimulation with specific tumor cells.

TIL clones have been generated using a limiting dilution technique from human primary melanoma. Furthermore, we were able to establish cell lines from a number of the same tumors. TIL clones were analysed using flow cytometry, proliferation and cytotoxicity assays, and specific ELISAs for cytokine detection.

We have identified eight TIL clones that specifically release TNF-α and IFN-γ after tumor cell stimulation. Both cytokines were only released after specific autologous stimulation, allogeneic tumor cells were not able to enhance cytokine production of TILs. Phenotypic analysis revealed that Six of these clones were $CD8^+$ T cells and two $CD4^+$ T cells. Four of the $CD8^+$ clones were *cytolytic against the autologous melanoma cell line, but non-cytolytic in non-MHC-restricted* cytotoxicity assays. The $CD4^+$ clones were non-cytolytic at all. All $CD4^+$ and all $CD8^+$ clones showed an enhanced proliferative response to low concentrations of IL-2 when cocultered with irradiated autologous tumor cells.

The current study explores the function of cytokine release as an indication of specific activation of human lyphocytes by autologous tumor antigen. $CD4^+$ and $CD8^+$ TIL clones could be activated by autologous melanoma cell lines to proliferate and to secrete TNF-α and IFN-γ. The cytotoxicity exerted against the autologous tumor could not be correlated with these effects. These findings suggest the presence of significant intratumoral concentrations of TNF-α and IFN-γ and the role of these cytokines in tumor regression. Furthermore, our findings support the relevance of the specific tumor/TIL interaction in the immune response against primary melanoma.

P156

HIGH EXPRESSION OF BETA$_2$-ADRENORECEPTORS IN VITILIGO.

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Differentiated keratinocytes established from vitiliginous epidermis showed a defect in $^{45}Ca^{++}$ uptake indicative of an increase in plasma membrane Ca^{++}-ATPase activity. Koizumi *et al.* measured an increase in intracellular Ca^{++} in normal human keratinocytes after exposure to beta-adrenergic agents. The aim of this study has been to determine whether the defect in Ca^{++} homeostasis in vitiligo can be explained by increased activity of catecholamines. Keratinocytes were established in MCDB-medium from lesional and non-lesional skin of a patient with vitiligo (skin type III) and from an age-matched (skin type III) control. Both differentiated and non-differentiated cells were examined for the presence of beta$_2$-adrenoreceptors in the presence of low Ca^{++} (0.1×10^{-3}M) and high Ca^{++} (1.5×10^{-3}M). Binding experiments were performed with saturating levels of ^{3}H-CGP-12177, a beta-adrenergic antagonist. Controls for non-specific binding were determined by the addition of propranolol (5 μmol). Normal human differentiated keratinocytes reveal high levels of beta$_2$-adrenoreceptors (ie. 7,000/cell). Non-differentiated keratinocytes, in 0.1×10^{-3}M Ca^{++} from both lesional and non-lesional vitiligo skin showed at least a 25% increase in beta$_2$-adrenoreceptors over skin type III controls. However, in differentiated keratinocytes, only cells established from depigmented skin showed a significant increase (50%) in receptors and a decrease in $^{45}Ca^{++}$-uptake. Differentiated keratinoctyes established in medium containing high calcium (1.5×10^{-3}M) revealed normal levels of beta$_2$-adrenoreceptors compared to skin type III controls. Our results with beta$_2$-adrenoreceptors could explain (a) the problem with calcium homeostasis in vitiligo, (b) implicate catecholamines in the pathophysiology of this disease, and (c) show that keratinocytes are involved in skin depigmentation.

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THE PHOTO HEN'S EGG TEST - A NEW MODEL FOR PHOTOTOXICITY

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Toxicologists originally introduced the hen's egg test as a screening method for mucocutaneous toxicity as an alternative to the rabbit's eye test (Draize Test). In an adapted version the Photo hen's egg test (PHET) was created to replace animal models as a phototoxic test system. It is much more advanced than the widely used in-vitro models like cultures of yeasts, bacteria and other cells of various origins.

In the PHET the embryo's yolk sac blood vessel system is exposed to test substances and UVA irradiation simultaneously. Gross morphological changes such as discoloration of the membrane and haemorrhage as well as lethality are monitored. During an observation period of 48 hours not only the degree of damage but also the time course of its development can be studied. Testing 10^{-3} molar promethazine in PSS irradiated with 5 J/cm^2 UVA (320-400 nm) some damage occurred immediatedly after irradiation and increased to a maximum at 24 hours. Haematoporphyrin also induced phototoxic damage while tetracycline proved to be negative in this test. At the tested doses neither drug nor UV irradiation were toxic by themselves.

This new model can provide insight into the mechanisms of phototoxicity and serve as a screening model for new substances.

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THE EFFECTS OF UVB IRRADIATION ON PROTO-ONCOGENE EXPRESSION IN HUMAN EPIDERMIS.

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It is well known that neoplastic transformation may take place when proto-oncogenes are activated by point mutation, gene amplification and translocation. Ultraviolet irradiation (UVR) is the main causative agent of non-melanoma skin cancer and one possible mechanism for DNA damage due to UVR is by the activation of proto-oncogenes. Recently transient overexpression of c-Ha ras and myc was observed in a human keratinocyte cell line *in vitro*. However, it is still not known whether similar activations occur in human skin after UVR *in vivo*.

Two groups of 6 volunteers were irradiated on sites on the buttock with UVB (300nm ± 5nm) at 0.5 and 2 x each subject's minimal erythema dose (MED). Biopsies of irradiated and unirradiated skin were taken 5 hrs (group 1) and 24 hrs (group 2) post irradiation. C-myc and c-Ha ras transcripts of the epidermis were investigated by a quantitative in situ hybridization technique. DNA synthesis in the epidermis was also measured by the BrdU labelling method.

At 5 hrs post irradiation c-myc expression of the epidermis irradiated with 2 x MED was significantly increased and the labelling index was significantly decreased. At 24 hrs post irradiation c-myc expression of the skin irradiated with 2 x MED remained higher than that of unirradiated skin. The labelling index of skin irradiated with 0.5 x MED was significantly higher than that of unirradiated skin and skin irradiated with 2 x MED. These results suggest that the c-myc gene may play an important role in photocarcinogenesis.

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A QUANTITATIVE IMMUNOFLUORESCENCE ASSAY FOR UV-INDUCED THYMINE-THYMINE DIMERS IN EPIDERMAL PROLIFERATIVE CELLS BASED ON FLOW CYTOMETRY.

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We report on a quantitative immunofluorescence assay for cyclobutyldithymine dimers (T<>T) based on flow cytometry. This method has proven to be applicable to cultured human fibroblasts, keratinocytes and melanocytes, to isolated human lymphocytes and to epidermal cells isolated after irradiation of hairless mice *in vivo*. Experiments with *in vivo* irradiation of human skin are currently being carried out.

