Cellular and Molecular Biology™ 47 (1), 179-188
Printed in France.

0145-5680/01
2001 Cell. mol. Biol.™

Review

THYMIC T-CELL TOLERANCE OF NEUROENDOCRINE FUNCTIONS: PHYSIOLOGY AND PATHOPHYSIOLOGY

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Received March 2, 2000; Accepted May 25, 2000

Abstract - Intimate interactions between the two major systems of cell-to-cell communication, the neuroendocrine and immune systems, play a pivotal role in homeostasis and developmental biology. During phylogeny as well as during ontogeny, the molecular foundations of the neuroendocrine system emerge before the generation of diversity within the system of immune defenses. Before reacting against non-self infectious agents, the immune system has to be educated in order to tolerate the host molecular structure (self). The induction of self-tolerance is a multistep process that begins in the thymus during fetal ontogeny (central tolerance) and also involves anergizing mechanisms outside the thymus (peripheral tolerance). The thymus is the primary lymphoid organ implicated in the development of competent and self-tolerant T-cells. During ontogeny, T-cell progenitors originating from hemopoietic tissues (yolk sac, fetal liver, then bone marrow) enter the thymus and undergo a program of proliferation, T-cell receptor (TCR) gene rearrangement, maturation and selection. Intrathymic T-cell maturation proceeds through discrete stages that can be traced by analysis of their cluster differentiation (CD) surface antigens. It is well established that close interactions between thymocytes (pre-T-cells) and the thymic cellular environment are crucial both for T-cell development and for induction of central self-tolerance. Particular interest has focused on the ability of thymic stromal cells to synthesize polypeptides belonging to various neuroendocrine families. The thymic repertoire of neuroendocrine-related precursors recapitulates at the molecular level the dual role of the thymus in T-cell negative and positive selection. Thymic precursors not only constitute a source of growth factors for cryptocrine signaling between thymic stromal cells and pre-T-cells, but are also processed in a way that leads to the presentation of self-antigens by (or in association with) thymic major histocompatibility complex (MHC) proteins. Thymic neuroendocrine self-antigens usually correspond to peptide sequences highly conserved during the evolution of their corresponding family. The thymic presentation of some neuroendocrine self-antigens does not seem to be restricted by MHC alleles. Through the presentation of neuroendocrine self-antigens by thymic MHC proteins, the T-cell system might be educated to tolerate main hormone families. More and more recent experiments support the concept that a defect in thymic tolerogenic function is implicated as an important factor in the pathophysiology of autoimmunity.

Key words: Thymus, T-cell tolerance, neuroendocrine protein families, autoimmunity

INTRODUCTION

Two important and specific properties characterize a normal functioning of the immune system. First, the generation of diversity of the immune response against foreign antigens (non-self) results from the random recombination in somatic cells of gene segments coding for the variable part of immunoglobulin (Ig) antibodies (Ab) (77) and T-cell receptors (TCR) for the antigen (TCR) (29). Secondly, even before being able to recognize and react against non-self antigens, the immune system must be educated to tolerate self-antigens. With memory, diversity and self-tolerance constitute the corner stones of immunophysiology. The induction of self-tolerance is not an automatic genetically-programmed process, but is an acquired multi-step phenomenon which is initiated within the thymus during T-cell differentiation (20).

In the last thirty years, close interactions were shown to exist between the major systems of cell-to-cell

Abbreviations: **Ab**: antibodies; **APC**: dedicated antigen-presenting cells; **CD**: cluster differentiation; **FTOCs**: fetal thymic organ cultures; **IDC**: interdigitating cells; **Ig**: immunoglobulin; **IGFBPs**: IGF-binding proteins; **LIF**: leukemia inhibitory factor; **MHC**: major histocompatibility complex; **NKA**: neurokinin A; **NOD**: non-obese diabetic; **NT**: neurotensin; **OT**: oxytocin; **TCR**: T-cell receptors; **TEC**: thymic epithelial cells; **Tg**: thyroglobulin; **TNC**: thymic nurse cells; **VP**: vasopressin signaling, the nervous, endocrine and immune systems. Neuroendocrine-immune interactions are fundamental both for the control of homeostasis, as well as for normal development in different species. The objective of this review is to show how such intimate relationships between the neuroendocrine and immune systems already occur within the thymus, the primary organ involved in T-cell lymphopoiesis. A special emphasis will be developed about neuroendocrine influences exerted on early T-cell differentiation, as well as about the cellular and molecular mechanisms by which the T-cell system is educated to tolerate neuroendocrine protein families. Recent experimental observations will be reviewed that strongly argue for an impairment of thymic T-cell tolerance as an important component for the pathogenesis of autoimmune endocrine disorders such as Type 1 diabetes and Type 3 thyroiditis (Graves' disease). Finally, a rationale hypothesis will be presented that supports the design of a tolerogenic approach based on thymic self-antigens in the prevention of autoimmune diseases.

ONTOGENY AND HISTOPHYSIOLOGY OF THE THYMUS

The thymus is a median organ in the superior mediastinum just dorsal to the sternum, and its general shape resemble the leaves of the 'thyme' plant. The major part of the thymic parenchyme is constituted by epithelial cells (TEC) which derive from (a) endoderm of the third pharyngeal pouch, and (b) ectoderm of the corresponding branchial clefts (11,81). Inclusion of ectoderm explains the heterogeneity of TEC, as well as the similar phenotype of medullary and outer cortical TEC. Interactions between the epithelial rudiment and mesenchymal cells derived from the cephalic neural crest cells are necessary for the development of proper thymic structure (6). Some human diseases and corresponding animal models are characterized by a defective thymic development which leads to primary immune deficiencies (64). The DiGeorge's syndrome includes the congenital absence (or hypoplasia) of thymus, parathyroids, and defects in the heart and truncal vessels (13). This syndrome seems to result from the failure of migration of the cephalic neural crest (12). Mice in which the homeobox *Hoxa-3* has been disrupted present thymic aplasia, parathyroid hypoplasia, and frequent defects in the heart and great vessels (46). Wild animals with immunodeficiencies most closely resembling those of DiGeorge's syndrome are nude mice with hairlessness and lack of thymic development both resulting from defect in epithelial cells. The nude phenotype is caused by mutations in a gene on murine chromosome 11 that encodes the transcription factor winged-helix nude (*whn*). In the absence of *whn*, the thymus anlage still develops but is filled with primitive epithelial cells that do not specialize and segregate into subregions (55).

Thymic nurse cells (TNC) are large epithelial cells found in the subcapsular and outer cortex of the thymus in different species. TNC contain a large number of internalized thymocytes (immature or pre-T-cells) that are not phagocytosed, but are engulfed within caveoles delineated by TNC plasma membrane (83). TNC-associated thymocytes display a high mitotic index. Functionally, TNC are involved in T-cell selection since a TNC-derived cell line was shown to induce *in vitro* deletion of thymocytes bearing transgenic TCR (62). Ultrastructural analyses have shown that TNC possess the intracellular machinery necessary for antigen processing and presentation (61).

