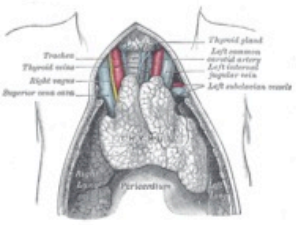
fnrs
LA LIBERTÉ DE CHERCHER

Université de Liège

GIGA

New Insights into  Physiology

BIS Annual Meeting – 8 November 2013
University Hospital of Liege
Auditorium Bacq & Florkin

vzw/asbl
Belgian Immunological Society

The 2013 Meeting of the Belgian Immunological Society has been organized with the generous help of the following sponsors:

Diamant sponsor

BIO-RAD

Gold sponsor

BD

Helping all people
live healthy lives

Bronze sponsors

affymetrix
eBioscience

filterService S.A./N.V.

analis

Westburg
Your partner of choice

ImTec
DIAGNOSTICS NV

VWR
We Enable Science

FOREWORD

First of all, I wish to express my most sincere gratitude to the keynote speakers who have immediately accepted to deliver a lecture during this 2013 Meeting of the Belgian Immunological Society (BIS) dedicated to the novel insights into thymus physiology. Three of them (T. Boehm, G. Anderson and P. Peterson) participated in the European FP6 Integrated Project Euro-Thymaide (2004-2008), and all of them are important opinion leaders that are recognized worldwilde in the field of thymus biology.

It is really fascinating to see how, in about 30 years, the thymus has gained a primary importance in the field of immunology. For centuries, this mysterious organ has searched for a definitive place in physiology. First considered to be the seat of soul and courage in the Ancient Greece according to the etymological origin of its name, this organ was then thought to be a component of the endocrine system until the demonstration of its immunological function by Jacques FAP Miller in the beginning of the '60s. Nowadays, besides its role in the generation of TCR diversity, the thymus occupies a prominent place in programming self-tolerance. Indeed, the presentation in the thymus of self-peptides by proteins of the major histocompatibility complex both promotes negative selection of self-reactive T cells issued from the lottery of TCR recombination, as well as generation of self-specific thymo-dependent regulatory T (tTreg) cells. Moreover, a defect in the thymic tolerogenic function is more and more implicated as the primary event driving the development of organ/cell-specific autoimmunity. The pharmacological 'boosting' of thymopoiesis also offers novel perspectives for reconstitution of our system of defences in various conditions of immune deficiency.

It is important to note that thymus research has a long history in Liege with the studies performed by C. Gregoire, J. Boniver, M-P. Defresne and C. Humblet. This is also the case in the EUREGIO with the seminal Thymus Rolduc Workshops previously held in an ancient monastery of Kerkrade close to Maastricht.

I am also grateful to the members of the BIS Board who accepted that the thymus would be the central topic of the 2013 BIS Annual Meeting. To the best of my knowledge, I am not sure this topic has been previously selected for a whole BIS Meeting.

I also thank the young immunologists (from Belgium, The Netherlands and even Portugal) who submitted thirty-five proposals for presentation of their most recent research data. Without their participation, this kind of meeting would not have much significance. Young colleagues, enjoy this day and do not hesitate to engage discussions with the invited speakers during the lunchtime, they are fantastic gentle woman and men!

Vincent Geenen, MD, PhD
Research Director at FSR (Belgium)
GIGA-I^3 Immunoendocrinology
University of Liege

PROGRAM OF THE DAY

9h – 9h45	Registration, morning coffee and display of posters
9h45 – 10h	Welcome address by Vincent GEENEN and Oberdan LEO (President of BIS)

MORNING SESSION – *Chairpersons: Bart Vandekerckhove (UGhent) & Vincent Geenen (ULg)*

10h	**Thomas BOEHM** *(Max Planck Inst. of Immunobiology, Freiburg, Germany)* "The thymus: 500 million years in the making"
10h30	**Graham ANDERSON** *(Babraham Institute, Cambridge, UK)* "Development and function of the thymus medulla"
11h	**Benedita ROCHA** *(INSERM U1020, Necker Research Inst., Paris, France)* "Thymocytes may persist and differentiate without any input from bone marrow progenitors"

Oral presentation of selected abstracts

11h30	Thymic microenvironmental crosstalk involved in the malignant transformation of thymocytes MH Ghezzo, MT Fernandes, PM Rodriguez, NL Alves & **NR dos Santos** (Univ. Algarve, Faro, Portugal)
	Interleukin-7 receptor a-dependent thymopoiesis regulates murine intrathymic invariant NKT cell migration **MB Drennan**, F Pattyn, K De Wilde, S Govindarajan, SM Schlenner, C Ware, S Nedospasov, HR Rodewald & D Elewaut (Ghent Univ. Hospital)
	Pro-death function of the NIK kinase in TNF-α-induced thymic involution L Boutaffala, M Bertrand, C Remouchamps, G Seleznik, C Bénézech, M Frings, A Hupalowska, S Marchetti, F Mair, J Tracy, C Ganeff JE Ricci, R Becher, J Piette, P Knolle, P Vandenabeele, J Caamano, M Heikenwalder & **E Dejardin** (ULg)

12h15	Lunch – **Poster session** – Visit of exhibitor stands
14h30	General Assembly of the Belgian Immunological Society

AFTERNOON SESSION – *Chairpersons: Dominique Bullens (KULeuven) & Michel Moutschen (ULg)*

Oral presentation of selected abtracts

14h45 Follicular regulatory T cells are impaired in patients with multiple sclerosis
T Dhaeze, B Broux, Liesbet M Peeters, B Van Wijmeersch, P Stinissen & N Heelings (Univ. Hasselt)

Human peripheral blood contains high levels of phosphoantigen-reactive Natural Killer/Th1-like semi-invariant Vγ9Vδ2 T cells
T Dimova, M Brouwer, C Donner, F Gosselin, A Marchant, O Leo & **D Vermijlen** (ULB)

Neutralizing TNF restores glucocorticoid sensitivity in a mouse model of neutrophilic airway hyperinflammation
L Dejager, K Dendoncker, J Souffriau, F Van Hauwermeiren, M Willart, T Naessens, M Eggermont, M Ballegeer, S Vandevyver, B Lambrecht, H Hammad, J Grooten & C Libert (VIB & Univ. Ghent)

15h30 **Knut PETKAU** *(Bio-Rad Flow Cytometry Specialist)*
S3 – Simplified and affordable cell sorting for researchers

15h45 **Pärt PETERSON** *(Inst. Molecular Pathology, University of Tartu, Estonia)*
"Anti-cytokine autoantibodies in AIRE deficiency"

16h15 **Adrian LISTON** *(Genetics of Autoimmunity, VIB-KULeuven, Belgium)*
"Molecular control over thymus involution"

16h45 Awarding of the two best posters

17h00 Closure of the meeting

Keynote Lectures

&

Biographical Sketches

The thymus: 500 million years in the making

Thomas BOEHM

Max-Planck Institute of Immunobiology, Freiburg, Germany

The evolutionary emergence of vertebrates was accompanied by the invention of adaptive immunity. This is characterized by extraordinarily diverse repertoires of somatically assembled antigen receptors and the facility of antigen-specific memory, leading to more rapid and efficient secondary immune responses. Adaptive immunity emerged twice during early vertebrate evolution, once in the lineage leading to jawless fishes (such as lamprey and hagfish) and, independently, in the lineage leading to jawed vertebrates (comprising the overwhelming majority of extant vertebrates, from cartilaginous fishes to mammals). Recent findings on the immune systems of jawless and jawed fishes impact on the identification of general principles governing the structure and function of adaptive immunity. Here, we discuss aspects of cellular immunity of vertebrates with special reference to the thymus and the principal lineages of T cells.

Thomas Boehm received his M.D. from the University of Frankfurt Medical School, Germany, and trained in paediatrics and biological chemistry. He then moved to the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK. Subsequently, he held professorships at the University of Freiburg, Germany, and the German Cancer Centre in Heidelberg, Germany, before taking up his current position at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg. He works on T cell and thymus development in vertebrates and the evolution of adaptive immune systems.

Development and function of the thymus medulla

Graham ANDERSON

MRC Centre for Immune Regulation, University of Birmingham, United Kingdom

The thymic medulla represents a specialized microenvironment that contains $CD4^+$ and $CD8^+$ thymocytes generated as a result of positive selection in the thymic cortex. While it is clear that the medulla acts as a repository for newly generated thymocytes, the role it plays in late stage thymocyte differentiation, as well as the mechanisms controlling its own development, remain unclear. Medullary thymic epithelial cells (mTEC) are thought to represent an important cellular source of signals that contribute to the development of a self-tolerant T-cell population. Notably, mTEC defined by expression of the AutoImmune REgulator (Aire) gene, arise during embryonic development at stages prior to abT-cell selection, indicating that the mechanisms controlling initial medulla formation to impose tolerance upon the nascent T-cell repertoire do not involve signals from conventional abT-cells. We recently showed that invariant $V\gamma5^+\gamma\delta TCR^+$ thymocytes, the precursors of Dendritic Epidermal T-cells, are involved in initial embryonic thymus medulla formation through their expression of RANKL to drive RANK-mediated mTEC development. In turn, interactions with mTEC expressing Skint-1 control Vγ5 T-cell development, highlighting the reciprocal links between invariant gdT-cells and the thymus medulla. We now show that the involvement of invariant T-cells in mTEC development extends to CD1d-dependent iNKT-cells, which also provide RANKL to induce $Aire^+$ mTEC development in the adult. Further, mTEC control iNKT-cell development, drawing parallels to links between gdT-cells and mTEC. Thus invariant subsets of both the αβ and γδ T-cell lineages are linked to the thymus medulla. Finally, by analyzing the requirement for mTEC in the context of thymocyte differentiation, we show that while maturation of conventional $CD4^+$ thymocytes can occur in the absence of an mTEC compartment, $Foxp3^+$ Regulatory T-cell (T-Reg) development is mTEC dependent, with mTEC playing an essential role in controlling the generation of $Foxp3^-CD25^+$ T-Reg precursors. Overall, our data reveal interplay between the innate immune system and mTEC development, and identify a differential requirement for mTEC in the control of conventional versus T-Reg development.

Graham Anderson is Professor of T-Lymphocyte Biology and a Theme Lead in the MRC Centre for Immune Regulation. His research focuses on T-cell development and thymus development and function, as well as the regulation of T-cell responses in lymphoid tissues. He gained a BSc (Hons) in Anatomical Studies from the University of Birmingham in 1990, and then studied for a PhD in Immunology as a Wellcome Prize PhD student with John Owen and Eric Jenkinson. He is currently holder of an MRC Programme Grant as Principal Investigator.

Thymocytes may persist and differentiate without any input from bone marrow progenitors

Laetitia Peaudecerf[1], Sara Lemos[1], Alessia Galgano[1], Gerald Krenn[1], Florence Vasseur[1], James P. Di Santo[2], Sophie Ezine[1] and Benedita ROCHA[1]

[1]*INSERM, Unit 1020, Faculty of Medicine Descartes Paris V, Paris, France*
[2]*Innate Immunity Unit, Pasteur Institute, Paris, France*

Thymus transplants can correct deficiencies of the thymus epithelium caused by the complete DiGeorge syndrome or FOXN1 mutations. However, thymus transplants were never used to correct T cell-intrinsic deficiencies, since it is generally believed that thymocytes have short intrinsic lifespans. This notion is based on thymus transplantation experiments, where it was shown that thymus resident cells were rapidly replaced by progenitors originating in the bone marrow. In contrast, here we show that neonatal thymi transplanted into interleukin-7 receptor deficient hosts harbour populations with extensive capacity to self-renew, and maintain continuous thymocyte generation and export. These thymus transplants reconstitute the full diversity of peripheral T cell repertoires one month after surgery, the earliest time point studied. Moreover, transplantation experiments performed across major histocompatibility barriers show that allogeneic transplanted thymi are not rejected, and allogeneic cells do not induce graft-versus-host disease; transplants induced partial or total protection to infection. These results challenge the current dogma that thymocytes cannot self-renew, and indicate a potential use of neonatal thymus transplants to correct T cell- intrinsic deficiencies. Finally, as found with mature T cells, they show that thymocyte survival is determined by the competition between incoming progenitors and resident cells.