A monoclonal antibody, specific for T<>T in single stranded DNA, is bound to T<>T and subsequently labelled with FITC in partially denatured DNA *in situ*. In order to relate DNA damage to DNA content in individual cells, total DNA is stained with the intercalating dye 7-amino-actinomycine D (7-AAD).

FITC fluorescence increases linearly with UV dose. Selection on DNA content enables assessment of damage levels in separate phases of the cell cycle. This opens the possibility to quantify T<>T in an epidermal subpopulation of (proliferative) cells by selection on G0/G1 phase, S phase or G2M phase cells.

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BINDING OF CIPROFLOXACIN (CPX) AND ITS PHOTOPRODUCTS TO HUMAN SKIN.

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CPX, a broad-spectrum antimicrobial agent, has been reported to induce photosensitivity reactions. In previous experiments we have found a photodegradation of CPX with the formation of numerous photoproducts. Now we investigated the binding of CPX and its photoproducts to skin components. Skin, including epidermis and dermis, was obtained from human thighs and lyophilized. CPX (10^{-6}–10^{-3}mol/l in double destilled water as well as in aqueous suspensions of lyophilized skin) was irradiated with 100 J/cm^2 UVA from an UVB-free source, or was left non-irradiated. Binding of CPX to lyophilized skin was determined by equilibrium dialysis. CPX and its photoproducts were recovered by high performance liquid chromatography (HPLC). Non-irradiated CPX was bound to the same extent as irradiated, the binding being concentration dependent (n= 5): 14.3±1.8% (10^{-6}mol/l) to 32.0±3.0% (10^{-3}mol/l). After dialysis of irradiated 10^{-3}mol/l CPX solutions against lyophilized skin suspended in phosphate buffer three photoproducts were detected: one, more polar than CPX was bound to 47.3±1.6%, and two less polar products were bound each to 75.0%. After irradiation of lyophilized skin suspended in CPX solutions and subsequent equilibrium dialysis neither the parent drug nor any of the photoproducts could be recovered in the dialysate, indicating their complete binding. Thus, photoproducts of CPX are bound more readily to skin components than the parent drug. Furthermore, direct irradiation of lyophilized skin in the presence of CPX enhances drug binding considerably. As skin is a natural target of UV radiation, this effect may be of importance with regard to the development of photosensitivity reactions to CPX.

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ULTRAVIOLET (UV) A IRRADIATION REDUCES BASAL UNSTIMULATED INTRACELLULAR CYCLIC ADENOSINE MONOPHOSPHATE (cAMP) WITHOUT AFFECTING β_2-ADRENOCEPTOR DENSITY NOR AFFINITY. U. Bleise, E. Haen, and *B. Przybilla, Walther-Straub-Institut für Pharmakologie und Toxikologie, Ludwig-Maximilians-Universität, München, FRG and *Dermatologische Klinik und Poliklinik, Ludwig-Maximilians-Universität, München, FRG.

UVA irradiation of human peripheral blood leukocytes (PBL) has been demonstrated to decrease histamine release (HR) induced by anti-IgE or Ca-Ionophore (Int Arch Allergy Appl Immunol 88, 136-138, 1989). This effect may be relevant for phototherapy of e.g. atopic exzema. As HR is inhibited by β_2-sympathomimetic agents, that stimulate cAMP formation, we studied β_2-adrenoceptors on PBL from 10 healthy volunteers before and after UVA exposure. PBL were exposed to 50 or 100 J/cm^2 UVA or left non-irradiated. β_2-adrenoceptor density and affinity were determined in a radio-receptor assay with ^{125}Iodocyanopindolol (1.0-150 pmol/l). The intracellular formation of cAMP was tested with 5 concentrations of isoproterenol (10^{-8}-10^{-4} mol/l) yielding basal (E_0) and maximum cAMP content (Emax) as well as the effective isoproterenol concentration to achieve 50% of maximal cAMP increase (EC_{50}). Exposure to 50 or 100 J/cm^2 UVA did not alter receptor density nor affinity. However, E_0, Emax as well as Emax expressed as a percentage of E_0 were dose-dependently reduced after UVA exposure (E_0: 0/50/100 J/cm^2: 5.7/3.4/2.9 pmol/10^6 cells [median]; Emax: 0/50/100 J/cm^2: 7.9/4.2/3.0 pmol/10^6 cells; Emax expressed as a percentage of E_0: 0/50/100 J/cm^2: 140/115/106%). The concentration of cAMP in irradiated buffer solutions did not change. Thus, UVA exposure affects the β_2-adrenergic signal transduction beyond the β-receptor. At present it remains speculative, whether the UVA effect on cAMP turnover and the decrease of stimulated HR are interrelated.

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DUAL EFFECT OF ULTRAVIOLET B ON UVA-INDUCED PHOTOTOXICITY. Bernadette Eberlein, Franziska Rueff, Thomas Bergner, Bernhard Przybilla, Dermatology Clinic and Policlinic, Ludwig-Maximilians-University Munich, FRG

Depletion of the stratospheric ozone layer is a matter of serious concern, as it may lead to an increase of ultraviolet (UV) B intensity on the earth's surface. We evaluated the effect of additional UVB irradiation on UVA-induced phototoxicity of ketoprofen in a photohemolysis test. Suspensions of human erythrocytes from 10 donors were incubated with 10^{-3} mol/l ketoprofen, a phototoxic nonsteroidal anti-inflammatory drug. These samples were exposed to 0/20/40/80 mJ/cm^2 UVB from TL/12 light bulbs, followed by irradiation with 0/6 J/cm^2 UVA from an UVASUN 5000. Hemolysis in the supernatants was determined by reading of absorbance at 550 nm with a photometer, and photo-induced hemolysis was calculated as percentage of complete hemolysis. Photohemolysis due to 6 J/cm^2 UVA alone was 66 %. UVB did not induce photohemolysis at doses $\approx$ 80 mJ/cm^2. Combined exposure to UVB and UVA enhanced UVA-induced photohemolysis in samples from 5/10 donors at 20, 40 and 80 mJ/cm^2 UVB. However, with erythrocytes from the other 5 donors 20 or 40 mJ/cm^2 UVB inhibited the UVA-induced photohemolysis by 69% or 38% (median). Co-incubation of the samples with ascorbic acid (10^{-3} M) resulted in a profound inhibition of UVA-induced and UVB-enhanced photohemolysis. These results indicate a dual effect of UVB: Whereas high UVB doses enhance UVA-induced phototoxicity, the effect of lower UVB doses depends on individual, yet unknown characteristics of the target cells. The effects of a moderate increase of UVB intensity on the earth's surface have to be evaluated in a differentiated way.