The thymic stroma also contains non-lymphoid bone marrow-derived cellular elements: macrophages and dendritic/interdigitating cells (IDC). Macrophages are dispersed throughout thymic cortical and medullary parenchyme, while IDC are located at the cortico-medullary junction. The expression by macrophages and IDC of major histocompatibility complex (MHC) class I and class II molecules is linked to their activity as dedicated antigen-presenting cells (APC).

Thymic lymphoid cells (thymocytes) form a 'passenger' cell population of the thymus. First from yolk sac and fetal liver, then from bone marrow, T-cell progenitors are attracted and migrate within the thymus. This organ provides an appropriate and specific microenvironment for T-cell maturation. In the thymic network, several types of interactions between thymocytes and parenchymatous cells trigger T-cell proliferation, TCR gene random rearrangements, differentiation end expression of the first specific cluster differentiation (CD) T-cell marker, CD2 in humans and Thy-1 in mice. The pathways of T-cell differentiation may be followed by the differential expression of the adhesion molecule CD44 (Pgp-1) and the α chain of the IL-2 receptor, CD25, the CD3/TCR complex proteins and the co-receptor proteins, CD4 and CD8. Most steps of T-cell differentiation occur in the cortex, while thymic medulla contains mainly mature T-cells. From 100 T-cell progenitors which migrate into the thymus, about only 10 mature T-cells will leave it in a state of functional competence and self-tolerance (68). The thymus is primarily a graveyard for T-cells harboring a randomly rearranged TCR oriented against self-antigens encountered and presented in the thymus.

THE THYMIC REPERTOIRE OF NEUROENDOCRINE SELF-ANTIGEN PRECURSORS

Thymic neurohypophysial self-antigen (see complete review in 22)

Though the galactogogue action of thymic extracts had already been reported at the beginning of this century (57), it is only in the early 50's that oxytocin (OT) was identified as the primary mediator of galactokinesis (16). OT and vasopressin (VP) are nonapeptides that are synthesized by distinct neurons of the hypothalamic paraventricular and supraoptic nuclei. Hypothalamic *OT* and *VP* transcription is followed by mRNA translation into larger precursors that are processed during their axonal transport towards the neurohypophysis. This processing gives rise to the active neurohormones OT and VP, and their associated 10-kDa binding proteins neurophysins. From the neurohypophysis, OT and VP are released with neurophysins in the bloodstream. According to the neuroendocrine type of cell-cell signaling, they are transported in the bloodstream to their target receptors in the mammary myoepithelial gland and myometrium for OT, and in the kidney collecting tubules and vessel smooth muscle cells for VP.

The oxytocic activity of thymic extracts was not further characterized until 1986 when immunoreactive (IR) OT and neurophysin were identified in the human thymus (19). The other neurohypophysial hormone VP is also detected in the human thymus but IR VP concentrations are much lower (0.01-0.06 ng/g VP *versus* 2.2-18.4 ng/g OT). By immunocytochemistry (ICC) and by *in situ* hybridization, TEC/TNC from different species were shown to express neurohypophysial genes, and the use of specific monoclonal (m) Abs against OT and VP revealed a dominance of OT immunoreactivity. However, *OT* expression and OT synthesis in TEC/TNC is not associated with the secretion of the nonapeptide or neurophysin in the supernatant of human TEC primary cultures. As another argument against a classic neurosecretion of thymic OT, the peptide is not located in secretory granules but is diffuse in the cytosol, in vesicles of the endoplasmic reticulum, or associated with cytokeratin filaments (84). Similar ultrastructural features were also described for *OT*- and *VP*-expressing murine splenic eosinophils (56).

The hypothesis that thymic OT could behave as the self-antigen of the neurohypophysial family was investigated through different types of experiments. Using affinity chromatography with a mAb to the monomorphic part of MHC class I proteins, a 55-kDa protein was identified in a preparation of human TEC plasma membranes. This protein was stained both with mAb to MHC class I and with a polyclonal Ab to neurophysin. This protein is thought to be a hybrid protein with a neurophysin domain (10 kDa), and a MHC class I heavy chain domain (45 kDa). According to this interpretation, the processing of thymic OT would implicate MHC pathways for targeting to the TEC plasma membrane. By analogy with the situation in the hypothalamo-neurohypophysial axis, the neurophysin domain of the thymic OT precursor could bind and transport OT until the external limits of OT-synthesizing TEC. If this hypothesis were confirmed, two significant advantages would appear in thymic T-cell tolerance of the neurohypophysial family: 1/ the absence of a MHC allelic restriction such as in the peripheral antigen presentation by professional APC, and 2/ the presentation to immature T-cells of the classic structure of neurohypophysial peptides (a cycle of six amino acids closed by a disulfide bridge, and a linear C-terminal part of three residues). The antigenic behavior of thymic OT was further confirmed by the fact that the immunological recognition of membrane OT by specific mAbs markedly enhances the secretion of interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) in primary cultures of human TEC (48). The treatment of such cultures with a mAb to VP did not induce any significant effect on IL-6 and LIF secretion arguing for the absence of VP presentation by thymic TEC. Additional observations about the tolerogenic function of thymic OT will be presented below.