Anti-cytokine autoantibodies in AIRE deficiency

Pärt PETERSON, Jaanika Kärner and Kai Kisand

Molecular Pathology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) is a monogenic autoimmune disease caused by mutations in the Autoimmune Regulator (AIRE). AIRE is a transcriptional regulator expressed in thymic epithelial cells where it promotes the expression of tissue-specific antigen genes. APECED patients commonly have chronic mucocutaneous candidiasis, hypoparathyroidism, Addison's disease and multiple other autoimmune disorders including type 1 diabetes. The autoantibodies in patient sera recognize many organ-specific targets. In particular, they are reactive to type I IFNs; IFNalpha and IFNomega and to Th17 cell-associated cytokines; IL-17 and IL-22. These autoantibodies are neutralizing, occur in high titers, are disease-specific and, in addition to APECED, have been only reported in patients with thymomas. We found that the immunoglobulins responsible for neutralizing type I IFNs belong to IgG with dominant subtypes of IgG1 and IgG4, the latter possibly having immune regulatory rather than inflammatory function. We also found neutralizing autoantibodies to IL-17 in aged Aire-deficient mice, which is the first antigen to which reactivity is seen in both human and mouse AIRE-deficiency states. We conclude that autoimmunization against IFNs and Th17 cytokines in AIRE-deficiency is closely related to the disease initiation.

Pärt Peterson is Professor of Molecular Immunology at the University of Tartu, Estonia. He graduated in molecular biology at the University of Tartu and defended his PhD on molecular immunology in 1996 at the University of Tampere, Finland. He spent his postdoctoral period in the Institute of Medical Technology where his research interest focused on the Autoimmune Regulator
(AIRE) gene, the key factor in central thymic tolerance. He also co-discovered DNA methyltransferase 3-like (DNMT3L) gene, a regulator of DNA methylation and epigenetic imprinting He obtained the degree of Docent at the University of Tampere and in 2000 became a Fellow of the Finnish Academy, after which he has continued his research on AIRE and APECED syndrome. In 2003-2009 he was supported by The Wellcome Trust as International Senior Fellow and started research group as Professor of Molecular Pathology at the University of Tartu, and later on in 2008, as Professor of Molecular Immunology. In 2009 he was elected as the Research Professor of the Academy of Estonia. Pärt Peterson is the recipient of the Estonian Science Prize (2008), and has more than 100 research publications on various aspects of immunology and molecular biology.

Keeping the thymus together – the role of microRNA and epithelial structure in preventing thymic involution

Adrian LISTON

VIB and KULeuven, Genetics of Autoimmunity, Leuven, Belgium

The thymus is the organ devoted to T cell differentiation, maturation and export to the periphery. Unique among organs, the thymus undergoes multiple rounds of natural atrophy and redevelopment. Atrophy of the thymus, also known as involution, is temporally induced by stress stimuli, ranging from infection to pregnancy, and chronically induced during healthy aging. The genetic and structural control over age-related thymic involution is poorly understood, despite the influence the phenomenon has on the peripheral T cell pool. In this talk, the mechanisms by which the thymus maintains integrity despite all of these potential triggers will be discussed. Such mechanisms include the modulation of involution-inducing signals by microRNA and the development of a tight three-dimensional network of epithelial and endothelial cells to maintain a consistent feed of precursor thymocytes.

Publications

Aikaterini S. Papadopoulou#, James Dooley#, Michelle A. Linterman, Wim Pierson, Olga Ucar, Bruno Kyewski, Saulius Zuklys, Georg A. Hollander, Patrick Matthys, Daniel H. Gray, Bart De Strooper and Adrian Liston. #Equal first authors. 'The thymic epithelial microRNA network elevates the threshold for infection-associated thymic involution via miR-29a mediated suppression of the IFN-α receptor.' 2012. *Nature Immunology*. 13 p181.

Olga Ucar, Lars-Oliver Tykocinski, James Dooley, Adrian Liston and Bruno Kyewski. 'An evolutionarily conserved mutual interdependence between Aire and microRNAs in promiscuous gene expression'. 2013. *European Journal of Immunology*. 43(7) p1769.

Saulius Zuklys, Carlos E. Mayer, Saule Zhanybekova, Heather Stefanski, Gretel Nusspaumer, Jason Gill, Thomas Barthlott, Stephan Chappaz, Takeshi Nitta, James Dooley, Ruben Nogales-Cadenas, Yousuke Takahama, Daniela Finke, Adrian Liston, Bruce R. Blazar, Alberto Pascual-Montano and Georg A. Holländer. 'MiRNAs control the maintenance of thymic epithelia and their competence for T lineage commitment and thymocyte selection'. 2012. *Journal of Immunology.* 189(8) p3894.

James Dooley and Adrian Liston. 'Molecular control over thymic involution: from cytokines and microRNA to aging and adipose tissue' 2012. *European Journal of Immunology*. 42(5) p1073.

Adrian Liston trained in immunology and genetics at the Australian National University in Canberra and the University of Washington in Seattle. In 2009 he was recruited to start the Autoimmune Genetics Laboratory at the VIB and University of Leuven. The laboratory has several major lines of research including the thymus (Papadopoulou et al, Nature Immunology 2012), regulatory T cells (Pierson et al, Nature Immunology 2013) and peripheral tolerance (Linterman et al, Nature Medicine 2011). He is an ERC grant holder and the laboratory is supported by the JDRF and FWO.

Oral Presentations of Selected Abstracts

Thymic microenvironmental crosstalk involved in the malignant transformation of thymocytes

Marinella N. Ghezzo,[1] Mónica T. Fernandes,[1] Pedro M. Rodrigues,[2] Nuno L. Alves,[2] &
Nuno R. dos Santos[1]

[1]IBB-Institute for Biotechnology and Bioengineering, CBME-Centre for Molecular and Structural Biomedicine, University of Algarve, 8005-139 Faro, Portugal; [2]Infection and Immunity Unit, CAGE Laboratory, Institute for Molecular and Cellular Biology, University of Porto, 4150-180 Porto, Portugal;

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive T-cell precursor malignancy, occurring more frequently in children, and fatal in the absence of chemotherapy. Expression profiling, surface marker analyses, and T-cell receptor gene rearrangement analyses have shown their relatedness to thymocytes of different developmental stages. Despite the extended knowledge about the microenvironmental factors involved in thymocyte maturation, little is known about the microenvironmental requirements for T-ALL initiation and maintenance. Similar to immature thymocytes, T-ALL cells have been shown to depend on interleukin-7, Notch ligands and chemokines for their progression. To gain insights into the role of the thymic microenvironment in T-ALL, we have set out to characterize the stromal cell alterations in thymic lymphomas from the TEL-JAK2 transgenic mouse model of T-ALL. To this end, immunofluorescence on tissue sections was performed with antibodies against markers for thymic stromal cells (thymic epithelial cells, fibroblasts, endothelial cells, and macrophages). In contrast to wild-type thymi, which show a clear demarcation between keratin 5-positive medulla and keratin 8-positive cortex, thymic lymphomas presented areas of both keratin 5 and 8 positivity and areas without any or little keratin staining. Gene expression qPCR analysis of thymic lymphomas confirmed the reduced expression of keratin 8 and 5 genes, as compared to wild-type thymi. Enzymatic dissociation of thymic lymphomas followed by cell surface marker staining of isolated cells, and flow cytometry confirmed the alterations in the thymic epithelium, revealing a proportional decrease in cells expressing the BP1 cortical marker and an increased expression of Aire, CD80 and UEA1 medullary markers. Keratin-negative areas were stained conspicuously with CD45, indicating the presence of malignant thymocytes, and were stained abundantly with ER-TR7 mAb, which stains fibroblasts. Furthermore, staining of the endothelial cell marker CD31/PECAM was abundant in thymic lymphomas, indicative of extensive vascularization. Despite the absence of notable thymic epithelial cellular outgrowth in thymic lymphomas, we addressed the question whether thymic epithelial cells (TEC) could influence thymocyte malignant transformation. To this end, TEL-JAK2 transgenic mice were bred with *Foxn1* nude mutation carriers, which present TEC defects in a dose-dependent manner. *Foxn1* mutant heterozygotes are known to display very mild thymic defects, yet TEL-JAK2;*Foxn1*$^{+/nude}$ mice developed T-cell leukemia with a statistically significant longer latency, as compared to littermate transgenic mice carrying two *Foxn1* alleles. Endpoint tumor load was however not significantly different between the two cohorts, which may indicate that a mild thymic stromal defect delayed disease initiation but not its progression. These results indicate that as yet unidentified FoxN1-dependent thymic epithelial proteins aid thymocytes to become malignantly transformed by the TEL-JAK2 fusion oncoprotein.

Interleukin-7 receptor α-dependent thymopoiesis regulates murine intrathymic invariant natural killer T cell migration

Michael B. Drennan*[1], Filip Pattyn†, Katelijne De Wilde*, Srinath Govindarajan*, Susan M. Schlenner‡, Carl Ware§, Sergei Nedospasov¶, Hans-Reimer Rodewald & Dirk Elewaut*

** Laboratory for Molecular Immunology and Inflammation, Department of Rheumatology, Ghent University Hospital, Ghent, B-9000, Belgium.*
† Center for Medical Genetics, Ghent University Hospital IK5, Ghent, B-9000, Belgium.
‡ Department of Microbiology and Immunology, University of Leuven, Leuven 3000, Belgium.
§ Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, California 92037.
¶ Laboratory of Molecular Immunology , Engelhardt Institute of Molecular Biology, Lomonosov Moscow State University, Russian Academy of Sciences, 32 Vavilov street, Moscow 119991, Russia.
||Department for Cellular Immunology, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany.

[1]Corresponding author: michael.drennan@ugent.be

Invariant natural killer T cells (iNKT) are one of the few examples of an innate T cell lineage that reside in the thymus following differentiation yet the mechanisms governing intrathymic iNKT cell migration remain poorly understood. Here we show that the intrathymic migration of iNKT cells is developmentally regulated and can be defined by expression of the G-protein-coupled transmembrane receptors Cx3CR1 and CxCR3, in contrast to that seen within the conventional CD4 T cell lineage. Furthermore we show that the intrathymic migration of iNKT cells is regulated in a non cell-autonomous manner by the lymphoid cytokine $LT\alpha_1\beta_2$. Surprisingly, $LT\alpha_1\beta_2$ expression within mature cellular lineages in the thymus served no role in regulating intrathymic iNKT cell migration but was dependent upon IL-7Rα-dependent fetal thymopoiesis in the developing embryo. We therefore conclude that IL-7Rα-positive precursors to the T lymphocyte lineage regulate the intrathymic migration of iNKT cells in the postnatal thymus.

Pro-death function of the NIK kinase in TNF-alpha-induced thymic involution

Layla Boutaffala [1], Mathieu Bertrand [2], Caroline Remouchamps [1], Gitta Seleznik [3], Cécile Bénézech [4], Marianne Frings [5], Anna Hupalowska[1], Sandrine Marchetti [6], Florian Mair [7], Jay Tracy [3], Corinne Ganeff [1], Jean-Ehrland Ricci [6], Buckhard Becher [7], Jacques Piette [8], Percy Knolle [5], Peter Vandenabeele [2], Jorge Caamano [4], Mathias Heikenwalder [9] & **Emmanuel Dejardin** [1]

[1]Laboratory of Molecular Immunology and Signal Transduction, GIGA-Research, University of Liège, 4000 Liège, Belgium, [2]Department for Molecular Biomedical Research, VIB, 9052 Gent, Belgium, [3]Institute of Neuropathology, University Hospital Zürich, 8091 Zürich, Switzerland, [4]School of Immunity and Infection, IBR-MRC, Centre for Immune Regulation, University of Birmingham, Birmingham B15 2TT, UK, [5]Institute of Molecular Medecine and Experimental Immunology, University of Bonn, Germany, [6]INSERM U1065, Centre Méditéranéen de Médecine Moléculaire, 06204 Nice, France, [7]Institute of Experimental Immunology, University of Zurich, Switzerland, [8]Laboratory of Virology, GIGA-Research, University of Liège, 4000 Liège, Belgium, [9]Institute of Virology, Technische Universität München (TUM)/ Helmholtz Zentrum München, D-81675 München, Germany

The thymus is composed of two main stromal compartments, the cortex and the medulla, which are filled with specialized thymic epithelial cells called cTEC and mTEC, respectively. The differentiation of mTEC requires a subset of TNFR family members like RANK, CD40 or LTbR known to activate the so-called alternative, or non-canonical NF-κB pathway. This pathway triggers the activation of two kinases that are NIK (NF-κB-Inducing Kinase) and IKKα that are required for the conversion of a NF-κB precursor complex p100/RelB into a transcriptionally active p52/RelB dimer. Yet, the role of the TNFR family members and the alternative NF-κB pathway in cTEC homeostasis is still unclear. We found a new role for NIK in cell death of cTEC and thymic involution. Indeed, using a mouse model of T cell mediated acute inflammation, we observed that NIK displays a pro-death function downstream of LTβR and TNFR1 in the cortical compartment. More importantly, we demonstrated that the pro-death activity of NIK was independent of the alternative NF-κB pathway, as opposed to its role in the medullary compartment. Mechanistically, we found that NIK phosphorylated RIP1 and stabilized the death complex RIP1/FADD/Caspase-8.

Therefore, NIK not only contributes to the development of the immune system and to self-tolerance, but also to TNFR1-mediated thymic involution but independently of the NF-κB alternative pathway.