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PHOTOTOXIC PROPERTIES OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN THE VISIBLE LIGHT RANGE. Thomas Bergner, Lorenz Becker, Bernadette Eberlein, Bernhard Przybilla, Dermatology Clinic and Policlinic, Ludwig-Maximilians-University, Munich, FRG

Photosensitivity reactions due to nonsteroidal anti-inflammatory drugs (NSAID) are well-known side effects of these drugs. 8 NSAID (carprofen, diclofenac, ibuprofen, ketoprofen, naproxen, piroxicam, phenylbutazone) were assessed in a photohemolysis test with regard to phototoxic effects in the visible light (VL) range. Washed human erythrocytes were incubated with the compounds at 10^{-5} to 10^{-3} M. These suspensions were exposed to up to 50 J/cm^2 from an experimental VL lamp, emitting at most 5% UVA. To control for UVA-induced effects, additional samples were exposed to 2.5 J/cm^2 UVA from an UVA source, and to the VL lamp while being covered by a GG 400 (transmission at 390 nm: 3%) or GG 420 filter (transmission at 400 nm: « 10^{-5} %). Hemolysis in the supernatants was determined by reading of absorbance at 550 nm with a photometer. Photo-induced hemolysis was calculated as percentage of complete hemolysis. Maximum photohemolysis found after exposure to the VL lamp was: tiaprofenic acid, 100%; naproxen, 97%; carprofen, 89%; phenylbutazone, 85%; ketoprofen, 83%; piroxicam, 18%; ibuprofen and diclofenac did not cause photohemolysis. The magnitude of hemolysis depended on the radiation dose and the drug concentrations. 2.5 J/cm^2 of UVA induced minor hemolysis only with tiaprofenic acid, carprofen or ketoprofen (maximum 35%). Beneath the GG 400 (GG 420) filter, maximum photohemolysis induced by the VL lamp was 76% (0%) with naproxen, 70% (66%) with phenylbutazone and 40% (36%) with carprofen. There was no photohemolysis with the other compounds. Phototoxic effects in the VL range may be of importance with regard to clinical photosensitization and its prevention.

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INTER-INDIVIDUAL VARIATION FOR PROTECTION AGAINST OXIDATIVE STRESS INCLUDING UVA RADIATION IN NORMAL AND *ATAXIA TELANGIECTASIA* SKIN CELLS. Lee Ann Applegate[1], Patrick Lüscher[2], Edgar Frenk[1] and Rex Tyrrell[2], University Dept. Dermatology[1] and Swiss Institute for Exp. Cancer Research[2], Lausanne, Switzerland

Ultraviolet-A radiation (UVA, 320-380 nm) constitutes a significant oxidative stress and induces potentially, hazardous free radical damage. Besides the constitutive mechanisms against oxidative stress (e.g. glutathione, superoxide dismutase, catalase) mammalian cells respond to UVA by a transient enhancement in the synthesis of specific gene products. The induction of heme oxygenase (HO), appears to be a mechanism by which cells can be protected against UVA radiation and other forms of oxidant stress. We have found a wide variation in the sensitivity of skin fibroblasts from different individuals to oxidising stress (UVA radiation and H_2O_2) both in terms of colony forming ability and gene activation. In cells from "normal" individuals, the fluences of UVA radiation required to reduce survival by 50% and 90% range from 13-51 and 37-120 KJ/m^2, respectively. Cell populations are reduced to 10% survivors by 30 min treatment with H_2O_2 in the concentration range 0.75-2.6 mM. Cell populations from five *Ataxia telangiectasia* (AT) patients are hypersensitive to oxidative stess showing 50% and 10% survival following UVA radiation in the fluence range 11-14 and 28-32 KJ/m^2, respectively and 10% survival following H_2O_2 treatment between 0.28-0.8 mM. Heme oxygenase induction also varies extensively between individuals as regards the fold-increase of mRNA over basal level expression of the gene (15-70 fold) and the kinetics of mRNA accumulation 2 to 8 hours following treatment which returns to basal levels by 24 to 48 hours. Expression of HO mRNA in cells from different AT patients are also heterogeneous in their response but seem to be similar to the control cells regarding fold-induction and kinetics of gene expression. In conclusion, defense against oxidative stress seems to vary considerably between cell populations derived from different humans. The correlation between susceptibility to cancer induction and levels of cellular defence remains to be established.

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DISTRIBUTION AND ULTRASTRUCTURAL LOCALIZATION OF THE CARCINOEMBRYONIC ANTIGEN (CEA) FAMILY IN NORMAL SKIN AND CUTANEOUS TUMORS. Dieter Metze*, Ranjit Bhardwaj**, Gerhard Kolde*, Susanne Daniel***, Fritz Grunert***, *Dept. Dermatol., **Dept. Exp. Dermatol., Univ. Münster, ***Inst. for Immunobiol., Univ. Freiburg, FRG.

Carcinoembryonic antigen (CEA) comprises a family of closely related glycoproteins, e.g. the intestinal type of CEA, non-specific crossreacting antigen (NCA) or biliary glycoprotein (BGP). CEA-like proteins have been demonstrated in sweat glands and cutaneous tumors. To determine the expression of the different CEA glycoproteins in normal and neoplastic skin a panel of highly specific antibodies was applied for routine immunoperoxidase staining and a postembedding immunogold technique for electronmicroscopy.

Sweat glands could be demonstrated to coexpress the intestinal type of CEA and NCA. Immunoelectron microscopy revealed these glycoproteins in the Golgi apparatus of secretory cells, along the microvilli or in multivesicular bodies of the sweat ducts. Sebaceous glands were exclusively labelled with an antibody crossreacting with BGP, preferentially in vesicular structures of higher differentiated sebocytes. Most of the reactive tumors expressed NCA only, whereas syringoma, porocarcinoma and others showed staining for both, NCA and the intestinal type of CEA. At ultrastructural level the neoplastic reactivity could be correlated to a labelling of intracellular vesicles, vacuoles, microvilli or cell membranes according to cellular differentiation.

In conclusion, these findings suggest an active biosynthesis of different CEA glycoproteins in normal and neoplastic skin. The ultrastructural labelling pattern indicates an important role of CEA for the formation of microvilli as well as for surface polarity and cellular differentiation corroborating that CEA functions as an adhesion molecule.

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ABERRANT EXPRESSION OF P53 TUMOUR SUPPRESSOR PROTEIN IN MALIGNANT MELANOMA AND DYSPLASTIC NAEVI. JM McGregor, CC-W Yu[1], DAL Levison[1], DM MacDonald, Depts. of Dermatology and Histopathology[1], UMDS (Guys' Campus), London, SE1 9RT, UK.