Thymic insulin-related genes

The presence of a thymic insulin-like peptide was first reported in 1965 (58) on the basis that AKR female mice spontaneously develop hypoglycemia and thymic hyperplasia associated with lymphoid leukemia. Marked hypoglycemia was induced by injection of mouse thymic extracts into female AKR mice, and this biological activity exceeded the hypoglycemic potency of similarly prepared pancreatic extracts. To the best of our knowledge, this thymic insulin-like peptide was not further characterized until independent observations showing that insulin-like growth factor (IGF) (28) and insulin (35) are expressed in the thymus. Using a panel of Abs directed against distinct insulin-related polypeptides, ICC analyses revealed that IGF-2 is the dominant member of the insulin family expressed by TEC/TNC in different species (21). The components of the IGF axis, including IGF-binding proteins (IGFBPs), have been characterized in the human thymus. Human TEC express different members of the IGF axis, with a predominance of IGF-2 and IGFBP-2 to -6 (36,37). With RT-PCR and specific primers, thymic *IGF2* transcription was found to be controlled by the same promoters as in other fetal and adult extrahepatic tissues (36,37). IR (pro)insulin was not detected in the thymic parenchyme, whereas IR IGF-1 was detected in thymic stromal cells with a macrophage-like morphology and distribution. The expression of IGF and IGF receptor genes was investigated by RT-PCR during ontogeny of the murine thymus. IGF-1, IGF-1R, M6P/IGF-2R genes are expressed in the thymus both in fetal and postnatal life, whereas IGF-2 transcripts decline after birth but remain detectable on the seventh week. By *in situ* hybridization, IGF-2 mRNAs were located in the outer cortex and medulla of the postnatal thymus, in accordance with the distribution of IR IGF-2 (38). In the human thymus, IGF-2, IGF-1 and (pro)insulin concentrations are: 96.7 ± 10.6 ng/g, 42.9 ± 5.0 ng/g, and <0.01 ng/g wet weight, respectively. No secretion of IGF-2 or IGF-1 could be evidenced in primary cultures of human TEC. By ICC and confocal microscopy, a significant part of IR IGF-2 (but neither IR IGF-1, nor IR proinsulin) was detected at the outer surface of human TEC plasma membrane. A thymic hyperplasia is observed in transgenic mice overexpressing *IGF2* under the control of the MHC H-2K^b promoter (78). Alltogether, these observations argue that the thymic insulin-like factor described by Pansky and coworkers (58) corresponds to IGF-2. The close homology between IGF-2 and proinsulin may explain a cross-reactivity with polyclonal Abs to insulin used in 1965. Similarly, the hypoglycemic effects of thymic extracts may result from the binding of thymic IGF-2 to insulin receptors. Moreover, the syndrome of hypoglycemia and lymphoid leukemia associated with thymic hyperplasia in AKR female mice might result from overexpression of *IGF2* in hyperplastic thymic epithelium leading to IGF-2 secretion into the bloodstream and disturbed thymic T-cell lymphopoiesis.

Other components of the thymic repertoire of neuroendocrine-related precursors

Neurokinin A (NKA) is the peptide of the tachykinin family expressed in human and rat TEC under the control of the preprotachykinin A gene (*PPT-A*) (17). NKA is known to exert IL-1-like mitogenic effects on murine thymocytes (71). The β and γ forms of *PPT-A* mRNA also encodes substance P (SP) in the brain, but this tachykinin is not detected in thymic epithelium suggesting a differential processing or translation of *PPT-A* mRNA in neurons and TEC. Interestingly, while IR NKA has been identified in TEC, IR SP was detected only in nerve profiles associated with thymic blood vessels. Since high affinity SP receptors have been described in association with vascular structures of rat thymic medulla (70), it is likely that neuronal SP regulates local blood flow.

IR neurotensin (NT) and somatostatin have been identified in sparse stromal cells of the chicken thymus (Sundler et al., 1978). Primary cultures of human TEC contain ± 5 ng/g IR NT/10^6 cells, of which 2.5-5% is associated with TEC plasma membranes. However, IR NT was not detected in the culture medium further questioning the classic neurosecretory model for thymic epithelium. Using anti-MHC class I affinity chromatography followed by HPLC analysis, one peak of IR NT was eluted at the same position as synthetic NT_{1-13}, together with two other NT C-terminal smaller fragments (79).

THYMIC NEUROENDOCRINE PEPTIDES AND T CELL DEVELOPMENT

Thymic OT and focal adhesion

The active role of thymic OT in a cryptocrine type of signaling between TEC and thymocytes was first evidenced by the expression of specific neurohypophysial binding sites by murine pre-T RL12-NP and cytotoxic CTL-L_2 cell lines. These binding sites behave as functional receptors since they transduced neurohypophysial signals through the phosphoinositide pathway (47). The molecular identity of these neurohypophysial receptors remains to be further precised (OT or a V1-subtype) and this point is under current investigation. Nevertheless, the KD of these receptors concords with the concentration of IR OT quantified in the human thymus, and mitogenic properties of neurohypophysial peptides are associated with the increase of inositol phosphates in pre-T-cells (47). A very recent study has underlined the importance of phosphoinositide 3-kinases in T-cell development and activation, as well as neutrophil migration (but without any significant role in the development and function of B cells) (65).

The observation of numerous points of focal adhesion between OT-producing TEC and pre-T-cells (84) prompted us to investigate the potential implication of the recently discovered focal adhesion-related kinase p125^{FAK} (30). Protein tyrosine phosphorylation is known to be an early event in T-cell activation. Western blot analysis of RL12-NP proteins probed with with anti-phosphotyrosine

(PY-20) revealed a number of proteins the phosphorylation of which increased after OT or VP treatment. OT-mediated phosphorylation was rapid and reached a maximum within 1-5 min. OT also was more potent than VP to induce phosphorylation in RL12-NP cells. Two of these proteins were precipitated with anti-FAK mAb 2A7 and were identified one as $p125^{FAK}$ and the other as a coprecipitated 130-kDa protein (most probably $p130^{CAS}$) (50). A neurohypophysial V1 antagonist inhibited OT-induced phosphorylation of $p125^{FAK}$, which demonstrates the specificity of this action but also questions the identity of the natural neurohypophysial ligand for V1 receptors expressed by T-lymphocytes. Another protein phosphorylated by OT in pre-T-cells was identified as paxillin, a 68-kDa protein located at focal adhesion sites in association with $p125^{FAK}$. Since T-cell differentiation depends on close interactions and adhesion between thymic stromal cells and thymocytes (7), the implication of focal adhesion kinases in this process surely deserves to be further investigated. Altogether, these observations largely document the model of cryptocrine signaling (18) proposed to distinguish the chemical communication between TEC/TNC and immature T-cells that migrate and differentiate at their contact.

Thymic IGFs and T-cell differentiation

Murine fetal thymic organ cultures (FTOCs) are an appropriate *in vitro* model for the study and manipulation of T-cell differentiation (4,34,63). The thymus removed from murine embryos on the 14^{th} fetal day contains the epithelial rudiment and only immature T-cell progenitors. After seven days in culture, immature T-cells differentiate along usual pathways. So, FTOCs closely mimic physiological conditions of *in vivo* T-cell differentiation. Briefly, the phenotype of early T-cell progenitors is double negative for the expression of CD4 and CD8 ($CD4^-CD8^-$). Then, they become double positive ($CD4^+CD8^+$), acquire CD3, and finally turn into the single positive cells expressing either CD4 or CD8. With the characterization of the thymic IGF axis, several observations have been reported supporting the implication of IGFs in T-cell development. Thymocytes express both types of IGF receptors (IGF-1R and M6P/IGF-2R) (26,41,80). Administration of IGF-1 stimulates stimulates lymphopoiesis and modulates the regeneration of T-lymphocytes in a rat model of dexamethasone-induced apoptosis (10,31). The thymus of *IGF2* transgenic mice displays an increase thymic cellularity, with a higher number of the $CD4^+$ T-cell subset (42). FTOC treatment with an anti-IGF-2 mAB, an anti-IGF-1R, or an anti-M6P-IGF-2R polyclonal Ab induced a blockade of T-cell differentiation at the $CD4^-CD8^-$ stage. This was evidenced by an increase of $CD4^-CD8^-$ cells and a parallel decrease in the percentage of $CD4^+CD8^+$ thymocytes. Anti-IGF-2 Ab also induced an increase in $CD8^+$ cells suggesting that thymic IGF-2 might have a role in determining differentiation into the CD4 or CD8 lineage. The strongest effects upon T-cell proliferation and differentiation were observed in FTOCs treated with anti-IGF-1R mAb. Anti-IGF-1 Ab decreased the percentage of $CD4^-CD8^-$ cells and increased the frequency in $CD4^+CD8^+$. Though the proinsulin gene is transcribed in the murine thymus (35,76), FTOC treatment with anti-(pro)insulin did not exert any significant effect on T-cell differentiation. As shown by these data, the intrathymic IGF-mediated signaling plays an active role in T-cell differentiation during ontogeny (38).