Follicular regulatory T cells are impaired in patients with multiple sclerosis

Dhaeze Tess [1], Broux Bieke [1], Peeters M Liesbet [1], Van Wijmeersch Bart [1,2,3], Stinissen Piet [1], & Hellings Niels [1]

[1] *Hasselt University, Biomedical Research Institute and transnationale Universiteit Limburg, School of Life Sciences, Agoralaan, Building C, 3590 Diepenbeek, Belgium*

[2] *REVAL Rehabilitation Research Center, Dpt. Healthcare, PHL University College, Agoralaan, Building A, 3590 Diepenbeek, Belgium*

[3] *Revalidatie & MS-Centrum, Boemerangstraat 2, 3900 Overpelt, Belgium*

Background + objective

Both autoreactive T cells and autoantibodies are identified as key players in the immunopathogenesis of multiple sclerosis (MS). These overt autoimmune responses may results from disturbances in regulatory T cells ($CD4^+CD25^+FoxP3^+$ T cells) important for the maintenance of peripheral tolerance. Recently a new subset of regulatory T cells was discovered in mice. These follicular regulatory T cells (T_{FR}, $CD4^+CXCR5^{hi}PD\text{-}1^{hi}FoxP3^+$) participate in germinal responses by controlling the maturation of B cells and the production of (auto)antibodies. First, the frequency, phenotype and function of T_{FR} in lymphoid tissue (tonsils) is compared to those in peripheral blood (PB), as PB samples are more accessible and are used for further studies. Second, the functionality of T_{FR} in MS patients is investigated, comparing frequency, phenotype and function to those of healthy controls (HC).

Methods

Mononuclear cells are isolated from paired PB and tonsil samples of HC and of PB of MS patients. Next, the frequency and phenotype of T_{FR} ($CD4^+CD25^{hi}CD127^{lo}CXCR5^+PD\text{-}1^+$) was investigated using multicolor flow cytometry. To determine the suppressive activity, T_{FR} cells are purified from PB using high-speed cell sorting using the previously mentioned markers and cocultured with CFSE-labelled responder T cells ($CD4^+CD25^-CD127^+$).

Results

No significant difference was found between phenotype and suppressive activity of T_{FR} isolated from tonsils and PB (n=6 HC), indicating that T_{FR} measurements in blood are justified. A significant difference (p=0.0011) could be found in the percentage of T_{FR} between untreated MS patients (0.1151 ± 0.008784 % of $CD4^+$, n=96) compared to HC (0.1658 ± 0.01235 % of $CD4^+$, n=47). Furthermore, we have shown that T_{FR} from MS patients (n=13) show a reduced *in vitro* suppressive function compared to HC (n=13, 1:1 ratio p=0.0036).

Conclusion

We have shown that T_{FR} from PB have identical phenotypic and functional characteristics compared to T_{FR} from tonsils, indicating that T_{FR} population from PB reflect the T_{FR} in secondary lymphoid organs. Furthermore, we have shown that the frequency and *in vitro* suppressive function of PB derived T_{FR} from MS patients is reduced when compared to HC. These results suggest that impairment in T_{FR} could contribute to overt autoimmune reactivity in MS disease.

Human fetal peripheral blood contains high levels of phosphoantigen-reactive Natural Killer/Th1-like semi-invariant Vγ9Vδ2 T cells

Tanya Dimova[1], Margreet Brouwer[1], Catherine Donner[2], Françoise Gosselin[2], Arnaud Marchant[1], Oberdan Leo[1] & **David Vermijlen**[1,3]

[1] *Institute for Medical Immunology, Université Libre de Bruxelles, 6041 Gosselies, Belgium.*
[2] *Department of Obstetrics and Gynecology, Hôpital Erasme, 1070 Brussels, Belgium*
[3] *Department of Bio-pharmacy, Faculty of Pharmacy, Université Libre de Bruxelles, 1050 Bruxelles, Belgium*

Correspondence: dvermijl@ub.ac.be

Human γδ T cells expressing the T cell receptor (TCR) heterodimer of γ-chain variable region 9 and δ-chain variable region 2 (Vγ9Vδ2 T cells) are activated by microbe- and host-derived phosphorylated prenyl metabolites ('phosphoantigens'), of which the most active are microbial (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) and host isopentenyl pyrophosphate (IPP). Thus using their TCR as a pattern recognition receptor for phosphoantigens presented by the ubiquitously expressed butyrophilin BTN3A1, Vγ9Vδ2 T cells are able to develop potent antimicrobial immune responses or promote the killing of transformed host cells that upregulate IPP production. Vγ9Vδ2 T cells represent the main population of γδ T cells in adult human peripheral blood: about 50-90% of γδ T cells in the circulation express this combination of Vγ and Vδ chains. The high percentage in adult blood and their restricted TCR repertoire are thought to be due to interaction with pathogens producing phosphoantigens such as HMB-PP after birth. Cord blood γδ T cells only contain a small subpopulation of Vγ9Vδ2 T cells (10-20%) and show polyclonal TCR repertoires. In addition, the human fetal immune system has been described as immature, Th2-biased or tolerogenic. Here we show that, unexpectedly, human fetal peripheral blood at mid-gestation without infection contained an adult-like percentage of Vγ9Vδ2 T cells (60-90% of total γδ). They expressed a highly restricted TCR repertoire containing an invariant/public CDR3γ9 chain which was phosphoantigen-reactive. Furthermore, these fetal Vγ9Vδ2 T cells showed a pre-programmed Natural Killer (NK)/Th1-like phenotype. Our results show that the fetal blood T cell lineage contains a subset of pre-programmed NK/Th1-like T cells possessing a phosphoantigen-reactive semi-invariant TCR, indicate similarities with early waves of γδ T cell production in other species and provide fundamental insight into the ontogeny of human blood Vγ9Vδ2 T cells.

Neutralizing TNF restores glucocorticoid sensitivity in a mouse model of neutrophilic airway hyperinflammation

Dejager L.[1,2], Dendoncker K. [1,2], Souffriau J. [1,2], Van Hauwermeiren F. [1,2], Willart M.[1,2], Naessens T.[2], Eggermont M.[1,2], Ballegeer M. [1,2], Vandevyver S. [1,2], Lambrecht, B. [1,3], Hammad, H. [1,3], Grooten J. [2] & Libert C[1,2].

[1] *Inflammation Research Center, VIB, Ghent, Belgium*
[2] *Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium*
[3] *Department of Pulmonary Medicine, Ghent University, Ghent, Belgium*

Asthma is a heterogeneous disorder characterized by airway hyperresponsiveness and inflammation. Most patients are effectively treated with glucocorticoids (GCs), but some are refractory to the beneficial effects of GCs. Tumor necrosis factor (TNF) is involved in asthma pathology and therefore, anti-TNF based therapies have been designed. However, the effects on steroid resistance were never assessed, as these studies defined their study population by the severity of disease and not by the degree of reversibility after steroid administration.

We aimed to investigate the role of TNF on the responsiveness to GCs, by using soluble fusion proteins which neutralize TNF effects.

We used two different OVA-based mouse models of airway hyperinflammation. The first is GC sensitive and eosinophil-driven, whereas the second represents GC-insensitive, neutrophil-predominant asthma subphenotypes.

By testing different parameters, such as bronchial hyperreactivity, immune cell influx, cytokine synthesis and mucus secretion, we showed that TNF blockade restores the beneficial effects of GCs in the GC-insensitive model. Additionally, we showed that oxidative stress plays a crucial role in the GC insensitivity. Next, an adoptive transfer study indicated that the TNF-induced GC insensitivity mainly occurs in the non-myeloid compartment. Interestingly, TNF reduces the transrepression actions of GR at the level of TSLP (thymic stromal lymphopoietin).

We demonstrate that TNF reduces the responsiveness to GCs in a mouse model of neutrophilic airway hyperinflammation, resulting in reduced GR-mediated repression of TSLP in epithelial cells. Thus, blockade of TNF may offer new strategies for therapeutic intervention in GC-insensitive asthma.

Keywords: asthma, glucocorticoid insensitivity, TNF

Poster Presentations

(listed by alphabetical order)

Expansion of CD16^{+} CD56^{+} NK cells in vericyte® NK cell growth medium

L. Brohée[1], R. Bastin[1], S. Wingert[2], P. Netter[2], C. Watzl[2], P. Delvenne[1] & N. Jacobs[1]

[1]*GIGA-I3, Experimental Pathology-Virology, University of Liege, Belgium*

[2]*Leibniz Research Center for Working Environment and Human Factors, IfADo, Dortmund, Germany*

Natural Killer (NK) cells play a key role in host resistance to virus and tumour. These cells are potent killers of virus infected and tumour cells via a direct recognition of the target by activation receptor such as NKG2D or by inducing Fcγ receptor (FcγRIII, CD16) mediated antibody dependent cellular cytotoxicity (ADCC). Current NK cell-based cancer immunotherapy aims to produce large amounts of functional NK cells, unfortunately most culture media used for NK cell expansion induced the down-regulation of CD16 on NK cells. Here, we tested the impact of a new NK cell growth medium (Vericyte® from Medicyte) on CD16 expression. Sorted NK cells and peripheral blood mononuclear cells (PBMC) were cultivated in Vericyte® NK cell growth medium and cells issued from these cultures were characterized in term of expansion and phenotype at several time points. After 5 days of culture, an expansion of both NK cells and PBMC was observed and maintained at least until day 20 of culture. In PBMC cultures, we observe only a small preferential NK cell growth since NK cells were around 5-10% at beginning of the culture and this percentage increased to 15% at the end of the culture. However, these cells showed a high proliferative potential when we started the culture with sorted NK cells (the proportion of contaminant cells remain low, under 5%). NK cells expressed CD56 and NKp46 and interestingly after a decreased expression of CD16 on the cell surface at day 3, this receptor was up regulated and most of the cells are CD56^{bright} CD16^{bright} from day 7 to day 12. According FACS FCS/SSC dot plot, NK cells acquired morphology of large activated lymphocytes and some of them expressed activation markers such CD25. Finally, these cells were able to kill efficiently tumour cell line K562. Thus our data show that Vericyte® NK cell growth medium allows the expansion of functional CD16^{+} CD56^{+} NK cells. Cytokine production and ADCC function are under investigation.

***Trypanosoma brucei brucei* induced acute inflammation is principally mediated by IFNγ**

Jennifer Cnops, Carl Detrez & Stefan Magez

VIB Department of Structural Biology, Laboratory of Cellulat and Molecular Immunology, VUB, Brussels, Belgium

In murine *Trypanosoma brucei brucei* infection the onset of inflammation occurs rapidly during the first days post infection, as witnessed by hepatosplenomegaly and a burst of pro-inflammatory cytokine levels in the serum. IFNγ is one of the major cytokines driving this acute inflammatory reaction. Using IFNγ reporter mice we show that the liver is the main organ responsible for IFNγ production. Hepatic NK cells react within 24 hours and are the main producers of IFNγ during the first 3 to 5 days post infection (pi). Subsequently CD8 T cells are activated and take over IFNγ secretion. After about 8 days pi, CD4 T cells seem to become the main source of IFNγ. IFNγ is partly responsible for 2 major pathologic events occurring during trypanosomiasis infection. Firstly, IFNγ- and IFNγR-deficient mice display diminished acute anemia compared to WT mice, in which red blood cell serum levels drop to 50% at day 6 pi. While $CD4^{-/-}$ and $CD1d^{-/-}$ mice exhibit a similar WT phenotype, $CD8^{-/-}$ mice exhibit a phenotype which is similar to $IFN\gamma^{-/-}$ and $IFN\gamma R^{-/-}$ mice, implicating CD8 T cells in IFNγ production. Secondly, IFNγ seems to play a role in the characteristic B cell depletion occurring during murine trypanosomiasis infection, as $IFN\gamma^{-/-}$ and $IFN\gamma R^{-/-}$ mice exhibit less depletion and less apoptosis in splenic B cell subsets. Therefore we hypothesize IFNγ-driven inflammation to be a cause of B cell depletion during murine trypanosomiasis infection.