P53 mutations are the most frequent genetic alterations found in human malignancies to date. Wild type p53 is a tumour suppressor protein, crucial for the negative control of cell cycling and requiring loss of function mutations for tumorigenesis. Such mutations result in stabilisation of the cellular p53 protein which is normally rapidly degraded. Immunohistochemical detection of nuclear p53 thus provides strong evidence for mutation in the p53 gene. To determine the role of p53 mutation in the pathogenesis of malignant melanoma we employed an immunohistochemical technique using the antiserum CM-1, a polyclonal antibody raised against recombinant human p53, on formalin fixed, paraffin embedded tissue.

P53 immunoreactivity was observed in 34%(2/6) superficial spreading malignant melanomas, 75% (13/18) invasive malignant melanomas and 60%(8/12) metastatic melanomas. Foci of p53 immunoreactivity were also seen in 25%(3/12) dysplastic naevi and 14%(1/7) Spitz naevi. P53 immunoreactivity was not observed in 25/25 benign melanocytic naevi nor in 2/2 spindle cell naevi of Reed examined.

The molecular biology of malignant melanoma is currently poorly understood; this study provides strong evidence that p53 gene mutation, resulting in loss of tumour suppressor function, may play a crucial role in the biology of melanoma. Furthermore, p53 immunoreactivity observed in a minority of dysplastic naevi and Spitz naevi may provide an explanation for the increased potential of these melanocytic naevi for malignant transformation.

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SKIN FIBROBLASTS ENHANCE THE TUMORIGENICITY OF HUMAN NEOPLASTIC CELLS TRANSPLANTED SUB-CUTANEOUSLY INTO NUDE MICE. N. Simon*, A. Noël*, B. V. Nusgens+, Ch. M. Lapière+ and J.M. Foidart*, Laboratory of Biology* and Experimental Dermatology+, University of Liège, Sart Tilman, Belgium.

The aim of our investigation is to substantiate the effect of fibroblasts (F) on the development of cancers. When injected sub-cutaneously into nude mice even in high number (3x10E6 cells), human breast adenocarcinoma MCF7 cells failed to induce tumors. In the presence of an extract of basement membrane proteins, matrigel, tumor growth was promoted in 75% of the animals with a latency period of 3 to 5 weeks for, respectively 1.5 x 10E6 and 3.5 x 10E5 cells. Injection of MCF7 cells (3.5 x 10E5) and F (10E6) induced tumors in 60% of the animals with a latency period of 10 weeks. By injecting 3.5 x 10E5 MCF7 cells with matrigel and F, 100% of the animals presented a tumor with a significantly decreased latency period to 20 days. Their volume of these tumors was significantly increased. A similar effect could be reproduced by repeated injections of F- conditioned medium at the site of inoculum of MCF7 cells and matrigel. The enhancing effect of F might be related to the production of diffusible factors active in promoting angiogenesis, a limiting factor in tumor growth.

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DIFFERENTIAL EXPRESSION OF LAMININ ISOFORMS AND ß4 INTEGRIN EPITOPES IN THE BASEMENT MEMBRANE ZONE OF NORMAL HUMAN SKIN AND BASAL CELL CARCINOMAS. Stephan Sollberg, Juha Peltonen, and Jouni Uitto, Department of Dermatology, Thomas Jefferson University, Philadelphia, PA, USA.

The basement membrane zone biology of normal human skin and nodular basal cell carcinomas (BCC) was explored by immunofluorescence with monoclonal antibodies specific for A, B1 and B2 chains of classic laminin, for M chain of merosin, or for S chain in S-laminin. Immunostaining with antibodies for A, B1 and B2 chain epitopes revealed a strong and linear immunosignal in normal human skin and in six nodular BCC. However, M chain epitopes were abundantly expressed in nodular BCC, but at a very low level in normal skin. Furthermore, S chain epitopes were only found in nodular BCC, but not in normal skin. Staining for ß4 integrins, potential receptors for laminin, revealed a linear staining pattern in normal skin as well as in the superficial portions of the nodular BCC. However, the immunofluorescence pattern in the deeper portions of the lesions was scattered and interrupted. Thus, altered composition of the basement membrane of nodular BCC with respect to laminin isoforms and their interactions with putative cell surface receptors, the ß4 integrins, may change the containment of the tumor islands, contributing to the local aggressive behavior of nodular BCC.

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EXPRESSION OF MUTANT P53 PROTEIN IN BOWEN'S DISEASE. Phillip H McKee, Susan D Slater, Chee S Sim, Department of Histopathology, St Thomas's Medical School, London, UK.

Wild-type p53 gene is situated on chromosome 17p and has tumour-suppressor function. Its mutation is one of the commonest genetic abnormalities identified in human neoplasms. It has been detected in carcinoma of the breast, colon, lung, urinary bladder, bone and skin. Mutant p53 protein is associated with a greatly extended half-life and therefore (in contrast to the wild-form) can be detected by immuno-cytochemical methodologies in tissue sections. The recently developed polyclonal rabbit-anti-p53 anti serum CM1 raised against recombinant human p53 mutant protein can be used on formalin-fixed, paraffin-embedded tissue sections.

In this study we have investigated 26 histologically proven cases of cutaneous Bowen's disease for the presence of mutant p53 protein using an indirect immunoperoxidase technique. Fifteen specimens (58 percent) showed positive nuclear labelling of dysplastic keratinocytes. Eleven specimens (42 percent) were immuno-negative. Positivity was in general diffuse in nature although on occasions it had a patchy distribution. It was not identified within the granular cell layer nor was it present in dyskeratotic or vacuolated keratinocytes. Adnexal epithelium if involved by the dysplastic process showed similar positive labelling. In six specimens (23 percent) positive nuclear labelling was identified within cytologically apparently "normal" keratinocytes in the adjacent epithelium. This may represent a potential marker of an early (visible) stage of neoplastic transformation and when present in histologically equivocal cases may provide additional data of prognostic significance.

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RELATION OF p53 TUMOR SUPPRESSOR PROTEIN EXPRESSION TO HPV DNA IN MALE GENITAL WARTS AND BOWENOID PAPULOSIS. A.Ranki, J.Lassus, D.Lane, and K-M.Niemi, Department of Dermatology, University of Helsinki, Helsinki, Finland, and Department of Biochemistry, University of Dundee, Dundee, U.K.

To demonstrate DNA spesific for HPV types 6, 11, 16, 18, 31,33,35 and 51 in male genital warts, we used digoxigenin (DIG-HPV)- and biotin-labeled (BIO-HPV) HPV DNA probes in *in situ* hybridization of paraffin-embedded sections. In HPV+ condylomata with no cellular atypia, HPV 6/11 was detected in 87%, and HPV 16/18 in 27%. In biopsies with cellular atypia, HPV types 6/11 were detected in 62%, HPV types 16/18 in 46%, and HPV types 31/33/35 or 51 in 50%.