THE NATURE OF 'SELF': THYMIC NEUROENDOCRINE SELF-ANTIGENS AND SELF-TOLERANCE

During their thymic differentiation, immature T-cells randomly rearrange the gene segments coding for the variable part of their TCR. Many of these TCR recombinations may be oriented against self-antigens expressed and presented by MHC proteins in the thymic microenvironment. The interaction of self-reactive T-cells with their cognate self-antigens is thought to lead to their negative selection either by programmed cell death (apoptosis) or by developmental arrest. Thymic clonal deletion was demonstrated using mouse mammary tumor virus (MMTV)-encoded superantigens (45), and with transgenic mice expressing a TCR specific for the male antigen H-Y (40). Self-antigens are not only involved in the induction of central T-cell self tolerance but also intervene in the process of T-cell maturation and positive selection (32). The 'avidity-affinity' hypothesis has been proposed to explain this major paradox of thymic physiology, i.e. how self-antigens are able to condition both the death and the survival of T-cells? According to this hypothesis, T-lymphocytes are deleted if their TCR is strongly engaged with a self-antigen at high concentrations (10^{-6} M) and they are positively selected if their TCR is barely engaged with self-peptide at low concentrations (10^{-12} M) (3,69). However, the affinity of a TCR for its cognate antigen is rather low (10^{-8} M at maximum). Thus, it is now of crucial importance to know the nature and the amount of peptide/MHC combinations that contribute *in vivo* to T-cell negative and positive selection (1).

As another explanation to this paradox, it has been proposed that the thymic repertoire of neuroendocrine-related precursors recapitulates at the molecular level the dual role of the thymus in T-cell selection (49). Thymic polypeptide precursors engage two distinct types of interactions with pre-T-cells depending on their behaviour either as cryptocrine growth signals or as self-antigens of their family. Cryptocrine signaling implies a high-affinity (10^{-12} M) but poorly selective binding of thymic neuroendocrine peptides to their cognate receptors expressed by pre-T-cells. Such cryptocrine signaling has been shown to exist and to be mediated by OT and IGF-2 in the thymus network. Recently, other authors have also discussed the point that thymocyte selection cannot be explained only by interaction with TCR (2). On the other hand, neuroendocrine self-antigens bind to their corresponding TCR with a moderate affinity (10^{-8} M), but with a high selectivity. This latter interaction is thought to induce the central T-cell tolerance of neuroendocrine families. Neuroendocrine self-antigens usually correspond to peptide sequences of neuroendocrine precursors highly conserved throughout evolution of a given hormone family. Moreover, a hierarchy of dominance has been shown in the organization of the neuroendocrine polypeptide repertoire expressed in the thymus. This hierarchy is highly significant since self-tolerance primarily concerns self-antigenic determinants that are dominant on self-proteins (9,67). Thus, even if some members of a family (i.e. VP or proinsulin) are detected at very low levels in the thymus, the thymic tolerogenic function firstly concerns their homologous dominant thymic growth factors (i.e. OT or IGF-2, respectively). Through the central tolerance of the dominant factor however, the tolerogenic influence could be extended to the whole family.

At this point, one may say that tolerogenic actions of OT and IGF-2 have not been definitively proved. However, OT-mediated functions are known to be stronger tolerated than the VP-mediated ones. Some cases of 'idiopathic' central diabetes insipidus result in fact from an autoimmune hypothalamitis directed against VP-producing neurones (33,66). Given the implication of OT as a reproductive hormone, a stronger tolerance of the OT lineage is crucial for the preservation of the species. This conclusion is indirectly supported by the frequence and the titers of Abs obtained from active immunization (equivalent to tolerance breakdown) against neurohypophysial hormones (VP>>OT). Similar conclusions were drawn from active immunization against insulin, IGF-1 and IGF-2. Thus, in the neurohypophysial peptide family, while OT behaves as the **self**-antigen, VP is strongly suspected to be the **auto**antigen targeted by the autoimmune process leading to some forms of central diabetes insipidus. In the insulin hormone family, insulin is a major autoantigen of the autoimmune process against insulin-secreting islet β cells, and insulin immunogenicity might result from its very low expression in the thymus network.

Using RT-PCR, *in situ* hybridization and ICC, we recently investigated the ontogeny of neurohypophysial gene expression in the thymus of Balb/c mice. Transcripts of *OT* and *VP* were detected without any visible modulation in the thymus already from fetal day (FD) 14 until day 7 after birth. In the murine thymus, neurohypophysial trancripts are located in cells with an epithelial morphology and are absent in the lymphoid compartment. Because of the microscopic size of thymic rudiments before FD 14, it was not possible to analyze the earlier thymic expression of neurohypophysial genes. Nevertheless, the comparison with previous reports shows that the transcription of neurohypophysial genes in the rodent thymus precedes their expression in the magnocellular neurones of the hypothalamic-neurohypophysial axis. At the peptide level, this difference is more evident since IR OT is detected in the thymus on FD 15, whereas ICC labels IR OT in the hypothalamus only on FD 20. Thus, the expression of neurohypophysial genes in the murine thymus coincides with the appearance of T-cell progenitors and precedes their hypothalamic transcription. This observation is highly significant with regard to the physiological role proposed for thymic OT both in T-cell lymphopoiesis and in central tolerance of the hypothalamo-neurohypophysial functions. Indeed, it is logical that the induction of central self-tolerance precedes the appearance of antigenic epitopes in target organs susceptible to autoimmune aggression.