Primary human cytomegalovirus infection induces the expansion of virus-specific activated and atypical memory B cells

Nicolas Dauby[*], Sandra Lecomte[*], Corinne Liesnard [†], Catherine Donner [‡] & Arnaud Marchant[*]

**) Institute for Medical Immunology (IMI), Université Libre de Bruxelles (ULB), Rue Adrienne Bolland, B-6041 Gosselies, Belgium*

†) Departments of Virology and ‡) Obstetrics and Gynecology, Hôpital Erasme, Université Libre de Bruxelles (ULB), Route de Lennik 808, B-1070 Brussels, Belgium

Corresponding author: ndauby@ulb.ac.be

Neutralizing antibodies play a central role in the control of cytomegalovirus (CMV) dissemination. However, little is known about the quality of the response of B lymphocytes to primary CMV infection. In this study, we show that primary CMV infection induces a sustained expansion of activated ($CD27^+CD20^+CD21^{low}$) and atypical ($CD27^-CD20^+CD21^{low}$) memory B cells (MBC), two subsets previously associated with chronic exposure to high antigen loads. Both activated and atypical MBC expressed an effector phenotype. The highest levels of activation markers were expressed by activated MBC whereas atypical MBC expressed high levels of inhibitory receptors, suggesting functional exhaustion. Fluorescent antigen labeling indicated that activated and atypical MBC were enriched in CMV-specific cells. We conclude that primary CMV infection mobilizes a large pool of memory B cells that includes activated and atypical MBC. The functional regulation of CMV-specific MBC may limit the production of antibodies and the control of viral dissemination.

Interleukin-6 inhibits the differentiation of Th2 responses *in vivo* and *in vitro*

D. Debuisson, A. Mayer, S. Denanglaire, O. Leo & F. Andris

Laboratoire d'Immunobiologie, Université Libre de Bruxelles, Belgium

Interleukin (IL)-6 plays an important role in the differentiation of several T helper (Th) cell types. IL-6 promotes the differentiation of naive T cells into follicular helper T (Tfh) and Th17 lineages, and inhibits the generation of Th1 and regulatory T (Treg) cells. Contradictory effects of IL-6 on Th2 cell differentiation have been reported in the literature. IL-6 has been demonstrated to induce Th2 differentiation *in vitro* through the activation of c-Maf and NFATc2. On the opposite, IL-6-KO mice developed exacerbated asthma, suggesting a negative role of this cytokine on Th2 development.

The objective of this project is to study the role of APC-derived IL-6 on Th2 differentiation.

We observed that inoculation of antigen-pulsed IL-$6^{-/-}$ bone marrow derived dendritic cells (BMDCs) strongly promoted Th2 responses *in vivo* and exacerbated recruitment of eosinophils in an experimental asthma model, suggesting that IL-6 production at the time of T cells priming might contribute to dampening Th2 inflammation responses.

To further investigate the molecular mechanisms by which IL-6 mediates its inhibitory effect, we developed an *in vitro* model of Th2 polarization in the presence and absence of IL-6. Our data suggest that although IL-6 does not affect early IL-4 production *in vitro*, it strongly decreases IL-4 secretion at later times in Th2-polarized cells. This is correlated with a progressive decline in GATA-3 expression in Th2 cells upon IL-6 treatment.

Experiments are in progress to decipher the mechanism by which IL-6 downregulates GATA-3/IL-4 expression in CD4 T cells.

Collectively, these findings suggest a novel regulatory pathway limiting the development of an exacerbated Th2-driven inflammatory response by IL-6.

Regulatory T cells control Th1 priming by inhibiting the CD70/CD27 pathway

Dhainaut M.[1], Coquerelle C.[1], Uzureau S.[1], Acolty V.[1], Oldenhove G.[1], Galuppo A.[1,2], Pays E.[1], Borst J.[3] & Moser M.[1]

[1]*Department of Molecular Biology, Université Libre de Bruxelles, Gosselies, Belgium*

[2]*Center for Microscopy and Molecular Imaging, Gosselies, Belgium*

[3]*Department of Immunology, Netherlands Cancer Institute, Amsterdam,The Netherlands*

Although naturally occurring Tregs are essential to prevent auto-immune disorders, the molecular basis of their suppressive function is still ill defined. Based on several observations showing that Tregs selectively control the development of Th1-type responses, we sought to determine the effect of suppression on the Th1-prone interleukin-12 and CD70 costimulatory molecule.

Our data show both *in vivo* and *in vitro* that Tregs inhibited the CD27/CD70 pathway, while sparing IL-12 production. Treg depletion *in vivo* resulted in increased CD70 expression on dendritic cells (DCs) and enhanced IFNγ production that was prevented by injection of neutralizing mAb to CD70. *In vitro,* Tregs inhibited CD70 expression on activated splenic DCs in a CD27-dependent manner. Surprisingly, CD70 down-regulation correlated with intercellular transfer of intact CD27 from Tregs to DCs. Indeed, when cultured with retrovirally transduced T cells expressing a (intracellular) GFP- and (extracellular) HA-tagged CD27, DCs acquired both GFP and HA. The analysis of the CD27 and CD70 localisation by confocal microscopy using retrovirally transduced BMDCs (mCherry-CD70) and T cells (CD27-GFP) revealed the formation of tunneling nanotubes that spanned from DCs to T cells and supported vesicular traffic of CD27 to DCs in a CD70-dependent process. Whether both molecules are internalized and degraded in DCs remains to be determined.

Our data thus suggest a novel mechanism, in addition to CTLA-4 inhibition of CD80/CD86 costimulation, by which $CD27^{+}$ Tregs might tune DC function by regulating the surface expression of CD70.

Lymphotoxin-β Receptor Signaling in Microenvironmental Cells Supports T-Cell Leukemogenesis

Mónica T. Fernandes,[1,2] Marinella N. Ghezzo,[1,2] , Vanda Póvoa,[3] Ana R. Ribeiro,[4,5] Nuno L. Alves,[4] João T. Barata[3] & Nuno R. dos Santos[1]

[1]*IBB-Institute for Biotechnology and Bioengineering, CBME-Centre for Molecular and Structural Biomedicine, University of Algarve, 8005-139 Faro, Portugal;*

[2]*PhD Program in Biomedical Sciences, Department of Biomedical Sciences and Medicine, University of Algarve, 8005-139 Faro, Portugal;*

[3]*Cancer Biology Unit , Instituto de Medicina Molecular, Lisbon University Medical School, 1649-028 Lisbon, Portugal;*

[4]*Infection and Immunity Unit, CAGE Laboratory, Institute for Molecular and Cellular Biology, University of Porto, 4150-180 Porto, Portugal;*

[5]*Doctoral Program in Biomedical Sciences, Institute for Biomedical Sciences Abel Salazar, University of Porto, 4050-313 Porto, Portugal.*

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematopoietic malignancy which arises from the combination of genetic and epigenetic alterations in thymic T-cell precursors and extracellular signals provided by the microenvironment. It was previously found that RelB expression in non-leukemic, non-hematopoietic stromal cells promoted T-cell leukemogenesis in the EμSRalpha-TEL-JAK2 transgenic (TJ2-Tg) mouse model. Since the RelB transcription factor is activated in thymic stromal cells by cell surface receptors of the TNFR superfamily, we hypothesized that an evolving crosstalk between leukemic thymocytes expressing $LT\alpha_1\beta_2$ and/or LIGHT and thymic stromal cells expressing their cognate receptor (LTβR) takes place and favors leukemogenesis. In this line, we have found that the gene encoding LTβR was expressed in thymic lymphomas and the LTα, LTβ, and LIGHT-encoding genes were overexpressed in TJ2-Tg leukemic T cells. Through the use of a pharmacological inhibitor, we have also found that overexpression of *Lta*, *Ltb* and *Light* genes in leukemic cells depended on canonical NF-κB signaling. Supporting the notion that lymphotoxin signaling plays a role in T-cell leukemia, inactivation of the *Ltbr* gene in mice resulted in a statistically significant delay in TEL-JAK2-induced leukemia onset (median survival of 22 weeks for TJ2-Tg;$Ltbr^{-/-}$ mice vs. 15 weeks for TJ2-Tg;$Ltbr^{+/-}$ littermates). LTα and LTβ mRNA expression was also detected in human T-ALL cell lines and primary samples from T-ALL patients, indicating that these proteins may be involved in human disease. $LT\alpha_1\beta_2$ protein expression at the surface of leukemic cells was low to undetectable but was inducible by PMA plus ionomycin treatment. Furthermore, cell surface lymphotoxin expression was found to be upregulated either when LTβR was absent from the microenvironment or when cells were cultured *ex vivo*, indicating that lymphotoxin-LTβR interaction occurs when leukemic T cells contact stromal cells. In sum, our data support the notion that leukemic cells expressing $LT\alpha_1\beta_2$ are able to activate LTβR signaling in stromal cells, thus prompting these cells to foster leukemogenesis.

This work was supported by national Portuguese funding through FCT – Fundação para a Ciência e Tecnologia, project ref. Pest-OE/EQB/LA0023/2013.

Developmental and structural changes reduce thymocyte precursor number and precede thymic involution

James Dooley[1,2*], Dean Franckaert[1,2*], Susan M. Schlenner[1,2], Nathalie Heirman[1,2], Jason Gill[3], Georg A. Hollander[3] & Adrian Liston[1,2]

[1] *VIB, Leuven 3000, Belgium*
[2] *Department of Microbiology and Immunology, University of Leuven, Leuven 3000, Belgium*
[3] *Pediatric Immunology, University of Basel, Basel Switzerland*

Abstract

The thymus is the organ devoted to T cell differentiation, maturation and export to the periphery. Unique among organs, the thymus undergoes multiple rounds of natural atrophy and redevelopment. Atrophy of the thymus, also known as involution, is temporally induced by stress stimuli, ranging from infection to pregnancy, and chronically induced during healthy aging. The genetic and structural control over age-related thymic involution is poorly understood, despite the influence the phenomenon has on the peripheral T cell pool. Here we have investigated the FVB/N mouse strain, which displays premature thymic involution and T cell output skewed towards the CD4 lineage. We find multiple structural and architectural features that precede thymic involution, with the division of the thymus into discrete lobules and disruption of the relationship between thymic epithelial cells and the vasculature responsible for the importation of hematopoietic precursors. Correlated with the disruption of the epithelium-endothelium relationship was a reduction in precursor thymocytes, providing a potential mechanism for thymic involution. These structural features, reminiscent of the human thymus, are controlled by weak polygenic loci. Skewed T cell output, by contrast, is a discrete trait, largely controlled by a single locus centered around TCRβ on chromosome 6 and active in the hematopoietic lineage. These results suggest a series of developmental steps that may precede thymic involution during aging.

Role of adenosine in intestinal homeostasis

Violaine François, Hussein Shehade, Nicolas Preyat, Muriel Moser & Guillaume Oldenhove

Université Libre de Bruxelles, Laboratory of Immunobiology-IBMM, 6041 Gosselies

Purpose/Objective

Intestinal T cells are chronically activated by antigenic stimuli derived from diet or commensal flora, as well as by extracellular ATP (released by damaged, activated cells or by intestinal bacteria). We hypothesize that mechanisms controlling extracellular ATP levels must operate to avoid excessive immune responses. Our objectives were: (i) to analyze the expression and activity of CD39 and CD73, two ectonucleotidases able to hydrolyze ATP in immunosuppressive adenosine, (ii) to identify the factors that regulate their expression and (iii) to evaluate the role of adenosine signaling pathway in the control of intestinal inflammation.

Material and methods

The analyses were performed in C57BL/6 wild-type mice and in genetically deficient mice. To induce inflammation in the small intestine, mice were inoculated orally with the parasite *Toxoplasma gondii.*

Results

We observed that small intestine $CD4^+$ (including $Foxp3^-$) and $CD8^+$ T cells expressed higher levels of CD39 and CD73 as compared to peripheral T cells and produced adenosine from AMP *in vitro*, in a CD73 dependent manner. CD73 expression was down-regulated on T cells from conditional TGF-β $RII^{-/-}$ mice and mice fed with vitamin A deficient diet, indicating that TGF-β and retinoic acid (RA) at the intestinal interface promoted CD73 up-regulation. *In vitro* experiments showed that RA synergized with TGF-β signaling by increasing SMAD3 phosphorylation. Using a model of oral infection with *Toxoplasma gondii*, we monitored CD73 expression on T cells and their capacity to generate adenosine from AMP. We found that, during acute infection, CD73 was downregulated and correlated with impaired T cells capacity to generate adenosine. Administration of BAY-606583, a specific A2B adenosine receptor agonist ameliorated *T. gondii*-induced inflammation, suggesting a critical role for adenosine in maintaining intestinal homeostasis in steady state and infectious conditions.

Conclusions

We showed that, at the steady state, intestinal T cells express high levels of CD73 and generate adenosine. TGF-β and RA synergize to sustain CD73 by a SMAD3 dependent manner. Our results suggest that, during intestinal inflammation, immunosuppressive adenosine generation is affected and could contribute to immunopathology.