We then looked for the presence of p53 tumor suppressor protein in keratinocytes with antibodies against full length p53 and against conformationally mutated loss-of-function protein. Normally, functionally active p53 protein is not detected due to short half-life. We also developed a double method to simultaneously visualize HPV DNA and p53 protein in the same cells. We found quantitative differencies in the cytoplasmic accumulation of p53 gene product (suggestive of mutant p53) in a positive correlation to the presence of HPV DNA, especially to types 31,33,35. Also, an association to cellular atypia was seen in all types of condylomata but not in Bowenoid papulosis (BP). However, during a follow-up of 2 years, only 1 of 17 BP lesions persisted. Our results thus speak against the concept that p53 degradation is enhanced by HPV 16 or 18 E6 protein.

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ORAL METAPLASIA OF ADULT HUMAN EPIDERMAL KERATINOCYTES GROWN IN VITRO IN THE PRESENCE OF RETINOIC ACID. Daniel Asselineau and Michel Darmon, Centre International de Recherches Dermatologiques Galderma (CIRD Galderma), Sophia Antipolis, 06565 Valbonne, France

A striking effect of retinoic acid (RA) is its ability to alter cell fate during development. The mucous metaplasia produced by treating chick embryo skin with RA is a classical example of this property. It has so far been impossible to reproduce with adult keratinocytes grown in vitro such a metaplasia, although RA has been shown to block terminal epidermal differentiation, to induce an increased synthesis of mucopolysaccharides, and to induce markers of non-keratinized epithelia such as K19 and K13 keratins. When adult human keratinocytes are grown on dermal lattices at the surface of the culture medium, they are able to form a normal keratinized epidermis. But, when excess RA is added to the culture medium, a stratified non-keratinized (parakeratotic) epithelium is formed. The distribution of tissue- and differentiation-stage specific markers in this epithelium shows that it has close resemblance with the oral epithelium. Moreover, when this tissue is transferred into a normal medium (no RA added), a new epithelium is formed beneath the "old" one at the expense of basal cells, and this epithelium has an epidermal orthokeratinized phenotype, whereas the "old" epithelium remains unchanged. These observations show that the reversibility of the changes produced by RA are only apparent, since the metaplastic tissue remains unchanged and the new tissue is derived from still uncommitted cells. Altogether, our studies suggest that adult keratinocytes treated in vitro by RA, although unable to transform into mucous cells, undergo a metaplasia into a wet stratified epithelium closely resembling oral epithelia.

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HAIR CYCLE HAS NO EFFECT ON DISTRIBUTION AND PERSISTENCE OF A TOPICALLY APPLIED CARCINOGEN IN MOUSE SKIN. C.L. Wilson, T.-T. Sun+, R.M. Lavker, Dept. of Dermatol., Univ. of Pennsylvania, Philadelphia, PA; + Depts. of Dermatol & Pharmacol., New York Univ., NY, NY.

Our recent finding that putative follicular stem cells reside in the bulge of the outer root sheath (ORS) raises the question of their involvement in the formation of experimentally induced skin tumours. Early mouse skin carcinogenesis studies indicated that tumour yield was greater when initiation was in the telogen phase of the hair cycle as opposed to anagen. This difference was explained by increased retention of carcinogen by telogen follicles. These retention studies did not examine the bulge region of the hair follicle. Therefore, we have re-examined the effects of hair cycle on the distribution and persistence of a topically applied initiator (benzo(a)pyrene; BP) in SENCAR mice. Varying doses of BP in acetone (200-4000 nmoles) were applied to the dorsal skin during early anagen, or in telogen. Mice were then biopsied at 1, 6, 24 hrs, 3, 5, and 7 days; the distribution of BP (an auto-fluorescent compound) in frozen sections of skin was visualized with a fluorescence microscope. At the earliest times (1, 6 hrs), in both anagen and telogen skin, fluorescence was noted in the entire sebaceous gland, epidermis and upper follicle. Within the follicle, fluorescence was only seen in the ORS down to the level of the bulge. In anagen follicles, fluorescence was never observed in the lower ORS or matrix keratinocytes of the bulb. At 24 hrs fluorescence was markedly reduced from all areas except the stratum corneum, where it persisted for 5 days. By 7 days no fluorescence could be detected. The distribution of BP was similar for all doses applied. These observations indicate that the hair cycle has no influence on distribution or persistence of topically applied carcinogen. Thus, the known effect of the hair cycle on tumour yield may be related to the proliferative state of cells at the time of initiation. Failure to detect the carcinogen below the bulge implies that the proliferating keratinocytes in the bulb are not the site of initiation.

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VARIABLE δ GENE EXPRESSION IN NORMAL HUMAN SKIN AND SOME PATHOLOGICAL SKIN CONDITIONS.

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Two main subsets of γδ T-cells have been identified to date. The first type observed on the great majority of human peripheral blood γδ T-cells express a δ chain using the variable δ2 segment paired with a Vγ9 segment. The second population of γδ T-cells utilize a Vδ1 rearrangement which is usually paired with a Vγ element other than Vγ9. The present study was designated to investigate the T-cell receptor diversity (TCR) of γδ T-cell subpopulations in normal human skin and some pathological skin conditions in which an increase of γδ T-cells was detected by our previous investigations.

Biopsies of normal human skin and some pathological skin conditions including Cutaneous Leishmaniasis (CL), Langerhans' Cell Histiocytosis (LCH) and Woringer-Kolopp disease were investigated by an alkaline phosphatase anti-alkaline phosphatase (APAAP) technique using the following monoclonal antibodies: anti-CD3 (Dakopatts), anti-γδ TCR, (T Cell Sciences), anti-Vδ1 (A13, Dr. Moretta,Istituto per la Ricerca sul Cancro, Genova, Italy), anti-Vδ2 (BB3, Dr.Moretta)

In normal human skin γδ T-cells generally expressed the Vδ2 segment in both the dermal and epidermal compartment. In CL TCR γδ bearing cells were predominantly Vδ2+ and only few were Vδ1+. Vδ2+ cells were generally localized in the dermis and only occasionally within the epidermis. In contrast Vδ1+ cells were almost exclusively detected within the epidermal layer. In LCH Vδ1+ cells were the prevalent population of γδ T-cells identified in both dermis and epidermis. In Woringer-Kolopp disease neither of these subsets was detected.

These findings: 1) indicate a prevalent expression of the Vδ2 segment by γδ T-cells in normal human skin; 2) demonstrate a preferential microanatomy distribution of γδ T-cells subsets in lesional skin of CL; 3) suggest a possible role of the Vδ1 subset in LCH.

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PRIMARY CUTANEOUS CD30+ve T-CELL LYMPHOMAS DO NOT CONTAIN HTLV-1 OR EBV DNA SEQUENCES.