THYMUS DYSFUNCTION AND AUTOIMMUNITY

Though the relationship between lymphoepithelial structures and autoimmunity has been suspected in 1962 by Burnet and Mackay (8), the question of a defective central T-cell self tolerance in the pathophysiology of autoimmune diseases has not been intensively investigated. Also, Burnet (8) proposed that the emergence of 'forbidden' self-reactive T-cell clones could play a major role in the pathophysiology of autoimmunity. In this perspective, neonatal thymectomy prevents the emergence of diabetes in an animal model of autoimmune Type 1 diabetes, the Bio-Breeding (BB) rat (52). In clinical practice also, thymectomy induces a significant improvement in patients suffering from autoimmune myasthenia gravis (5,15). In both cases, the benefit of thymectomy can be explained by the removal of the defective thymic censorship. Such a trouble in thymic self-tolerance would be responsible for a continuous release and enrichment of the peripheral T-cell pool with intolerant and 'forbidden' self-reactive lymphocytes. The transplantation of the defective thymus from non-obese diabetic (NOD) mice to athymic nude mice induces autoimmune insulitis in the recipient (25). On the contrary, the development of autoimmune diabetes was shown to be prevented by the transplantation of thymus from diabetes-resistant to diabetes-prone BB rats (24). Recently also, insulitis and sialitis developed in athymic nude mice grafted with pure thymic epithelium from NOD mice (75). Some studies also suggest that thymic epithelium not only mediates negative selection of self-reactive T-cells but also selects regulatory T-cells helping in maintaining tissue-specific self-tolerance (51).

Autoimmune Type 1 diabetes (23)

More and more, the breakdown of immunological tolerance of insulin-secreting islet β cell is thought to be a major event in the pathophysiology of autoimmune Type 1 diabetes and several putative β cell autoantigens have been identified. In this cohort of autoantigens, insulin (and/or its precursor proinsulin) is specific of the islet β-cell-related autoantigens and has been repeatedly shown to play an important role in the development of this chronic devastating disease. To investigate the hypothesis of a defect in thymic T-cell self-tolerance of the insulin family in Type 1 diabetes, a comparative study of thymic IGFs and insulin gene expression was performed in BB rats. Noteworthy, a defect of thymic epithelium was previously observed in BB rats and was proposed to intervene in the dysfunction of thymic T-cell differentiation and in the susceptibility to autoimmune diabetes (14). The absence of thymic *IGF2* expression was evidenced in more than 80% of diabetes-prone BB rats, while IGF-1 and proinsulin mRNAs were detected in Wistar-Furth, diabetes-resistant and diabetes-prone BB rats. This defect was thymus-specific since diabetes-prone BB rat brains and livers express readily detectable IGF-2 mRNA. The defect was shown both at the IGF-2 transcript and protein levels. The absence of *IGF2* expression in the thymus of young and adult diabetes-prone BB rats might have a role in the defect of central self-tolerance of the insulin hormone family and contribute to the pathophysiology of autoimmune Type 1 diabetes (36,37,39).

Autoimmune Type 3 thyroiditis (Graves'disease)

The pathogenesis of autoimmune thyroid diseases involves several thyroid antigens including human sodium iodide symporter, thyrotropin receptor (TSH-R), thyroid peroxydase (TPO) and thyroglobulin (Tg) (82). Auto-Abs to TSH-R exerting thyroid stimulation by recognition of TSH-R are responsible for the state of hyperthyroidism in autoimmune Type 3 thyroiditis or Graves'disease (43). Thyroid-associated orbitopathy and pretibial myxedema are commonly associated in Graves'disease and TSH-R and/or a TSH-R variant (27) was shown to be expressed in orbital tissues (59) and in pretibial fibroblasts of patients with Graves'disease (74). T-cell recognition of TSH-R peptide sequence 158-176 is thought to be an early event in the initiation of the autoimmune process leading to Graves'disease (72). During their thymic development, T-cells can be educated to tolerate thyroid-related epitopes since a TSH-R variant (60), TSH-R itself, and other thyroid antigens (53,73) are expressed in the human thymus. The failure of thymic T-cell tolerance of thyroid antigens has not been demonstrated, but this hypothesis is supported by the increased thymic size and density observed by computed tomography in patients with Graves'disease (53).

CONCLUSIONS AND PERSPECTIVES IN TOLEROGENIC VACCINATION

The thymic repertoire of neuroendocrine self-peptide precursors recapitulates at the molecular level the dual physiological role played by the thymus in T-cell positive and negative selection. Consequently, this model provides an appropriate answer to the major thymic paradox in T-cell development and deletion. During the last fifteen years, the advancement of our common knowledge in thymus physiology has been impressive. It is not unreasonable to consider that the natural tolerogenic properties of the thymus could be useful in organ transplantation (including xenotransplantation) the ultimate goal of which is complete tolerance between the donor and the recipient (54). In addition, more and more experimental observations suggest that a defect in the active establishment of thymic T-cell self-tolerance is implicated as a crucial event in the pathogenesis of organ-specific autoimmune diseases. As already claimed by Lederberg in 1959 (44), the most efficient way to deal with autoimmunity is to delete it. Of course, it is neither useful nor even ethical to propose thymectomy for the prevention or treatment of chronic autoimmune diseases. However, the identification of neuroendocrine self-antigens that are presented by thymic MHC proteins could be useful, at least theoretically, to provoke deletion or inactivation of 'forbidden' self-reactive T-cell clones that have escaped the induction of thymus self-tolerance. While autoantigens are the drivers of autoimmune process, thymic self-antigens could be used to reprogram self-tolerance. In addition to classic immunogenic vaccination, perhaps will we have soon at our hand tolerogenic vaccination for the prevention of autoimmunity, the heavy tribute mainly paid by the human species for the specific efficiency of its complex system of immune defenses.

Acknowledgments – Our studies in this review have been supported by the National Fund of Scientific Research (NFSR) of Belgium, by Fondation Léon Fredericq of Liège University Medical School, by the Special Research Fund of Liège University, by the Fédération Belge contre le Cancer, by the Association Belge du Diabète, and by the Juvenile Diabetes Foundation International (JDFI).