Cyclophosphamide treatment induces rejection of established P815 mastocytoma by enhancing CD4 priming and intra-tumoral infiltration of P1E/H-2K^{d}-specific CD8^{+} T cells

Gwendoline Rahir[1], Nathalie Wathelet[1], Aurélie Hanoteau[1], Coralie Henin[1], Guillaume Oldenhove[1], Adrien Galuppo[1], Hanane Lanaya[1], Charles R. Mackay[2], Benoit Van den Eynde[3] & Muriel Moser[1]
G.R. and N. W. should be considered as co-first authors

[1]*Department of Molecular Biology, Université Libre de Bruxelles, Gosselies, Belgium*
[2]*Faculty of Medicine, Monash University, Clayton, Victoria, Australia*
[3]*Ludwig Institute for Cancer Research, UCL, Brussels, Belgium*

There is increasing evidence that the effect of chemotherapy on tumor growth is not cell autonomous but relies on the immune system. The objective of this study was therefore to decipher the cellular and molecular mechanisms underlying the role of innate and adaptive immunity in chemotherapy-induced tumor rejection. Treatment of DBA/2 mice bearing P815 mastocytoma with cyclophosphamide induced rejection and long-term protection in a CD4- and CD8-dependent manner. A population of inflammatory-type dendritic cells was dramatically expanded in the lymph nodes of mice that rejected the tumor and correlated with increased CD4^{+} T cell activation and infiltration, in tumor bed, of CD8^{+} T lymphocytes specific for the P1E antigen, encoded by mutated methionine sulfoxyde reductase gene. Our data point to a major role of CD4^{+} T cells in inducing chemokine expression in the tumor, provoking migration of tumor-specific CXCR3^{+} CD8^{+} T lymphocytes.

Impact of *T. vivax* (trans)-sialidase in animal trypanosomiasis: detection, localization and inhibition of the enzyme using Nanobody technology

Haynes C.[1,2,3], Remout H.[1], La Greca F.[1,2], Ameloot P.[3], Callewaert N.[3] & Magez S.[1,2]

[1]*Department of Structural Biology, VIB, Brussels, Belgium*
[2]*Laboratory of Cellular and Molecular Immunology, Free University of Brussels (VUB), Brussels, Belgium*
[3]*Department for Molecular Biomedical Research, University of Ghent, Ghent, Belgium*

Trypanosoma vivax is a protozoan extracellular parasite that infects animals and mostly affects cattle, in Africa and South America, leading to dramatic economic losses in the concerned countries. The major pathological feature is the high level of anemia that seriously affects the hosts.

T.vivax bloodstream form was shown to express sialidase (SD) activity, by which the SD enzyme is able to cut off terminal sialic acid (SA) residues from sugar entities. Knowing that red blood cells (RBCs) possess high amounts of this SA on their surface and that reduction in this SA level serves as a measure for the aging of the cell, we hypothesize that SD might be responsible for the drastic anemia levels observed during infection. The structure and function of this SD remains mostly unknown. Also its accessibility towards the immune system, its role in the induction of pathology and possible drug target applications are to be discovered.

In the study presented here there are two major goals: (I) the in depth characterization of the *T.vivax* SD, (II) the development of strategies for the inhibition of SD activity during infection.

From the available sequences of putative *T.vivax* SD genes, 3 were generated synthetically in order to produce recombinant SD protein (rSD) in yeast. Subsequently, activity of the rSD was assessed and confirmed. Furthermore, a nanobody® (Nb) library against rSD was generated and after screening for inhibitory Nbs, a potential candidate was retrieved. In the mean time, different conditions were tested for crystallization of the *T.vivax* rSD and using the first needle crystals, a structure of 2.7Å was obtained. Next, the inhibitory potential of the anti-rSD Nb and its impact on pathology will be assessed *in vivo*.

The capacity of Th2 lymphocytes to deliver B cell help requires expression of the transcription factor STAT3

Mélanie Hercor [*], Nathalie Mari [*], Sébastien Denanglaire, Oberdan Leo & Fabienne Andris

*co-first authors

Laboratoire d'Immunobiologie, Université Libre de Bruxelles, Belgium

$CD4^+$ T cell help for B cells is crucial for effective antibody responses. Although follicular T helper (Tfh) cells have emerged as the main providers of T cell help to B lymphocytes during the germinal center reaction, much less is known about the helper capacities of other effector $CD4^+$ T cells. The purpose of the present study was to evaluate the acquisition of B cell help capacity of canonically-derived T helper 2 (Th2) cells, a Th cell subset originally considered as responsible for B cell help *in vivo*.

Our data showed that naïve T cells developing under Th2-prone conditions acquired some features of T follicular helper cells, including BCL6 expression, IL-21 secretion and the capacity to promote B cell responses. Interestingly, developing Th2 cells co-expressed activated forms of both STAT6 and STAT3 transcription factors and STAT3 expression was required for their adequate B cell help capacity both *in vitro* and *in vivo*.

Moreover, expression of STAT3 in Th2 cells enhanced IgG1 to IgE class switch ratio *in vivo*, a finding with important implications for understanding the molecular basis of allergic diseases.

Collectively, our observations are compatible with the concept of a substantial plasticity for conversion of Th2 cells into Tfh *in vivo* and highlight a major role of STAT3 in the acquisition of Tfh functional features in Th2 cells.

A novel factor promotes human T cell development in a cell free system

MJAJ Huijskens, M Walczak, N Koller, BLMG Senden-Gijsbers, GMJ Bos & WTV Germeraad

Department of Internal Medicine, Division of Haematology, Maastricht University Medical Center, Maastricht, The Netherlands

Previously we have established human DN2-3 T-cells in an *in vitro* feeder system from mobilized hematopoietic stem cells (HSC) that further differentiate to mature T-cells in humanized mice. The current cell-based systems use transfected stromal cell lines such as TSt-4/DLL4. For clinical application, production needs to be performed under GMP compliant conditions that prohibit the use of feeder cells. A feeder free culture system was optimized where HSCs are differentiated to T-cells in the presence of immobilized DLL4 in combination with specific cytokines. CD7+ DN1 cells can be generated from CD34+HSCs, albeit with limited potential. In the search for candidate factors that improve *in vitro* T-cell development, gene expression profile analysis was performed on TSt-4/DLL4 and OP-9/DL1 stromal cells with and without progenitor T-cells. 1475 genes were differently expressed between TSt-4/DLL4 and OP-9/DL1. Interestingly, TSt-4/DLL4 +/- progenitors revealed a difference in gene expression of 54 genes, indicating their possible role during crosstalk. One factor (under patent evaluation) promoted CD7+iCD3+CD5+ DN2-3 cells development together with an increased expansion up to 1000 fold compared to the control. Although *in vivo* T lineage commitment still needs to be confirmed, these results show a promising GMP compliant culture method for the production of precursor T-cells for adoptive T-cell therapy by patients having undergone stem cell transplantation for leukaemia.

Lipoprotein levels modulate the human monocyte phenotype and are altered in MS patients

Winde Jorissen[1], Jeroen Bogie[1], Bart van Wijmeersch[2], Lita Freeman[3], Alan T. Remaley[3], Piet Stinissen[1], Monique Mulder[2], Niels hellings[1], Tim Vanmierlo[1] & Jerome Hendriks[1]

[1] *Hasselt University, Biomedical Research Institute and Transnational University Limburg, Diepenbeek, Belgium*
[2] *Revalidation and MS Center, Overpelt, Belgium*
[3] *National Institutes of Health, Department of Laboratory Medicine, Clinical Center, Bethesda, United States*
[4] *Erasmus Medical Center, Vascular and Metabolic diseases, Rotterdam, the Netherlands*

OBJECTIVES

Monocytes/ macrophages have been identified as the major effector cells in multiple sclerosis (MS), causing demyelination and axonal damage. Recent studies show that lipoproteins modulate the inflammatory state of monocytes. Interestingly, LDL and HDL levels are altered in the blood of MS patients. Moreover, an association between plasma HDL levels and neurologic recovery in MS patients is reported. In atherosclerosis, HDL is known to beneficially influence the inflammatory phenotype of macrophages by influencing their cholesterol metabolism. We hypothesize that changes in lipoprotein levels in the blood and the cerebrospinal fluid (CSF) in MS patients have an influence on the phenotype of monocytes and microglia by regulating LXR activation which may subsequently affect disease progression in MS.

RESULTS

Our preliminary data indicate that HDL cholesterol levels are lowered in MS patients. Furthermore, higher amounts of small particles of both HDL and LDL, as well as lower amounts of large particles of HDL, are found to be present in the plasma of MS patients as compared to healthy individuals. VLDL levels and particle sizes were not affected. Interestingly, our data show that HDL suppresses the basal and/ or LPS induced expression of the pro-inflammatory markers TNFα, CD40, IFNγ, IL6 and IL1β in a dose-dependent manner *in vitro*.

CONCLUSIONS

In summary, our preliminary data indicate that lipoprotein levels in MS patients are altered. Furthermore, HDL modulates the monocyte phenotype in a dose-dependent manner. Further research is required to determine the exact role of lipoproteins on the phenotype and function of monocytes in MS patients.

***Leishmania donovani* B cell dysfunction is not mediated through B cell depletion**

Florence Kauffmann, Carl de Trez & Stefan Magez

Laboratory of Cellular and Molecular Immunology, Free University of Brussels (VUB), Brussels, Belgium

Visceral leishmaniasis (VL) is a zoonotic disease that is caused by the bite of phlebotomine sandflies. The disease affects approximately 500 000 individuals/year, resulting in 70 000 deaths/year. Clinical presentation of VL typically involves long-term low-grade fever, enlarged spleen and liver and weight loss, pancytopenia and polyclonal (IgG and IgM) hypergammaglobulinemia. Infection in the liver is controlled, whereas the spleen displays uncontrolled amastigote growth, massive micro-architecture remodelling, leading to severe immunosuppression. So far, no vaccine against VL has been commercialized. B cells have been demonstrated to play a negative role during VL. As such, B cell depletion was shown to enhance resistance to *Leishmania donovani* infections[1] and marginal zone (MZ) B cells were proven to suppress Ag-specific CD8 and CD4 T cell responses during the early stages of VL[2]. However, the mechanisms underlying B cell dysfunction during Leishmania infections are still poorly understood. Considering the phylogenetic acquaintance of *Leishmania spp.* to the *Trypanosoma spp.*, both of which belong to the Trypanosomatidae, we hypothesized that Leishmania parasites modulate the host immune system by exhausting the B cell compartment, as this occurs during *T. brucei* infections[3,4]. In this study, we investigated the impact of *L. donovani* infection on the distribution of various B cell subsets, in the liver and spleen of both C57BL/6 mice and BALB/c mice. The percentages of B cell subsets, including immature, transitional and mature (follicular and MZ in case of the spleen) B cells did not differ significantly between infected and control (non-infected) mice over the course of the infection (up to 8 weeks). No significant differences were found between infected and control mice at the level of T cell subsets ($CD4^+$ and $CD8^+$), NK cells, myeloid cells or granulocytes either. Although not one population seemingly changed during the course of infection in both C57BL/6 and BALB/c mice, the total cell amounts were higher in infected mice compared to control mice, which indicates a certain level of inflammation. Also, signs of infection, such as mild hepatosplenomegaly and the presence of parasites in liver and spleen were observed. Therefore, our data suggest that depletion of the B cell compartment is not a hallmark of experimental *L. donovani* infections.

1. Smelt, S. C., Cotterell, S. E., Engwerda, C. R. & Kaye, P. M. B cell-deficient mice are highly resistant to Leishmania donovani infection, but develop neutrophil-mediated tissue pathology. *J. Immunol.* **164,** 3681–8 (2000).

2. Bankoti, R., Gupta, K., Levchenko, A. & Stäger, S. Marginal zone B cells regulate antigen-specific T cell responses during infection. *J. Immunol.* **188,** 3961–71 (2012).

3. Radwanska, M. *et al.* Trypanosomiasis-Induced B Cell Apoptosis Results in Loss of Protective Anti-Parasite Antibody Responses and Abolishment of Vaccine-Induced Memory Responses. **4,** (2008).

4. Bockstal, V. *et al.* T . brucei Infection Reduces B Lymphopoiesis in Bone Marrow and Truncates Compensatory Splenic Lymphopoiesis through Transitional B-Cell Apoptosis. **7,** (2011).

Oncogenic human papillomavirus triggers crosstalk between Natural Killer cells and dendritic cells

I. Langers[1] , V. Renoux[1 2], E. Dortu[1], J. Boniver[1], P. Delvenne[1] & N. Jacobs[1]

[1] *University of Liège, GIGA-I[3]-Cancer, Experimental Pathology-Virology, Liège, Belgium.*
[2] *Current address: Lund University, Hematopoietic Stem Cell Laboratory, Lund, Sweden.*

Recently, we showed that natural killer (NK) cells are directly activated by virus-like particles (VLP) formed by the major capsid protein L1 of human papillomavirus (HPV)-16, responsible for more than 50% of uterine cervical cancer. These VLPs are licensed as the first prophylactic vaccine against this cancer. Since, the collaboration between NK cells and dendritic cells (DC) could be a key factor in host resistance to viruses and tumours, we evaluated their crosstalk in the presence of VLP.