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Primary cutaneous large cell lymphomas represent a heterogenous group which may express the CD30 antigen. This antigen is not lineage specific and may be induced by viral transformation on both B and T lymphocytes. We have analysed DNA extracted from peripheral blood mononuclear cells and 8 skin biopsies from 4 patients with primary cutaneous CD30 positive large cell lymphomas using restriction enzyme digestion, electrophoresis and Southern blot hybridisation with a cDNA probe for the CβT cell receptor (TCR) gene, a genomic probe for the Immunoglobulin (Ig) heavy chain gene, a full length HTLV-1 probe (λ23-3) and an EBV probe for the internal repetitive sequence (Bam HIW). DNA samples were also subjected to in vitro amplification using primers for a specific HTLV-1 pol gene sequence.

Analysis of Ig genes revealed a germline configuration while TCR genes were rearranged in all cutaneous DNA samples. Multiple biopsies from individual patients revealed identical TCR gene rearrangements. Analysis of peripheral blood mononuclear cells consistently revealed a germline configuration. No evidence of hybridisation was detected in EcoRI/Hind III digests with an HTLV-1 probe or in Bam H1 digests with an EBV probe. In addition specific HTLV-1 pol gene sequences could not be amplified from tissue DNA samples.

This study indicates that these primary cutaneous CD30 positive large cell lymphomas contain T cell clones. However we have found no evidence of EBV DNA or integration of HTLV-1 proviral DNA within cutaneous tissue, in contrast to previous reports.

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MODULATION OF MY7 ANTIGEN EXPRESSION BY CYTOKINES (INTERFERON ALPHA AND IL-1) IN EPIDERMIS OF T- CELL LYMPHOMA (CTCL). CORRELATION BETWEEN IN VITRO AND IN VIVO RESULTS.

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Basal keratinocytes (bK) express a myelomonocytic antigen MY7 (CD13) in normal conditions. In CTCL, this expression disappears but may be induced again if a clinical and histological improvement under Interferon alpha 2a (InF α) therapy is obtained. The aims of this study are firstly to investigate if an expression of MY7 antigen may be induced in vitro by InF α or by Interleukin 1 (IL-1) whose production is increased in epidermis of CTCL and secondly to appreciate the relation between this possible in vitro induction and the clinical response to treatment ?

We studied on reconstituted skin (RS) the MY7 expression by bK arising from 10 CTCL and 5 controls and its modulation by InF α and IL-1 using an indirect immunofluorescence technique. Concurrently, a clinical examination and an immunological study on cutaneous biopsies have been performed at 3 and 6 month for each patient under InF α therapy.

- In vitro, normal bK express MY7 antigen and this expression is not modified by the addition of InF α or IL-1 in the cell culture medium
- On the contrary, bK from CTCL do not express MY7 Ag (8 /10) but express it again with InF α (5 / 8) or IL-1 (4/8). The three RS which never express MY7 after incubation with InF α and IL-1 arise from three "bad-responders" to InF α therapy (no in vivo MY7 expression after 3 month of treatment). The 5 RS which express MY7 after incubation with InF α or IL-1 are related to 5 good clinical responses under treatment.

This study demonstrates that firstly, an MY7 expression by the bK from CTCL could be induced in vitro, not only with InF α but also with IL-1 suggesting that InF α might act not directly but by increasing the production of another cytokine and we are actualy extending this study to others cytokines. Secondly this modulation seems correlated with InF α efficacy in vivo. Our results have to be extended but this in vitro test could have an important predictive value.

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HUMAN T-CELL LYMPHOTROPIC VIRUS TIPE I (HTLV-I) IN CUTANEOUS T-CELL LYMPHOMA: DETECTION BY POLIMERASE CHAIN REACTION (PCR) ANALYSIS.

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HTLV-I is a human retrovirus associated with the adult T-cell leukemia, as previously described in Japan. Only rarely, specific anti-HTLV-I antibodies have been detected in patients with cutaneous T-cell lymphoma (CTCL), but the meaning of this association has not been clarified as yet. We investigated 30 CTCL patients (24 males and 6 females, middle age 52.4 years) for blood reactivity to HTLV-I antigen. By ELISA and Western Blot analyses all patients resulted negative. Peripheral blood lymphocytes of each patient were cultured in RPMI medium and stimulated by PHA and IL-2. In serial passages 10^7 cells were drawn and DNA was extracted. Each DNA sample was subjected to PCR analysis with the following primers: SK 43 and SK 44, recognizing the "tax" region of both HTLV-I and HTLV-II; SK 54 and SK 55, recognizing the "pol" region of HTLV-I. All amplification products were hybridized by the following probes labelled with P 32 in Southern blot analysis: SK 45, recognizing the "tax" region of HTLV-I and HTLV-II; SK 56, recognizing position 3426 through 3460 in the "pol" region of the HTLV-1 genome. In one patient (Z.B., a 44 year-old omosexual man, with stage II CTCL) the analysis was positive, whereas in the other 29 was negative. Our findings show, for the first time in Italy, the presence of HTLV-I in one patient with CTCL, and may suggest a causal role of this virus, at least in some cases, in the pathogenesis of CTCL.

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ANALYSIS OF T-CELL RECEPTOR GENES CONFIGURATION IN MYCOSIS FUNGOIDES.

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The routine histologic diagnosis of early patch and plaque stage of M.F. is difficult and may require numerous skin biopsies because of a polymorphous infiltrate closely resemble some chronic dermatitis. For this reason, many investigators have attempted to develop adjunctive techniques to aid in the evaluation of cutaneous lymphoid infiltrates. Recently, DNA hybridization has been found to be useful in the diagnosis of T-cell malignancy by demonstration the clonal nature of M.F. through the demonstration of consistent beta T-cell receptor (TCR) gene rearrangements. In this study we analyzed the configuration of the TCR beta, gamma and delta chain genes in 27 skin biopsies of patients affected by M.F. and the results have been correlated with the histologic features and associated immunophenotype. The immunophenotype of lymphocytes were CD3+, CD2+, CD4+, CD5+, CD7+/-, CD8-, CD25+/-, CD30+/-, BerAct8-, TCRd1-, BF1+. We detected clonal rearrangements of the TCR beta chain genes after restriction with BamHI and EcoRI in 22 from 27 patients. Rearrangements of the TCR gamma chain genes were demonstrated in 22 patients after restriction with BamHI and EcoRI. For the TCR delta chain genes we detected a mono or biallelic delection in all patients sample after restriction with BglII. However the three cases of suspected M.F. in stage I did not show any rearranged bands. These negative results could be due to the sensitivity of the Southern-Blot analysis in the very early stage of the disease. The presence of rearranged bands for gamma TCR genes in 90% of patients is very interesting to solve this problem. In fact the TCR gamma genes are ideal targets for PCR based detection of minimal residual disease.