REFERENCES

1. Allen, P.M., Peptides in positive and negative selection: a delicate balance. *Cell* 1994, **76**: 593-596.
2. Amsen, D. and Kruisbeek, A.M., Thymocyte selection: not by TCR alone. *Immunol. Rev.* 1998, **165**: 209-229.
3. Ashton-Rickardt, P.G. and Tonegawa, S., A differential-avidity model for T-cell selection. *Immunol. Today* 1994, **15**: 362-366.
4. Benoist, C. and Mathis, D., T-lymphocyte differentiation and biology. In: *Fundamental Immunology,* 4th ed., Paul, W.E. (ed.), Lippincott-Raven, Philadelphia, 1999, pp. 367-409.
5. Berrih-Aknin, S., Morel, E., Raimond, F., Safar, D., Gaud, C., Binet, J.P., Levasseur, P. and Bach, J.F., The role of the thymus in myasthenia gravis. Immunohistological and immunological studies in 115 cases. *Ann. NY Acad. Sci.* 1987, **505**: 50-70.
6. Bockman, D.E. and Kirby, M.L., Dependence of thymus development on derivatives of the neural crest. *Science* 1984, **223**: 498-500.
7. Boyd, R.L., Tucek, C.L., Godfrey, D.L., Izon, D.J., Wilson, T.J., Davidson, N.J., Bean, A.G.D., Ladyman, H.M., Ritter, M.A. and Hugo, P., The thymic microenvironment. *Immunol. Today* 1993, **14**: 445-459.
8. Burnet, F.M. and Mackay, I.R., Lymphoepithelial structures and autoimmune disease. *Lancet* 1962, **2**: 1030-1033.
9. Cabaniols, J.P., Cibotti, R., Kourilsky, P., Kosmatopoulos, K. and Kanellopoulos, J., Dose-dependent T-cell tolerance to an immunodominant self-peptide. *Eur. J. Immunol.* 1994, **24**: 1743-1749.
10. Clark, R., Strasser, J., Mc Cabe, S., Robbins, K. and Jardieu, P., Insulin-like growth factor-1 stimulation of lymphopoiesis. *J. Clin. Invest.* 1993, **92**: 540-548.
11. Cordier, A.C. and Haumont, S.M., Development of thymus, parathyroids and ultimobranchial bodies in NMRI and nude mice. *Am. J. Anat.* 1980, **157**: 227-263.
12. Couly, G., Lagrue, A. and Griscelli, C., Le syndrome de DiGeorge, neurocristopathie rhombencéphalique exemplaire. *Rev. Stomatol. Chir. Maxillofac.* 1983, **84**: 103-108.
13. DiGeorge, A.M., Congenital absence of the thymus and its immunological consequences: concurrence with congenital hypothyroidism. *Birth Defects* 1997, **4**: 116-120.
14. Doukas, J., Mordes, J.P., Swymer, C., Niedzwiecki, D., Mason, R., Rozing, J., Rossini, A.A. and Greiner, D.L., Thymic epithelial defects and predisposition to autoimmune diabetes in BB rats. *Am. J. Pathol.* 1994, **145**: 1517-1525.
15. Drachman, D.B., *Myasthenia gravis*. In: *The Autoimmune Diseases,* 3rd ed., Rose, N.R. and MacKay, I.R. (eds.), Acad. Press, San Diego, 1998, pp. 637-662.
16. du Vigneaud, V., Ressler, C., Swann, J.M., Roberts, C.W., Katsoyannis, P.G. and Gordon, S., The synthesis of oxytocin. *J. Am. Chem. Soc.* 1954, **76**: 3115-3123.
17. Ericsson, A., Geenen, V., Robert, F., Legros, J.J., Vrindts-Gevaert, Y., Franchimont, P., Brene, S. and Persson, H., Expression of preprotachykinin A and neuropeptide Y messenger RNA in the thymus. *Mol. Endocrinol.* 1990, **4**: 1211-1218.
18. Funder, J.W., Paracrine, cryptocrine, acrocrine. *Mol. Cell. Endocrinol.* 1990, **70**: C21-C24.
19. Geenen, V., Legros, J.J., Franchimont, P., Baudrihaye, M.F., Defresne, M.P. and Boniver, J., The neuroendocrine thymus: Coexistence of oxytocin and neurophysin in the human thymus. *Science* 1986, **232**: 508-511.
20. Geenen, V. and Kroemer, G., Multiple ways to cellular immune tolerance. *Immunol. Today* 1993, **14**: 573-575.
21. Geenen, V., Achour, I., Robert, F., Vandersmissen, E., Sodoyez, J.C., Defresne, M.P., Boniver, J., Lefèbvre, P.J. and Franchimont, P., Evidence that IGF-2 is the dominant thymic member of the insulin superfamily. *Thymus* 1993, **21**: 115-127.
22. Geenen, V., Kecha, O. and Martens, H., Thymic expression of neuroendocrine self-peptide precursors: Role in T-cell survival and self-tolerance. *J. Neuroendocrinol.* 1998a, **11**: 811-822.
23. Geenen, V. and Lefèbvre, P.J., The intrathymic expression of insulin-related genes: implications for pathophysiology and prevention of Type 1 diabetes. *Diabetes Metabol. Rev.* 1998b, **14**: 95-103.
24. Georgiou, H.M. and Bellgrau, D., Thymus transplantation and disease prevention in the diabetes-prone Bio-Breeding rat. *J. Immunol.* 1989, **142**: 3400-3405.
25. Georgiou, H.M. and Mandel, T.E., Induction of insulitis in athymic (nude) mice: the effect of NOD thymus and pancreas transplantation. *Diabetes* 1995, **44**: 49-59.
26. Goxe, B., Martens, H., Vandersmissen, E., Achour, I., Kecha, O. and Geenen, V., Interactions entre les cellules T et la famille insulinique: étude du récepteur de l'IGF-II. *Ann. Endocrinol.* Paris 1995, **56**: 399, abstract.
27. Graves, P.N., Tomer, Y. and Davies, T.F., Cloning and sequencing of a 1.3 kb variant of human thyrotropin receptor variant transcripts in retroocular and other tissues. *Biochem. Biophys. Res. Commun.* 1992, **187**: 1135-1143.
28. Han, V.K.M., D'Ercole, J. and Lund, P.K., Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science* 1987, **236**: 193-197.
29. Hedrick, S.M., Cohen, D.I., Nielsen, E.A. and Davis, M.M., Isolation of cDNA clones encoding T-cell-specific membrane-associated proteins. *Nature* 1984, **308**: 149-152.
30. Hildebrand, J.D., Schaller, M.D. and Parsons, J.T., Identification of sequences required for the efficient localization of the focal adhesion kinase, pp125FAK, to cellular focal adhesions. *J. Cell Biol.* 1993, **123**: 993-1005.

31. Hinton, P.S., Peterson, C.A., Dahly, E.M. and Ney, D.M., IGF-I alters lymphocyte survival and regeneration in thymus and spleen after dexamethasone treatment. *Am. J. Physiol.* 1998, **274**: R912-R920.

32. Hogquist, K.A., Jameson, S.C. and Bevan, M.J., The ligand for positive selection of T-lymphocytes in the thymus. *Curr. Opin. Immunol.* 1994, **6**: 273-278.

33. Imura, H., Nakao, K., Shimatsu, A., Ogawa, Y., Sando, T., Fujisawa, I. and Yamabe, H., Lymphocytic infundibulo-neurohypophysitis as a cause of central diabetes insipidus. *N. Engl. J. Med.* 1993, **329**: 683-689.

34. Jenkinson, E.J. and Owen, J.J., T-cell differentiation in organ cultures. *Semin. Immunol.* 1990, **2**: 51-58.

35. Jolicœur, C., Hanahan, D. and Smith, K.M., T-cell tolerance toward a transgenic β-cell antigen and transcription of endogenous pancreatic genes in the thymus. *Proc. Natl. Acad. Sci. USA* **91**: 6707-6711.