Interestingly, NK cells increased DC maturation induced by VLP as shown by an up-regulation of HLA-DR and CD86 on DC. Transwell experiments indicated that the expression of HLA-DR is cell-cell contact dependent. Moreover, in the presence of VLP and NK cells, DC produced higher amounts of IL12p70, while the production of the immunosuppressive cytokine IL10 remained unchanged. We also demonstrated that DC up-regulates the expression of NK activation markers (CD69 and HLA-DR) in the presence of VLP. Transwell experiments indicated that cell-cell contact is necessary for the expression of CD69 and HLA-DR. Preliminary results suggested that IL12 plays a role in the up-regulation of CD69 and HLA-DR. In the presence of DC activated by VLP, the function of NK was strengthened since they became more cytotoxic against HPV+ cell line and secreted more IFN-γ. Preliminary results also suggested that the secretion of IFN-γ is dependent on IL12.

Unlike it was reported for Langerhans cells, VLP containing L2 (the minor protein of capsid) did not inhibit the mutual activation of DC and NK cells in the presence of VLP.

Our results suggest that NK-DC crosstalk could play a role in the immune system activation induced by HPV-VLP during the current vaccination protocols against cervical cancer, but also in second-generation vaccines, which might contain HPV L2 protein.

Simultaneously Restorations of Foxp3(+)Treg Subsets, Type 1 Regulatory-Like Cells and B Cells by Anti-TNF Therapy for IBD

Zhe Li[†‡], Séverine Vermeire[‡], Dominique Bullens[†], Marc Ferrante[‡], Kristel Van Steen[‡§], Maja Noman[‡] Paul Rutgeerts[‡],Jan L. Ceuppens[†] & Gert Van Assche[†‡]

[†]Laboratory of Clinical Immunology, [‡]Division of Gastroenterology,Catholic University of Leuven, [§] Dept of Bioinformatics -Statistical Genetics, Université de Liège, Belgium

Background: A therapy with Infliximab (IFX, an anti-TNF monoclonal anti-body) increases circulating Foxp3 (+) T cells in patients (pts) with Crohn's disease (CD), ulcerative colitis (UC), rheumatoid arthritis (RA), psoriasis and Behçet's disease. Co-expression of CD45RA & Foxp3 distinguishes resting & active Treg (rTreg & aTreg) from Foxp3 (+) effector T cells (Teff). IFX also up-regulates blood total memory and pre-switch memory B cells in RA. In IBD, IgM (+) memory B cells are decreased. CD19(+) B cells in the inflamed intestinal mucosa predicts long lasting remission to IFX in CD. IL-10/IFNγ producing Tr1-like cells (Tr1L) have been characterized in human blood. Genetically modified B cells induce Tr1L in vivo. Recently resting B cells have a role in expanding Foxp3+Treg.We aimed to investigate the kinetics of these cells in pts with IBD during IFX therapy.

Methods: Blood was taken from healthy controls (HC, N=37) and pts with IBD (70 CD, 39 UC) before and during therapy (5mg/kg IV 0-2-6 and q8 wks). The 3 subsets of Foxp3 T cells, Tr1L and B cells were assessed by flow cytometry after staining for CD4, CD45RA, Foxp3, CD25, CD127 and CD19. Assessment of symptoms, endoscopic healing & histological improvement was used to distinguish responders (RS) from non-responders (NRS) at 4 to 12 weeks after start of therapy. Serum CRP was collected to monitor biological response.

Results: Pts with active IBD before therapy had low circulating rTreg(0.43±0.080, p<0.001), aTreg(0.62±0.12, p<0.001), Foxp3Teff(2.38±0.27, p=0.002) , Tr1L (4.79±0.68, p<0.001) and B cells (0.17±0.02, p=0.002) (N=25), compared with HC (1.47±0.16),(2.40±0.17),(3.75±0.34), (16.82±1.7) and (0.27±0.02) (N=37) (10e6/L blood mean±SEM for Tr1L, 10e9/L for others).

Compared with baseline before therapy, change of these cells after IFX treatment was seen in rTreg(RS: 1.57±0.21, p<0.001; NRS: 1.14±0.24, p<0.001), aTreg(RS: 2.70±0.26 , p<0.001; NRS: 1.48±0.33, p=0.0057), Foxp3+Teff (RS: 3.19±0.24, p=0.09; NRS: 3.02±0.41, p= 0.25),Tr1L(RS: 26.09±2.21, p<0.001; NRS: 8.92±1.00, p=0.013) and B cells (RS: 0.25±0.03, p=0.035;NRS:0.14±0.01, p=0.31) (N=59 in RS,15 in NRS).

Significant differences between RS and NRS were seen only for aTreg, Tr1L and B cells (p=0.0067, <0.001, <0.001), but not in rTreg and Foxp3Teff (p=0.32, 0.72).

Blood inflammatory biomarker (CRP) negatively correlated with rTreg, aTreg, Tr1L (as %of CD4T cells) and B cells (absolute number)(p=0.0011, r=- 0.32),(p<0.001, r=- 0.40),(p<0.001, r=- 0.39) and(p=0.044, r=- 0.21).

B cells positively correlated with rTreg, aTreg and Tr1L (absolute number)(p=0.002, r=0.31), (p<0.001, r=0.49) and (p<0.001, r=0.37).

Conclusions: Circulating Foxp3T cells, Tr1L and B cells are decreased in active IBD and an increase in these cells (except for Foxp3Teff) correlates with biological response to IFX and/or with the clinical response to IFX. The positive correlation between B cells and Foxp3Treg subsets or Tr1L suggests that there might be a cross talk between B cells and Tregs.

The ISGF3 complex is a Critical Regulator of Eomesodermin Expression in CD8 T Lymphocytes

Valérie Martinet[1], Sandrine Tonon[1], David Torres, Muriel Nguyen, Véronique Flamand, Oberdan Leo & Stanislas Goriely
[1]These authors contributed equally to the work.

WELBIO and Institute for Medical Immunology (IMI), Université Libre de Bruxelles, 8 rue Adrienne Bolland, B-6041 Charleroi-Gosselies, Belgium.

During acute infection or immunization, CD8 T lymphocytes undergo rapid expansion and acquire effector functions such as cytotoxicity and cytokine production. After this initial activation step, the pool of activated cells goes through a contraction phase, leaving behind a small fraction of memory cells. Cell fate decision is influenced by the balance between two transcription factors from the T-box family: T-bet, which induces the differentiation into terminal effector and "effector-memory" cells and Eomesodermin (Eomes), which favors the development of "central memory" T cells. The signals that influence this balance are poorly understood. In the current study, we observed that type I IFNs strongly upregulate the expression of Eomes both in vitro and in vivo. This effect occurs even in absence of antigenic stimulation. We demonstrate that Interferon Regulatory Factor (IRF) 9, a component of the ISGF3 complex, is directly implicated in this process in a T cell-intrinsic manner. Upon Listeria monocytogenes (LM) infection, we observed that Eomes upregulation in antigen-specific CD8 T cells was also affected in absence of IRF9 both at the effector and the memory phases. However, it did significantly affect neither memory cell phenotype nor their capacity to control a secondary infection. In contrast, we observed that IRF9-dependent Eomes expression was required for the expansion of $CD44^{+}CD62L^{+}CD122^{+}$ cells in aged mice. In conclusion, we demonstrate that the ISGF3 complex is an important regulator of Eomes that controls long-term homeostasis of CD8 T cell memory compartment.

Resident CD11b$^+$Ly6C$^-$, not neo-recruited inflammatory CD11b$^+$Ly6C$^+$ dendritic cells mediate allergic airway sensitization to house dust mite antigens

Claire Mesnil[1], Catherine Sabatel[1], Thomas Marichal[1], Marie Toussaint[1], Didier Cataldo[2], Pierre-Vincent Drion[3], Pierre Lekeux[1], Fabrice Bureau[1] & Christophe J. Desmet[1]

[1]*Laboratory of Cellular and Molecular Immunology, GIGA-Research Center and Faculty of Veterinary Medicine*
[2]*Laboratory of Tumors and Developmental Biology, GIGA-Research*
[3]*Laboratory of Preclinical and Biomedical Sciences, University Hospital Center, University of Liege, Belgium*

Conventional dendritic cells (DCs) of the lung are considered to be the prime initiators of airway allergy. DCs indeed are the most specialized antigen-presenting cells of the lung. They take up and transport inhaled antigens to the lung draining lymph nodes where they trigger the activation of allergen-specific T helper type 2 cells following initial allergen exposure. Yet, lung DCs are a heterogeneous cell population and it remains unclear whether specific DC subsets are preferentially involved in allergic airway sensitization.
Here, we assessed the respective pro-allergic potential of individual lung DC subsets using a systematic dichotomic approach in models of adoptive transfer. DC subsets were sorted from the lung of donor mice 24h after intranasal exposure to a sensitizing dose of house dust mite antigens (HDM). These cells were transferred immediately to naïve recipient mice without any further treatment. Ten days later, recipient mice were exposed to a low dose HDM and parameters of airway allergy and T cell response profiles were assessed. As a proof of principle for this approach, we first confirmed that the transfer of an entire pool of lung DCs from donor mice was able to induce allergic airway sensitization in naïve recipient animals. The cytokine profile of bronchial lymph node cells following allergen re-exposure indicated the induction of Th2- and Th17-type responses, and minor IFN-gamma production, as expected in HDM-induced airway allergy.
We next transferred recipient mice with either CD11b$^-$ or CD11b$^+$ lung DCs from donor mice. We observed that only CD11b$^+$ DCs were able to sensitize recipient animals, in which they primed allergen-specific type 2 adaptive responses and IL-17 production by bronchial lymph node cells. In contrast, CD11b$^-$ DCs primed IL-17 and IFN-gamma production, but not type 2 responses. Further separation of lung CD11b$^-$ cells, which contained bona fide CD11b$^-$CD103$^+$ DCs and CD11b$^-$CD103$^-$ cells (presumably macrophages) supported that these cell subsets primed IL-17 and IFN-gamma production, respectively.
We further dissected the contribution of CD11b$^+$ cells, which alone were able to mediate allergic sensitization. The pool of lung CD11b$^+$ DCs expands following allergen exposure, which is essentially due to the recruitment of Ly6C$^+$ inflammatory monocytes that may differentiate into inflammatory DCs. Because of this coinciding neo-recruitment, inflammatory DCs have been previously suspected of being responsible for allergic airway sensitization. We observed that Ly6C expression is stable on neo-recruited inflammatory DCs *in vivo* for at least 24 hours, the time lapse after which DCs are isolated from donor mice. We thus considered it a good marker to discriminate between resident CD11b$^+$Ly6C$^-$ DCs and inflammatory CD11b$^+$Ly6C$^+$ DCs. Transfer experiments indicated that only CD11b$^+$Ly6C$^-$ DCs but not inflammatory CD11b$^+$Ly6C$^+$ DCs were able to prime adaptive responses and to sensitize naïve recipient mice to airway allergy. In spite of significant allergen uptake in the lung, allergen-loaded CD11b$^+$Ly6C$^+$ DCs appear to mostly remain in the lung following HDM exposure, unlike CD11b$^+$Ly6C$^-$ DCs, which display efficient antigen transport activity to the lymph nodes. The reason for this differential behavior remains unclear, since both subsets express CCR7.
Altogether, our data support that resident non-inflammatory CD11b$^+$Ly6C$^-$ DCs are the prime inducers of airway allergic sensitization.

CX_3CR1^{hi} $Ly6C^-$ monocytes play a regulatory role during liver inflammation

Morias Y.[1,2], Abels C.[1,2], Laoui D.[1,2], Van Overmeire E.[1,2], Guilliams M.[3], Schouppe E.[1,2], Tacke F.[4], deVries C.[5], De Baetselier P.[1,2] & Beschin A.[1,2]

[1] *Myeloid Cell Immunology Lab, VIB, Brussels, Belgium*
[2] *Cellular and Molecular Immunology Unit, Vrije Universiteit Brussel, Belgium*
[3] *Department for Molecular Biomedical Research, VIB Ghent, Belgium; Laboratory of Immunoregulation and Mucosal Immunology, University Gent, Belgium*
[4] *Department of Medicine III, Rheinisch-Westfaelische Technische Hochschule (RWTH) University Hospital Aachen, Germany*
[5] *Department of Medical Biochemistry, Academic Medical Center, K1-113, University of Amsterdam, The Netherlands*

Two major subsets of blood monocytes can be distinguished: inflammatory $Ly6C^{hi}CX_3CR1^{int}$ and patrolling $Ly6C^-CX_3CR1^{hi}$ monocytes. Both $Ly6C^{hi}$ and $Ly6C^-$ monocytes are recruited to inflammation sites where they can differentiate into macrophages and/or DCs and exert protective or detrimental roles, depending on the pathogenic trigger. We use experimental African trypanosomiasis as a model to study the role of these monocytes during liver inflammation. We have reported that $Ly6C^{hi}$ monocytes are recruited to the liver of *Trypanosoma congolense* infected mice and differentiate in classically activated monocyte-derived cells (M1). By secreting TNFα, these cells contribute to liver cell damage, resulting in organ failure and early death. IL-10, as suppressor of M1 immune responses, plays a critical role in limiting liver injury and prolonging survival.