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THE HUMAN SKIN IMMUNE SYSTEM AND HECA-452: SKIN HOMING T LYMPHOCYTES.

Jan D. Bos', Els Tibosch', Onno J. de Boer', Pranab K. Das'*, & Steven T. Pals*. Departments of 'Dermatology and *Pathology, University of Amsterdam, Academisch Medisch Centrum, Amsterdam, The Netherlands.

The immigration of circulating T-cells into specific tissues is directed by the binding of adhesion molecules on lymphocyte subpopulations to vascular addressins, while emigration occurs through afferent lymphatics originating in the dermis. In skin, various adhesion molecules are expressed by endothelial cells, being upregulated during inflammation. Of these, endothelial leukocyte adhesion molecule (ELAM-1), weakly expressed in normal human skin (NHS), seems to be the counterstructure for 'cutaneous lymphocyte antigen (CLA)'. CLA is a 200-kd cell surface glycoprotein of which sugar moieties sialyl Le(a) and sialyl Le(x) are the putative epitopes recognized by the rat monoclonal antibody HECA (high endothelial cell antigen)-452. HECA-452 was originally described as a marker for lymphoid organ high endothelial cells, but 16% of peripheral blood derived T-cells react with this antibody. We studied the expression of HECA-452 on the cellular constituents of the skin immune system (SIS). By applying double immunohistochemical staining techniques, CD3+ T-cells were found to express HECA-452 in 41%. CD4+ T-cells expressed HECA-452 in 44%, and CD8+ T-cells in 31%. Keratinocytes, CD1a+ Langerhans cells (LC's) nor endothelial cells were found to express HECA-452 in significant numbers in NHS. Monocytes were found to express HECA-452 in 14% of CD68+ cells. HECA-452 expression was relatively low on T-cells subsets localized distant from NHS vessels, suggesting loss of the molecule during further migration after transendothelial paasage. However, intraepidermal T-cells expressed HECA-452 in similar percentages when compared to T-cells localized directly perivascular. Our findings support the assumption that HECA-452 expression by T-cells is associated with their homing into normal cutaneous structures.

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ANALYSIS OF T-CELL RECEPTOR GENES IN CHRONIC ACTINIC DERMATITIS: NO EVIDENCE OF CLONALITY. H du Peloux Menagé, S J Whittaker, Y L Ng, P G Norris, N P Smith, J L M Hawk. St John's Institute of Dermatology, St Thomas' Hospital, London, U.K.

The histology of the actinic reticuloid (AR) variant of chronic actinic dermatitis (CAD) mimicks that of cutaneous T-cell lymphoma and isolated reports of the development of dermal reticulosis in AR patients have suggested that CAD may have the potential for lymphomatous transformation, although a previous flow cytometric study showed no evidence of DNA aneuploidy in the cutaneous infiltrate of patients with AR. T-cell receptor (TCR) and immunoglobulin (Ig) gene rearrangement studies have proved more sensitive in the diagnosis of lymphoid neoplasia, and we have therefore investigated CAD for evidence of clonal lymphoid population.

We analysed the configuration of TCR and Ig genes in DNA extracted from skin biopsies and peripheral blood mononuclear cells of 10 patients with CAD including 6 with the AR variant. The DNA was subjected to electrophoresis and Southern blot hybridisation with DNA probes for the β, γ and δ TCR and Ig heavy chain genes. FACS analysis was performed on peripheral lymphocytes, and buffy coat smears were prepared for identification of atypical lymphocytes using a May-Grünwald Giemsa stain.

All patients had circulating lymphocytes with atypical nuclear morphology but none had classical Sézary cells. Total circulating CD_3+ lymphocyte count was normal but DR+CD_3+ cells were increased and predominance of CD8+ lymphocytes was noted, more marked in patients with AR, confirming previous work. In all cases TCR and Ig genes were in germ line configuration with no evidence of discrete rearranged bands.

Our results indicate that T-cell clones are not present in CAD, supporting the view that this disorder does not have neoplastic potential.

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PRESENCE OF A NEW T-CELL ACTIVATION MARKER CD27 IN NORMAL HUMAN AND INVOLVED PSORIATIC SKIN. Menno A. de Rie, Irina Cairo, René A.W. van Lier, and Jan D. Bos, Dep. of Dermatology and Lab. for Exp. and Clin Immunology (RAWvL), Univ. of Amsterdam, The Netherlands.

CD27 is a 55 kDa transmembrane glycoprotein that is expressed on 80% of normal resting peripheral blood T cells (PBTL). During T-cell activation, CD27 expression is initially upregulated and subsequently lost on the cellmembrane, but detectable in supernatant as soluble (sol)CD27. CD27 belongs to a family of receptor (R) molecules (i.e. Nerve Growth Factor-R, Tumor Necrosis Factor-R), but the function on T cells is unknown. We studied the relative expression of CD27 on CD2-positive T cells of normal human skin (NHS) as well as of involved psoriatic skin (PS), using CD-defined monoclonal antibodies in a double labeling procedure. Simultaneously, we used anti-CD25 (IL2-R) to detect the activational state of these T cells and anti-CD45RA to detect memory or naive state of differentiation. NHS T cells expressed CD25 in 40% (versus 10% on PBTL). NHS T cells expressed CD27 in 40% and CD45RA in 10% (as compared to 20% on PBTL). PS T cells expressed CD25 in 45% (versus 10% on PBTL), CD27 on 25% and CD45RA in 5%. These results indicate that T cells in PS, and to a lesser extent in NHS, are mainly activated and of memory subset. The activational state of T cells in PS is further supported by our finding of elevated sol CD27 levels in serum of psoriasis patients. The observed differences in CD27 expression of PBTL, NHS and PS support in vitro studies showing that CD27 expression changes during T-cell activation.

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ACCUMULATION OF CD7 NEGATIVE HELPER T CELLS IN INFLAMMATORY SKIN LESIONS M. Moll, S. Kukel, H.W. Kreysel, U. Reinhold, Department of Dermatology, University of Bonn, D-5300 Bonn, Germany.

CD7 is a 40 kD T cell-associated glycoprotein which appears early during T cell ontogeny. Several studies have confined that CD7 is expressed on most human thymocytes and peripheral blood T cells. However, all these studies indicate the existance of a minor proportion of circulating T cells (<10%) which do not express the CD7 antigen. We have isolated skin-infiltrating lymphocytes (SIL) from biopsies of 16 patients with inflammatory skin lesions (psoriasis, atopic dermatitis, lichen planus, pityriasis rubra pilaris, mycosis fungoides) and expanded in vitro in the presence of IL-2. Fluorocytometric analysis of skin-derived cells showed a high percentage of CD7 neg. T cells (5 - 75%) as compared to the blood. The majority of CD7 neg. SIL were of the CD4 helper phenotype. The possibility that absent CD7 expression is the result of down-regulation was investigated. Hihgly enriched CD7 neg. and CD7 pos. normal human T cells were exposed to different cell activating agents for several periods of time and T cell antigen expression was analyzed on a flow-cytometer. The antigen density on CD7 pos. cells increased time dependend after stimulation wherease none of the activating agents used did induce CD7 expression on neg. T cells. The data indicate that CD7 neg. T cells preferentially accumulate in inflammatory skin lesions and that cell activation is not the underlying course of absent CD7 expression. The question of whether some T cells lose CD7 expression or whether there is a distinct CD7 neg. lineage remains unanswered.