36. Kecha, O., Martens, H., Franchimont, N., Achour, I., Hazée-Hagelstein, M.T., Renard-Charlet, C., Geenen, V. and Winkler, R., Characterization of the insulin-like growth factor axis in the human thymus. *J. Neuroendocrinol.* 1999a, **11**: 435-440.

37. Kecha, O., Winkler, R., Martens, H., Collette, J., Lefèbvre, P.J., Greiner, D.L. and Geenen, V., Thymic insulin-related polypeptides in diabetes-prone Bio-Breeding rats. *Diabetologia* 1999b, **42**(Suppl. 1): A29, abstract 106.

38. Kecha, O., Brilot, F., Martens, H., Franchimont, N., Renard, C., Greimers, R., Defresne, M.P., Winkler, R. and Geenen, V., Involvement of insulin-like growth factors in early T-cell development: a study using fetal thymic organ cultures. *Endocrinology* 2000, **141**: 1209-1217.

39. Kecha-Kamoun, O., Achour, I., Martens, H., Colette, J., Lefèbvre, P.J., Greiner, D.L. and Geenen, V., Thymic expression of insulin-related genes in an animal model of autoimmune Type 1 diabetes. *Diab./Metabol. Res. Rev.* 2001, in press, accepted December 5, 2000.

40. Kisielow, P., Blüthmann, H., Staerz, U., Steinmetz, M. and von Boehmer, H., Tolerance in T-cell receptor transgenic mice involves deletion of non mature $CD4^+CD8^+$ thymocytes. *Nature* 1988, **333**: 742-745.

41. Kooijman, R., Scholtens, L.E., Rijkers, G.T. and Zegers, B.J.M., Type 1 insulin-like growth factor receptor expression in different developmental stages of human thymocytes. *J. Endocrinol.* 1995a, **147**: 203-209.

42. Kooijman, R., van Buul-Offers, S.C., Scholtens, L.E., Schuurman, H.J., Van den Brande, J.L. and Zegers, B.J., T-cell development in insulin-like growth factor-II transgenic mice. *J. Immunol.* 1995b, **154**: 5736-5745.

43. Larsen, P.R., Davies, T.F. and Hay, I.D., The thyroid gland. In: *Williams Textbook of Endocrinology*, Wilson, J.D., Foster, D.W., Kronenberg, H.M. and Larsen, P.R. (eds.), Saunders, Philadelphia, 1998, pp. 389-515.

44. Lederberg, J., Genes and antibodies. *Science* 1959, **129**: 1649-1652.

45. MacDonald, H.R., Glasebrook, A.L., Schneider, R., Lees, R.L., Pircher, H., Pedrazzini, T., Kanagawa, O., Nicolas, J.F., Howe, R.C., Zinkernagel, R.M. and Hengartner, H., T-cell reactivity and tolerance to Mls-encoded antigens. *Immunol. Rev.* 1989, **107**: 89-108.

46. Manley, N.R. and Capecchi, M.R., The role of *Hoxa-3* in mouse thymus and thyroid development. *Development* 1995, **121**: 1989-2003.

47. Martens, H., Robert, F., Legros, J.J., Geenen, V. and Franchimont, P., Expression of functional neurohypophysial peptide receptors by murine immature and cytotoxic T-cell lines. *Prog. Neuro. Endocrinol. Immunol.* 1992, **5**: 31-39.

48. Martens, H., Malgrange, B., Robert, F., Charlet, C., De Groote, D., Heymann, D., Godard, A., Soulillou, J.P., Moonen, G. and Geenen, V., Cytokine production by human thymic epithelial cells: control by the immune recognition of the neurohypophysial self-antigen. *Regul. Pept.* 1996a, **67**: 39-45.

49. Martens, H., Goxe, B. and Geenen, V., The thymic repertoire of neuroendocrine-related self-antigens: physiological implications in T-cell life and death. *Immunol. Today* 1996b, **17**: 312-317.

50. Martens, H., Kecha, O., Charlet-Renard, C., Defresne, M.P. and Geenen, V., Neurohypophysial peptides stimulate the phosphorylation of pre-T-cell focal adhesion kinases. *Neuroendocrinology* 1998, **67**: 282-289.

51. Modigliani, Y., Coutinho, A., Pereira, P., Le Douarin, N.M., Thomas-Vaslin, V., Burlen-Defranoux, O., Salaün, J. and Bandeira, A., Establishment of tissue-specific tolerance is driven by regulatory T-cells selected by thymic epithelium. *Eur. J. Immunol.* 1996, **26**: 1807-1815.

52. Mordes, J.P., Bortell, R., Doukas, J., Rigby, M., Whalen, B., Zipris, D., Greiner, D.L. and Rossini, A.A. The BB/Wor rat and the balance hypothesis of autoimmunity. *Diab.-Metabol. Rev.* 1996, **12**: 103-109.

53. Murakami, M., Hosoi, Y., Negishi, T., Kamiya, Y., Miyashita, K., Yamada, M., Iriuchijima, T., Yokoo, H., Yoshida, Y. and Mori, M., Thymic hyperplasia in patients with Graves'disease. Identification of thyrotropin receptors in human thymus. *J. Clin. Invest.* 1996, **98**: 2228-2234.

54. Murray, J.E., Organ transplantation and the revitalization of immunology. In: *Immunology, the Making of a Modern Science.* Gallagher, R.B., Gilder, J., Nossal, G.J.V. and Salvatore, G. (eds.), Acad. Press, San Diego, 1995, pp. 179-189.

55. Nehls, M., Kyewsky, B., Messerle, M., Waldschütz, R., Schüddekopf, K., Smith, A.J. and Boehm, T., Two genetically separable steps in the differentiation of thymic epithelium. *Science* 1996, **272**: 886-889.

56. Kumamoto, K., Matsuura, T., Amagai, T. and Kawata, M., Oxytocin-producing and vasopressin-producing eosinophils in the mouse spleen: immunohistochemical, immuno-electron-microscopic and *in situ* hybridization studies. *Cell Tissue Res.* 1995, **281**: 1-10.

57. Ott, I. and Scott, J.C., The galactogogue action of the thymus and corpus luteum. *Proc. Soc. Exp. Biol. Med.* 1910, **8**: 48.

58. Pansky, B., House, E.L. and Cole, L.A., An insulin-like thymic factor. A preliminary report. *Diabetes* 1965, **14**: 325-332.

59. Paschke, R., Metcalfe, A., Alcalde, L., Vassart, G., Weetman, A.P. and Ludgate, M., Presence of a non-functional thyrotropin receptor variant transcripts in retroocular and other tissues. *J. Clin. Endocrinol. Metabol.* 1994, **79**: 1234-1238.