We now document that $Ly6C^-$ monocytes were increasingly recruited to the liver during infection. These IL-10 producing cells do not own a gene signature reflecting an alternative, M2 activation status and do not differentiate into macrophages. We revealed that $Ly6C^-$ monocytes could suppress production of TNFα by $Ly6C^{hi}$ monocytes during infection, favor the differentiation of the latter cells towards M2-type macrophages and protect liver integrity. These data illustrate a regulatory role for $Ly6C^-$ monocytes in liver pathogenicity. Hereby, $Ly6C^-$ monocytes protect the liver from damage caused by a persistent pathogenic immune response.

Identification and functional analysis of molecular interaction partners of the glucocorticoid receptor in inflammation and cancer

Ioanna Petta[1,2,3], Sam Lievens [3,4], Lien Dejager [1,2], Sofie Vandevyver [1,2], Karolien De Bosccher [3,4], Jan Tavernier [3,4] & Claude Libert [1,2]

[1] *Department for Molecular Biomedical Research, VIB, 9052 Ghent, Belgium*
[2] *Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium*
[3] *Department of Medical Protein Research, VIB, 9000 Ghent, Belgium*
[4] *Department of Biochemistry, Faculty of Medicine and Health Sciences, Ghent University, 9000 Ghent, Belgium*

The Glucocorticoid receptor (GR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors, mediated by Glucocorticoids (GCs). In the absence of ligand, GR is sequestered in the cytoplasm in an inactive complex which is altered after addition of GCs. However these complexes are poorly characterized.

In many inflammatory diseases, it has been reported that the activity of the GR is reduced, leading to Glucocorticoid Resistance (GCR), a condition where the patients do not respond anymore to the administration of GCs. Tumor necrosis factor (TNF) is one of the strongest known pro-inflammatory cytokines and experimental results have already proven that TNF is involved in the process of GCR *in vitro* and *in vivo.*

Our hypothesis is that GR may form protein-protein interactions in the cytoplasm, which cause modifications of the GR leading to its decreased activity in the nucleus. It is possible that TNF may induce GCR by changing the protein-protein complexes that GR forms in the absence and presence of ligands.

In this study, we make use of MAPPIT (Mammalian Protein- Protein Interaction Trap), a high-throughput technology in order to analyze the GR interactome in different stimulatory conditions.

Our aim is to investigate the interaction partners of the GR in the absence of ligand, as well as upon Dexamethasone (Dex) stimulation, a synthetic agonist of the GR. Furthermore, we use TNF as inflammatory model, in order to elucidate how GR interactome is influenced in this inflammatory condition, giving insights into the mechanism of the GCR.

TNF-induced necroptosis is regulated by nicotinamide adenine dinucleotide in a sirtuin-dependent manner

Nicolas PREYAT & Oberdan LEO

Laboratory of Immunobiology, Institute for Molecular Biology and Medicine, Université Libre de Bruxelles, Gosselies, BELGIUM

npreyat@ulb.ac.be

Cellular necrosis has long been regarded as an incidental and uncontrolled form of cell death. However, a regulated form of cell death termed necroptosis has been uncovered recently. Necroptosis can be induced by extracellular cytokines like TNF, FASL or TRAIL, pathogens and several pharmacological compounds which share the property of triggering the formation of a molecular complex containing the kinases receptor-interacting protein 1 (RIPK1) and receptor-interacting protein 3 (RIPK3). This complex has been shown to initiate a series of intracellular events leading to cell swelling, loss of plasma membrane integrity and mitochondrial fragmentation ultimately leading to cell death. Of interest, most ligands known to induce apoptosis (including notably TNF, FASL or TRAIL) can also promote necroptosis under particular experimental settings, and the mechanisms regulating the decision of cells to commit to one form of cell death or the other are still poorly defined. We demonstrate herein that intracellular nicotinamide adenine dinucleotide (NAD^+) plays an important role in supporting cell progression to necroptosis. NAD^+ appears to regulate necroptosis in a sirtuin-dependent fashion, opening new avenues of intervention to protect cells and organs against necrotic insults including ischemic injuries.

Exercise induced bronchoconstriction in young athletes

Sven F Seys, G Marijsse, E Dilissen, S Aertgeerts, T Troosters, V Van Belle, K Peers, JL Ceuppens, LJ Dupont & DM Bullens

Pneumology, Department of Clinical and Experimental Medicine, Catholic University of Leuven (KU Leuven), Leuven, Belgium

Introduction

Exercise induced bronchoconstriction (EIB) is more prevalent in elite athletes compared to controls. It is however unclear how many young athletes suffer from EIB.

Methods

Football players (n=24), basketball players (n=15), swimmers (n=12) were recruited at the elite sport high school (12-14 years old) in Leuven (Belgium). Age-matched controls (n=7) were recruited among children performing sports at a recreational level. Eucapnic voluntary hyperventilation test was used to assess EIB according to previous standards. Subjects breathed a gas mixture (5% CO_2, 21% O_2 and 74% N_2) at a target rate of 85% of their maximal voluntary ventilation (MVV) per minute (assessed before the EVH test) for 6 minutes. Spirometry was performed at 1, 5, 10 and 15 min after the EVH challenge. EVH test was considered positive if the fall in FEV_1 ≥10%. Allergy for house dust mite, grass pollen, tree pollen, weeds, dog and moulds was assessed by skin prick test (considered positive if at least one SPT was positive).

Results

FEV_1 and FVC (%predicted) levels were not different among four groups. FVC (L) was significantly higher in swimmers compared to controls ($p<0.05$). Maximal fall in FEV_1 (%) was significantly higher in swimmers (mean: -8.8%) compared to football players (mean: -6.1%), basketball players (mean: -1.0%) and controls (mean: -3.6%) ($p=0.027$). EIB (fall in FEV_1 ≥10% at EVH test) was diagnosed in 4 out of 12 swimmers, 3 out of 20 football players, 1 out of 11 basketball player and 1 out of 7 control individual. Only 1 of these individuals (swimmer) had pre-existing asthma. Allergy was equally distributed among four groups: 7 out of 24 football players, 1 out of 7 controls, 5 out of 11 basketball players, 3 out of 11 swimmers ($p=0.94$).

Conclusion

Swimmers had highest prevalence of EIB. Maximal fall in FEV_1 was significantly higher in swimmers compared to other athletes and controls despite higher FVC levels. Competitive swimmers but not other athletes (only intense exercise) and controls are exposed to both intense exercise and airborne trichloramine. This might explain why airway hyperreactivity is more common in swimmers compared to other athletes.

Th1 /Th17 balance is controlled by HIF-1a under Hypoxia

Hussein Shehade, Violaine François, Muriel Moser & Guillaume Oldenhove

Université Libre de Bruxelles, Laboratory of Immunobiology-IBMM, 6041 Gosselies

Objective

During an immune response, inflammation can damage tissue microvasculature and reduce cellular oxygen supply. This process is referred as tissue hypoxia. When O_2 availability decreases, the transcription factor HIF-1a, a key metabolic sensor, is stabilized and regulates cellular adaptation to hypoxia. The objective of this project was to test whether HIF-1a regulates T cell fate during inflammation and to define the molecular mechanisms of this control.

Materials and methods

Naive $CD4^+$ T cells were cultured under Th1 and Th17 polarizing conditions and then transferred to 1% (Hypoxic) or 20% (Normoxic) O_2 environment. As strategy, we have used genetically deficient mice, bicistronic vectors allowing the overexpression of selected genes, flow cytometry and real time PCR to investigate the mechanisms involved in T helper adaptation to hypoxia. T cell transfer model of chronic colitis is ongoing to investigate the *in vivo* relevance of our observations.

Results

We found that, unlike Th17 cells, Th1 cells lose their effector function (as assessed by IFN-g production) when cultured under hypoxia. Of note, HIF-$1a^{-/-}$ Th1 cells were insensitive to hypoxia underlining a critical role for HIF-1a in Th1 inhibition by hypoxia. Similarly, the production of IFN-g by IL-$10^{-/-}$ Th1 was not altered in hypoxia, supporting a role for IL-10 probably by sustaining STAT3 phosphorylation. In support of this hypothesis, STAT3 was phosphorylated constitutively in both Th1 and Th17 cells under hypoxia, but not normoxia, and $STAT3^{-/-}$ Th1 cells produced similar amounts of IFN-g in normoxic and hypoxic conditions, suggesting that, in hypoxic Th1 cells, STAT3 may favor HIF-1a accumulation. The mechanism by which HIF-1a decreased Th1 activation could involve the inhibition of STAT4 activation as suggested by our preliminary data.

Conclusion

We found that hypoxic culture conditions result in decreased Th1 activation while sparing Th17. Our results suggest that IL-10 secretion by Th1 cells induces a STAT3 dependent feedback leading to HIF-1a accumulation, which may in turn inhibit the STAT4 signaling pathway.

Regulation of CD4 T cell repertoire diversity and avidity by dendritic cells and regulatory T cells *in vivo*

M. Stefkova[1], A. Galuppo[1], G. Kassiotis[2], O. Leo[1] & M. Moser[1]

[1]*Laboratory of Immunobiology, Institut de Biologie et Médecine Moléculaire, Université Libre de Bruxelles, 6041 Gosselies, Belgium*

[2]*Divisions of Immunoregulation, The MRC National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK*

mstefkov@ulb.ac.be

Recent studies conducted in mice and humans have demonstrated the importance of new parameters playing a role in the efficiency of an adaptive immune response. One of these parameters is the avidity of antigen receptors. The avidity allows the T cells to respond quickly to small amounts of antigen. In several studies, the presence of high avidity cells for an antigen was associated with better control of HIV replication and in therapies against cancer. All these observations are in favor of a more complex role of antigen-presenting cells in the development of an immune response and underline the importance of new parameters such as the avidity of the antigen receptors in the efficiency of the immune response. Regulatory T cells were very recently shown to play an important role in regulating the functional avidity of anti-viral T $CD8^+$ immune responses. Better understanding of how regulatory T cells can modulate the functional avidity seems to be essential.

To study the avidity of the TCR receptor, we used a mouse model (Antunes et al., 2008[1]) in which T cells transgenically express the TCRβ chain of a TCR specific to an MHC class II-restricted peptide $env_{122\text{-}141}$. Kassiotis' group showed that T cells expressing the combination of the TCRβ-transgenic chain and the TCR $V\alpha2^+$ displayed a higher avidity for $env_{122\text{-}141}$ than their TCR $V\alpha2^-$ counterparts, suggesting that this model is suitable to study the avidity of the TCR *in vivo*. Our objective was to test the role of natural regulatory T cells in the avidity of an immune response *in vivo*. Our results show that, mice immunized with dendritic cells pulsed with the env peptide displayed an antigen specific response of higher avidity when depleted of natural regulatory T cells (by injection of anti-CD25 mAb). This result suggests that regulatory T cells may play an important role in the clonal selection of T lymphocytes and favor low-affinity $CD4^+$ T cells. In our future experiments we will attempt to understand the mechanism by which regulatory T cells control the functional avidity of CD4 T cells and we will investigate whether the high-avidity T cells (TCR $V\alpha2^+$ cells) have the same CDR3 diversity upon depletion of regulatory T cells. In addition, we will also try to understand how co-stimulatory molecules expressed on dendritic cells may play a role in the functional avidity of CD4 T cells.

[1]Antunes, I., Tolaini, M., Kissenpfennig, A., Iwashiro, M., Kuribayashi, K., Malissen, B., Hasenkrug, K., and Kassiotis, G. (2008) Retrovirus-Specificity of Regulatory T Cells Is Neither Present nor Required in Preventing Retrovirus-Induced Bone Marrow Immune Pathology. Immunity *29*, 782–794.

Role of Gamma Delta T cells in HPV-induced Cancer Murine Model

D. Van Hede[1], R. Bastin[1], F. Francis[1], J. Arrese Estrada[2], Ambre Gau Okroglic[1], V. Renoux[1,4], E. Dortu[1], I. Langers[1], P. Delvenne[1], D. Vermijlen[3] & N. Jacobs[1]

[1]Laboratory of Experimental Pathology-Virology, Giga-Cancer/I3,[2] Department of Dermatopathology, University of Liège, Liège, Belgium; [3]Institute for Medical Immunology, Université Libre de Bruxelles, Gosselies, Belgium; [4]Current address: Lund University, Hematopoietic Stem Cell Laboratory, Lund, Sweden

High-risk human papillomavirus (e.g: HPV16, 18) infection is the etiological agent of cervical cancer, the third cause of cancer-associated death in women worldwide. Gamma delta T cells (γδT cells), a small population of T cells expressing a T cell receptor (TCR) composed of γ and δ chains. Anti-viral and anti-tumour activities for these cells have been described, but not in the context of HPV infection. The goal of this project is to determine the role of γδT cells in the immune response against HPV-induced tumours.