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A SPONTANEOUSLY PROLIFERATING T CELL LINE ESTABLISHED FROM A PATIENT WITH SÉZARY SYNDROME. R. Müller, U. Reinhold, S. Kukel, H. Abken, I. Oltermann, H. W. Kreysel, Department of Dermatology, Institute for Genetics, University of Bonn, D-5300 Bonn, Germany.

A human T cell line, designated Hol-7, was established from a patient with Sèzary syndrome, whose leukocyte count was 90.000/mm3 with >90% Sèzary cells. Freshly isolated blood lymphocytes showed proliferative response to interleukins 2, 4, and 7. After 1 month in culture a growing T cell line independent on exogeneous growth factors was obtained. Southern blot analysis showed a monoclonal rearrangement of the T cell receptor beta chain gene. The phenotype of Hol-7 was CD3+, CD4+, CD8-, CD7-, CD45RO+, CD45RA-, CD25+, HLA-DR-. Loss of CD7 surface expression was associated with absence of CD7 mRNA analyzed by Northern blot. Cultured T cells were analyzed for cytokine secretion after stimulation with mitogens and ionomycin/phorbolester. Mitogens did not induce cytokine secretion in Hol-7 cells. Ionomycin/phorbolester induced measurable concentrations of IL-2 and IFN-gamma whereas IL-4 and GM-CSF could not be detected. Cell free supernatants of stimulated as well as unstimulated growing Hol-7 cells mediated suppression of proliferative responses in allogeneic mixed lymphocyte culture in a dose-dependent manner. This indicates that tumor cells secrete a factor which may lead to down-regulation of immune responses.

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T-CELL RECEPTOR γδ-POSITIVE CUTANEOUS T-CELL LYMHPOMAS. Annette Wolff-Sneedorff, Gunhild Lange Vejlsgaard, Elisabeth Ralfkiaer, Department of Dermatology, Rigshospitalet and Department of Pathology, Gentofte Hosipital, University of Copenhagen, Denmark.

Examination of biopsy samples from 62 patients with or suspected of cutaneous T-cell lymphoma (CTCL) revealed two cases in which the neoplastic cells were positive for the T-cell receptor (TCR) γδ complex. One patient had mycosis fungoides and one patient had a pleomorphic lymphoma of medium and large-cell type. Both cases showed aggressive courses with dissemination to internal organs and short survival times.

The phenotypic examination showed that the neoplastic cells were positive with TCRδ1, CD3, CD25, CD29, CD45R0 and CD54. No staining was seen with antibodies against framework determinants or variable regions on the TCR αß heterodimer. Negative reactions were also seen with CD4, CD8, CD5, CD7, CD16, CD30 and CD57. The expression of CD2, CD45RA and Leu-8 was variable.

It is concluded that rare CTCL express TCR γδ chains. These malignancies may be related to and/or originate from the TCR γδ-positive T-cells seen in normal skin, and it is possible that their recognition may be important for clinical reasons.

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IN VITRO FUNCTIONAL STUDIES ON sIL-2R RELEASE BY SEZARY CELLS. Antonella Appino, Maria T. Fierro, Mauro Novelli, Francesco Lisa, and Maria G. Bernengo, Clinica Dermatologica, Università di Torino, Italy.

Serum levels of soluble IL-2 receptor (sIL-2R) are elevated in CTCL and correlates with the clinical course. sIL-2R release and its kinetic were evaluated in the supernatants obtained from Sèzary cells (SC) and normal PBL cultured under different conditions. A small amount of sIL-2R was constitutionally released by cultured normal lymphocytes (124 ± 211 U/ml) and markedly enhanced by mitogens (PHA+IL-2: 1860 ± 1361 U/ml). Cultured SC from 6 patients with active disease (AD)(> 90% SC in culture) after PHA stimulation failed to release a normal amount of sIL-2R (119 ± 66 U/ml); the addition of IL-2 to PHA slightly increased the sIL-2R production, but mean values remained significantly lower than in normal controls (230±136 U/ml, $p < 0.02$). Normal levels were found in PR and CR patients (0-50% SC in the culture). The behaviour of serum levels was inversely proportioned to that of cellular release. CD25 expression by AD patients was inducible by PHA+IL-2 stimulation, but positive cells were lower than those of normal controls (39±16% vs. 71±16%). The behaviour of cultured cells from PR and CR patients was superimposable to that of normal lymphocytes. Our results indicate that SC, as opposed to ATL and HCL, produce only a small quantity of sIL-2R; the increased serum values may be linked with their elevated number.

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BLOOD LYMPHOCYTES FROM PATIENTS WITH MYCOSIS FUNGOIDES ARE HIGHLY ADHERENT TO CULTURED ENDOTHELIAL CELLS. C M Watson, *N P Smith, *S Whittaker, D C Dumonde and K A Brown, Departments of Immunology and *Dermatology, St Thomas' Hospital, London.

The aim of this study was to investigate if the marked epidermotropism exhibited by blood mononuclear cells (MNC) in patients with mycosis fungoides is due in part to an abnormal adhesiveness of these cells for vascular endothelium. The binding properties of lymphocytes and monocytes was assessed in a quantitative monolayer adhesion assay using human umbilical vein endothelial cells (HUVEC) that were either untreated or pretreated with the cytokine, tumour necrosis factor (TNF).

Lymphocytes from 17 patients with mycosis fungoides were significantly more adherent than control subjects' cells to untreated endothelial monolayers (mean increase of 76±31%; $p<0.02$). These patients' lymphocytes were not as responsive as normal lymphocytes to TNF-stimulated HUVEC. Control subjects' lymphocytes demonstrated a mean increase in adherence of 168±75% to HUVEC preincubated with 10 U/ml TNF compared with a mean increase in adherence of 74±56% seen with the patients' lymphocytes. In contrast, monocytes from the patients displayed a similar basal adherence as control subjects' monocytes (mean adherence of 29±10% and 22±6%, respectively) but failed to exhibit an enhanced adherence to TNF-treated HUVEC.

We suggest that circulating lymphocytes from patients with mycosis fungoides may be predisposed to adhere to endothelium and that this effect may relate to their increased infiltration of skin in this disease.