60. Paschke, R. and Geenen, V., Messenger RNA expression for a TSH receptor variant in the thymus of a two-year child. *J. Mol. Med.* 1995, **73**: 577-580.

61. Penninger, J., Rieker, T., Romani, N., Klima, J., Salvenmoser, W., Dietrich, H., Stössel, H. and Wick, G., Ultrastructural analysis of thymic nurse cell epithelium. *Eur. J. Immunol.* 1993, **24**: 222-228.

62. Pircher, H., Brduscha, K., Steinhoff, U., Kasai, M., Mizuochi, T., Zinkernagel, R.M., Hengartner, H., Kyewsky, B. and Muller, K.P., Tolerance induction by clonal deletion of $CD4^+8^+$ thymocytes *in vitro* does not require dedicated antigen-presenting cells. *Eur. J. Immunol.* 1993, **23**: 669-674.

63. Plum, J., De Smedt, M., Leclercq, G. and Vandekerckhove, B., Influence of TGF-β on murine thymocyte development in fetal thymus organ culture. *J. Immunol.* 1995, **154**: 5789-5798.

64. Rosen, F.S., Cooper, M.D. and Wedgwood, R.J., The primary immunodeficiencies. *N. Engl. J. Med.* 1995, **333**: 431-440.

65. Sasaki, T., Irie-Sasaki, J., Jones, R.G., Oliveira-dos-Santos, A.J., Stanford, W.L., Bolon, B., Wakeham, A., Itie, A., Bouchard, D.,

Kozieradzki, I., Joza, N., Mak, T.W., Ohashi, P.S., Suzuki, A. and Penninger, J.W., Function of PI3Kγ in thymocyte a-development, T-cell activation and neutrophil migration. *Science* 2000, **287**: 1040-1046.

66. Scherbaum, W.A. and Bottazzo, G.F., Autoantibodies to vasopressin cells in idiopathic diabetes insipidus: evidence for an autoimmune variant. *Lancet* 1983, **1**: 897-901.
67. Schneider, S.C., Deng, H.K., Rottinger, B., Sharma, S., Stolima, M., Bonpane, C., Miller, A., Dubinett, S., Kyewski, B. and Sercarz, E., Tolerance and determinant hierarchy. *Novartis Found. Symp.* 1998, **215**: 54-66.
68. Scollay, R., Butcher, E.C. and Weissman, I.L., Thymus cell migration. Quantitative aspects of cellular traffic from the thymus to the periphery in mice. *Eur. J. Immunol.* 1980, **10**: 210-218.
69. Sebzda, E., Wallace, V.A., Mayer, J., Young, R.S.M., Mak, T. and Ohashi, P.S., Positive and negative selection induced by different concentrations of a single peptide. *Science* 1994, **263**: 1615-1618.
70. Shigematsu, K., Saavedra, J.M. and Kurihara, M., Specific SP binding sites in rat thymus and spleen: *in vitro* autoradiographic study. *Regul. Pept.* 1986, **16**: 147-156.
71. Söder, O. and Hellström, P.M., The tachykinins neurokinin A and physalaemin stimulate murine thymocyte proliferation. *Int. Arch. Allergy Appl. Immunol.* 1989, **90**: 91-96.
72. Soliman, M., Kaplan, E., Guimaraes, V., Yanagawa, T. and DeGroot, L.J., T-cell recognition of residue 158-176 in thyrotropin receptor confers risk for development of thyroid autoimmunity in siblings in a family with Graves'disease. *Thyroid* 1996, **6**: 545-551.
73. Spitzweg, C., Joba, W. and Heufelder, A.E., Expression of thyroid-related genes in human thymus. *Thyroid* 1999, **9**: 133-141.
74. Stadlmayr, W., Spitzweg, C., Bichlmair, A.M. and Heufelder, A.E., TSH receptor transcripts and TSH receptor-lie immunoreactivity in orbital and pretibial fibroblasts of patients with Graves'ophtalmopathy and pretibial myxedema. *Thyroid* 1997, **7**: 3-12.
75. Thomas-Vaslin, V., Damotte, D., Coltey, M., Le Douarin, N.M., Coutinho, A. and Salaün, J., Abnormal T-cell selection on nod epithelium is sufficient to induce autoimmune manifestations in C57BL/6 athymic nude mice. *Proc. Natl. Acad. Sci. USA* 1997, **94**: 4598-4603.
76. Throsby, M., Homo-Delarche, F., Chevenne, D., Goya, R., Dardenne, M. and Pleau, J.M., Pancreatic hormone expression in the murine thymus: localization in dendritic cells and macrophages. *Endocrinology* 1998, **139**: 2399-2406.
77. Tonegawa, S., Maxam, A.M., Tizard, R., Bernard, O. and Gilbert, W., Sequence of a mouse germ-line gene for a variable region of an immunoglobulin light chain. *Proc. Natl. Acad. Sci. USA* 1978, **75**: 1485-1489.
78. van Buul-Offers, S.C., de Haan, K., Reijnen-Gresnigt, M.G., Meinsma, D., Jansen, M., Oei, S.L., Bonte, E.J., Sussenbach, J.S. and Van den Brande, J.L., Overexpression of human insulin-like growth factor II in transgenic mice causes increased growth of the thymus. *J. Endocrinol.* 1995, **144**: 491-502.
79. Vanneste, Y., Ntodou Thome, A., Vandersmissen, E., Charlet, C., Franchimont, D., Martens, H., Lhiaubet, A.M., Schimpff, R.M., Rostène, W. and Geenen, V., Identification of neurotensin-related peptides in human thymic epithelial cell membranes and relationship with MHC class I molecules. *J. Neuroimmunol.* 1997, **76**: 161-166.
80. Verland, S. and Gammeltoft, S., Functional receptors for insulin-like growth factors I and II in rat thymocytes and mouse thymoma cells. *Mol. Cell. Endocrinol.* 1989, **67**: 207-216.
81. Von Gaudecker, B., Functional histology of the human thymus. *Anat. Embryol.* 1991, **183**: 1-15.
82. Weetman, A.P., Autoimmune thyroid disease. In: *The Autoimmune Diseases,* 3rd ed., Rose, N.R. and Mackay, I.R. (eds.), Acad. Press, San Diego, 1998, pp. 405-430.
83. Wekerle, H. and Ketelsen, U.P., Thymic nurse cells – Ia-bearing epithelium involved in T-lymphocyte differentiation? *Nature* 1980, **283**: 402-404.
84. Wiemann, M. and Ehret, G., Subcellular localization of immunoreactive oxytocin within thymic epithelial cells of the male mouse. *Cell Tissue Res.* 1993, **273**: 79-s87.