In order to study the role of γδT cells in HPV-induced lesions, we have established a mouse model by crossing transgenic mice expressing HPV16 oncogenic genes, which develop spontaneous skin lesions, with γδT cell-deficient mice (collaboration with Prof. Girardi, Yale University, USA). Surprisingly, depletion of γδT cells significantly delays development of HPV-induced lesions. In parallel, we observed by immunohistochemistry an increase of leukocyte infiltration (CD45+ cells) in HPV-induced lesions in absence of γδT cells. Then, we evaluated by flow cytometry the proportions of immune cell populations present in the mouse skin and we found a larger proportion of CD4+ T cells in HPV and HPV γδT cell-deficient mice compared to normal mice. Since γδT cells could induce angiogenesis when infiltrating tumors, we measured blood vessels density in mice skin sections and we observed a significantly increase of blood vessels density in HPV mice compared to γδT cells-deficient mice.

Our results suggest that γδT cells could promote cancer progression in the context of HPV-induced lesions. We will further characterise these cells to understand in which cellular and molecular mechanisms they are involved.

Hemozoin is a Prominent Inflammatory Virulence Factor in Malaria-Associated Acute Respiratory Distress Syndrome

K. Deroost[1], A. Tyberghein[1], N. Lays[1], S. Noppen[1], E. Schwarzer[2], E. Vanstreels[1], M. Komuta[3], J.-W. Lin[5], A. Pamplona[5], C.J. Janse[4], P. Arese[2], T. Roskams[3], D. Daelemans[1], G. Opdenakker[1] & P.E. Van den Steen[1]

[1]Rega Institute, University of Leuven, Belgium; [2]University of Torino, Italy; [3]University of Leuven, Belgium; [4]Leiden University Medical Centre, The Netherlands; [5]Universidade de Lisboa, Portugal

Malaria-associated acute respiratory distress syndrome (MA-ARDS) is a deadly complication of malaria and its pathophysiology is insufficiently understood. Both in humans and mouse models, MA-ARDS is associated with marked pulmonary inflammation. We investigated the role of hemozoin, a detoxification product of heme produced by the parasite during hemoglobin degradation, in MA-ARDS in different murine malaria models. By quantifying hemozoin in the lungs and measuring disease parameters of MA-ARDS, we demonstrate a highly significant correlation between pulmonary hemozoin levels, lung weight and alveolar edema. Histological analysis of the lungs demonstrated that hemozoin is localized in phagocytes and infected erythrocytes, and only occasionally in granulocytes. Species-specific differences in hemozoin production, as measured in individual schizonts, were associated with variations in pulmonary pathogenicity. Furthermore, we show that both pulmonary hemozoin and lung pathology are correlated with the number of infiltrating inflammatory cells, with increased pulmonary expression of cytokines, chemokines and enzymes, and with alveolar VEGF levels. The causal relation between hemozoin and inflammation was investigated by injecting *P. falciparum*-derived hemozoin intravenously in malaria-free mice and shows that hemozoin potently induces the pulmonary expression of pro-inflammatory chemokines (IP-10/CXCL10, MCP-1/CCL2, and KC/CXCL1), cytokines (IL-1β, IL-6, IL-10, TNF, and TGF-β) and other inflammatory mediators (iNOS, Hmox1, NOX2, and ICAM-1). Thus, hemozoin at the interface of host and parasite is a prominent inflammatory virulence factor in the pathogenesis of MA-ARDS.

Novel insights in glucocorticoid receptor dimer dependent biology

Sofie Vandevyver, Marlies Ballegeer, Filip Van Hauwermeiren, Lien Dejager & Claude Libert

Inflammation Research Center, VIB-UGent, Technologiepark 927, 9051 Ghent, Belgium

The Glucocorticoid receptor (GR) is a major anti-inflammatory protein, activated by binding of glucocortoids (GCs), known to protect against TNF-induced lethal shock. Previously, we demonstrated that GR dimers are crucial to inhibit JNK-2, via induction of MKP-1, and hence TNF-induced lethality. Here, we illustrate that GR dimers play a dominant role over GR monomers in the course of protecting against TNF-induced toxicity and lethality. We investigated the TNF-induced toxicity in $GR^{dim/dim}$ and $GR^{wt/wt}$ mice, which were adrenalectomized or pre-treated with a synthetic GC, Dexamethasone. We assessed different characteristics, including systemic inflammatory parameters, cell death in the intestine, intestinal permeability, and the intestinal mucus barrier. When no GCs are present, both GR dimers and monomers play a crucial role in the protection against TNF-induced toxicity. Furthermore, we illustrate that a lethal dose of TNF results in increased intestinal permeability and depletion of mucus in goblet cells in both $GR^{wt/wt}$ and $GR^{dim/dim}$ mice. Interestingly, when pre-treating mice with Dex, only $GR^{wt/wt}$ mice showed reduced to no intestinal permeability and replenishing of the goblet cells, whereas Dex pre-treatment in $GR^{dim/dim}$ mice had no effect. We conclude that upon the therapeutic administration of exogenous GCs, GR dimers are dominant over GR monomers for the protection against TNF-induced disruption of the intestinal mucus barrier and intestinal permeability.

Does CMV infection cause expansion of cytotoxic $CD4^+CD28^{null}$ T cells?

Marjan Vanheusden[1], Bieke Broux[1], Liesbet M. Peeters[1], Ine Pauwels[2], An Goris[2], Piet Stinissen[1] & Niels Hellings[1]

[1] *Hasselt University, Biomedisch Onderzoeksinstituut, School of Life Sciences, Diepenbeek, Belgium.*
[2] *KULeuven, Laboratory for Neuroimmunology, Section of Experimental Neurology, Leuven, Belgium.*

Background. $CD4^+CD28^{null}$ T cells arise during chronic activation of the immune system. $CD4^+CD28^+$ T cells lose CD28 after repeated antigenic stimulation (chronic infection/inflammation) or homeostatic proliferation as a response to thymic involution (immunosenescence). $CD4^+CD28^{null}$ T cells are present in the peripheral blood of a subset of both healthy controls (HC) and patients with multiple sclerosis (MS), a chronic disabling autoimmune disease of the central nervous system (CNS).
$CD4^+CD28^{null}$ T cells have an effector memory phenotype ($CD62L^-$, $CCR7^-$) with cytotoxic properties (perforin, granzyme B, NK-cell receptors). They accumulate in MS brain lesions and at least a subpopulation of these cells is autoreactive in nature. Therefore, these cells may actively contribute to the disease process of MS. So far, the cause of the expansion of $CD4^+CD28^{null}$ T cells and their contribution to MS disease pathology is poorly investigated. There is mounting evidence that the expansion occurs after infection with Cytomegalovirus (CMV), a double stranded virus of the β-herpes family.

Results. The role of CMV in the expansion of $CD4^+CD28^{null}$ T cells was examined on an immunological and genetic level. An association study between CMV serology and the percentage of peripheral blood $CD4^+CD28^{null}$ T cells in MS patients and healthy controls showed that expanded $CD4^+CD28^{null}$ T cells are significantly more present in CMV^+ persons ($p<0.001$ for MS and HC). CMV IgG titers positively correlated with the percentage of $CD4^+CD28^{null}$ T cells (MS: 0.54-0.63, $p<0.0001$. HC: 0.57, $p=0.01$). Furthermore, *in vitro* stimulation of PBMCs with CMV pp65 in combination with IL-2 led to an increase in $CD4^+CD28^{null}$ T cells in CMV^+ healthy donors with or without an *in vivo* expanded $CD4^+CD28^{null}$ population. When CMV pp65 was used alone, $CD4^+CD28^{null}$ T cells increased only in CMV^+ donors with an *in vivo* expanded population.
On the genetic level, a SNP (rs2523651) flanking the gene of MHC class I polypeptide-related sequence B (*MICB*), a ligand for the NKG2D receptor, was investigated via Taqman. From preliminary data an odds ratio of 1.5 ($p>0.05$, 95% CI 0.86-2.63) was calculated for the risk allele, indicating an increased risk for CMV infection. An increasing trend in the percentage of $CD4^+CD28^{null}$ T cells was also observed in carriers of the risk allele ($p>0.05$).

Conclusion/significance
These data suggest that CMV infection leads to the expansion of $CD4^+CD28^{null}$ T cells via the process of repeated antigenic challenge. The genetic background of the subject seems to be an important factor to determine the risk for CMV infection and thus ultimately the risk for expanded $CD4^+CD28^{null}$ T cells. While the exact contribution of $CD4^+CD28^{null}$ T cells to MS pathogenesis needs to be further documented, CMV vaccination to prevent the expansion of cytotoxic $CD4^+CD28^{null}$ T cells may be of benefit for people at risk of developing MS.

Keywords: CMV, $CD4^+CD28^{null}$ T cells, MS

Molecular imaging with macrophage CRIg-targeting Nanobodies for diagnosis and prognosis in a mouse model of rheumatoid arthritis

[1,2]Fang Zheng, [3]Stéphanie Put, [4]Luc Bouwens, [5,6]Tony Lahoutte, [3]Patrick Matthys, [1,2]Patrick De Baetselier, [1,7]Serge Muyldermans, [5]Nick Devoogdt*, [1,2]Geert Raes* & [1,2]Steve Schoonooghe*

[1] Vrije Universiteit Brussel, Laboratory of Cellular and Molecular Immunology, Brussel, Belgium; [2]VIB Laboratory of Myeloid Cell Immunology, Brussel, Belgium; [3] Katholieke Universiteit Leuven, Laboratory of Immunobiology, Rega Institute, Leuven, Belgium; [4] Vrije Universiteit Brussel, Cell Differentiation Unit, Diabetes Research Centre, Brussel, Belgium; [5] Vrije Universiteit Brussel, In Vivo Cellular and Molecular Imaging Center, Brussel, Belgium; [6] Vrije Universtiteit Brussel, Department of Nuclear Medicine, UZ Brussel, Brussel, Belgium. [7] VIB Department of Structural Biology, Brussel, Belgium (: ND, GR and SS share senior authorship)*

Rationale. An accurate and noninvasive tracer able to detect molecular events underlying the development of rheumatoid arthritis (RA) would be useful for RA diagnosis and drug efficacy assessment. A complement receptor of the Ig superfamily (CRIg) is expressed on synovial macrophages of RA patients, making it an interesting target for molecular imaging of RA. We aim to develop a radiotracer for the visualization of CRIg in a mouse model for RA using radiolabeled single domain variable antibody VHH fragments (a.k.a. Nanobodies).

Methods. qPCR was used to locate CRIg expression in collagen-induced arthritis (CIA) mice. A Nanobody, NbV4m119, was generated to specifically target CRIg. Flow cytometry, phosphorimaging and confocal microscopy were used to confirm NbVm119 binding to $CRIg^{+}$ cells. Single-photon emission computed tomography (SPECT/CT) was used to image arthritic lesions in inflamed paws using the ^{99m}Tc-NbV4m119 Nb.

Results. CRIg is constitutively expressed in the liver and was found to be upregulated in synovial tissues of CIA mice. SPECT/CT imaging revealed that ^{99m}Tc-NbV4m119 specifically targeted $CRIg^{+}$ liver macrophages in naïve wild type but not in $CRIg^{-/-}$ mice. In CIA mice, ^{99m}Tc-NbV4m119 accumulation in arthritic lesions increased according to the severity of the inflammation. In knees of collagen-challenged mice, ^{99m}Tc-NbV4m119 was found to accumulate even before the onset of macroscopic clinical symptoms.

Conclusion. SPECT/CT imaging with ^{99m}Tc-NbV4m119 visualizes joint inflammation in CIA. Furthermore, imaging could predict which mice will develop clinical symptoms during CIA. Consequently, imaging of joint inflammation with CRIg-specific Nanobodies offers perspectives for clinical applications in RA patients.

Keywords: Complement receptor of the Ig superfamily (CRIg, VSIG4 or Z39Ig), collagen-induced arthritis (CIA), single photon emission computed tomography (SPECT), nanobody, macrophage.

Corresponding author: Steve Schoonooghe, Laboratory of Cellular and Molecular Immunology, VIB Laboratory of Myeloid Cell Immunology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium. E-mail: sschoono@vub.ac.be. Phone: +3226291977. Fax: +3226291981.

First author: Fang Zheng, PhD student, Laboratory of Cellular and Molecular Immunology, VIB Laboratory of Myeloid Cell Immunology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium. E-mail: Fzheng@vub.ac.be. Phone: +3226291977. Fax: +3226291981.

NOTES