Application for the 2012 Delbert A. Fischer Research Scholar Award

Vincent GEENEN, MD, PhD

The Presentation of Neuroendocrine Self-peptides in the Thymus: A Necessity for an Integrated Evolution of the Neuroendocrine and Immune systems

Vincent GEENEN, MD, PhD

University of Liege Center of Immunology, Institute of Pathology CHU-B23,

B-4000 Liege-Sart Tilman, Belgium

Abstract

Immunoendocrinology was recognized as a scientific field early in the 20th century, soon after Paul Ehrlich identified immunology as a specific domain of scientific investigation. By the 1930s, Hans Selye introduced the concept of stress-induced and adrenal cortex-mediated thymus involution with secondary immunosuppression. The dissection of the intricate cellular and molecular interactions between the major systems of cell-to-cell signaling — the neuroendocrine and immune systems — restarted in the 1980s but this scientific domain received only gradual acceptance by the scientific community. Endocrinologists did not hesitate to widely open the door to this new field and provided the first robust experimental arguments for its fundamental relevance to physiology. The immune system may be considered as a sensory organ able to respond to different kinds of danger signals that are not detected by nervous cells. The immune response is not autonomous but regulated by the central and peripheral nervous systems, as well as by neuropeptides, vitamin D and neuroendocrine axes such as the corticotrope, somatotrope, thyrotrope and gonadotrope axes. During evolution, the thymus emerged concomitantly with recombinase-dependent adaptive immunity as an 'immune brain' or a 'master class' highly specialized in the orchestration of central immunological self-tolerance. This was an absolute requirement for survival of species because of the high potential of autotoxicity inherent to the stochastic generation of extreme diversity characterizing this novel adaptive type of immune defenses against non-self. The true nature of the thymus now appears to be a crossroad necessary for the integrated evolution of the major systems of cell-to-cell signaling, the neuroendocrine and immune systems. For most of them, the presentation of neuroendocrine self-antigens by thymic major histocompatibility complex (MHC) proteins is controlled by the autoimmune regulator (AIRE) gene/protein, and is responsible for negative selection of self-reactive T cells. In the same time, by still unexplained mechanisms, MHC presentation of the same self-peptides in the thymus promotes the selection of self-specific FOXP3+CD4+CD25+ natural regulatory T cells (nTreg) that are able to inhibit in periphery self-reactive CD4+ and CD8+ T cells having escaped the thymus censorship. Moreover, an impairment of these thymic selective processes is now established as a primary event driving the development of endocrine-specific autoimmunity. Our novel knowledge about thymus physiology and physiopathology already paves the way for the development of various innovative and efficient immunomodulating strategies in pharmacology.

A moving place for the thymus in the history of medicine

Figure 1 - Claude Galen

Claude Galen (129 – 210 or 216 AD) first described an organ located behind the sternum that he named 'thymus' because of its close resemblance with the leaf of the thyme plant (Fig. 1). For Galen, the thymus was the seat of soul, eagerness, and fortitude, and this old misconception most probably explains why some terms like '*troubles thymiques*' are still used in the French medical language to designate mood disorders such as those observed in unipolar and bipolar depressive diseases. Jacobus Berengarius Carpensis (1460-1530) then provided the first complete anatomical description of the thymus in his work entitled '*Anatomia Carpi. Isagoge breves perlucide ac uberime, in anatomiam humani corporis*'.

For a very long time, the thymus was considered as a useless vestigial organ that had become redundant during both phylogeny as well as human ontogeny after puberty. It is only in the early 1900s' that the first 'thymologist' J. August Hammar initiated in Sweden biomedical research focusing on this organ [1]. His pioneering work was followed by numerous studies that highlighted the important neuroendocrine regulation of the thymus, in particular by the hypothalamo-hypophysial axis, thyroid hormones, adrenal and sex steroids. For a long time, the thymus was considered as a gland and thus an intrinsic component of the endocrine system (Fig. 2). However, despite the identification of several thymic 'hormones', the endocrine model failed to apply to accurately describe the dialogue between thymic stromal cells and thymocytes (thymic T cells).

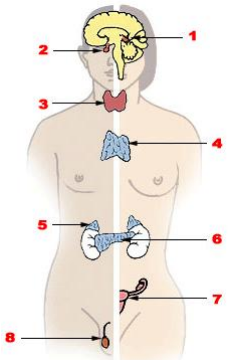

Figure 2 - The thymus when considered as an endocrine gland

1. Pineal gland
2. Hypophysis
3. Thyroid
4. *Thymus*
5. Adrenals
6. Pancreas
7. Ovary
8. Testis

Starting in 1959 and in parallel with the mounting evidence of its important role in immunity [2], one can distinguish the following milestones leading to our current knowledge in thymus physiology:

- Role of the thymus in mouse leukemia and in T-cell development [3,4].
- Developmental biology of and self-recognition by differentiating T cells in the thymus [5,6].
- Promiscuous transcription in thymus epithelium of genes encoding neuroendocrine-related and peripheral tissue-restricted antigens [7-11].
- Identification of the autoimmune regulatory (Aire) gene/protein as a transcription-like factor controlling promiscuous gene expression in thymus epithelium [12-14].
- Intrathymic selection of self-antigen specific natural regulatory T cells (nTreg) [15-17].
- Embryology of the thymus and deciphering of the lympho-stromal interactions required for T-cell differentiation in the primary lymphoid organ [18-20].

Emergence of the thymus in evolution

In all living species, the neuroendocrine and innate immune systems have evolved in parallel and still coexist without any apparent problem (Fig. 3). Indeed, Toll-like receptors (TLR) that are the most important mediators of innate immunity do not have the capacity of reaction against normal self. Some anticipatory immune responses already exist in jawless vertebrates (agnathans), and are mediated by diverse variable lymphocyte receptors (VLR), with 4-12 leucine-rich repeat modules assembled by a gene conversion process. Some 450-500 millions years ago, the emergence of transposon-like recombination activating genes *RAG1* and *RAG2* in jawed vertebrates (gnathostomes) promoted the development of adaptive immunity [21-23]. The appearance of *RAG1* and *RAG2* in the genome of jawed vertebrates (most putatively via horizontal transmission), and the subsequent appearance of the combinatorial immune system, has been assimilated to the immunology's 'Big Bang'. Gene recombination in somatic lymphoid cells is responsible for the random generation of the extreme diversity of immune receptors for antigens, B-cell- ($\pm 5 \times 10^{13}$ BCR combinations) and T-cell receptors ($\pm 10^{18}$ TCR combinations). Because of its inherent autotoxicity, the emergence of this sophisticated type of immune response exerted an evolutive pressure so powerful that, in concordance with Paul Ehrlich's concept and prediction of *'horror autotoxicus'*, novel structures and mechanisms appeared with a specific role in the setting-up of immunological self-tolerance. Of note, the first thymus appeared in cartilaginous-jawed fishes but was preceded by thymus-like lympho-epithelial structures in the gill baskets of lamprey larvae as very recently demonstrated [24]. These structures named '*thymoids*' express the gene encoding forkhead box N4 (FOXN4), the orthologue of FOXN1.

FOXN1 is the transcription factor specific for the differentiation of thymus epithelium in jawed vertebrates, and *Foxn1* mutation is responsible for the 'nude' phenotype in mouse. Therefore, FOXN1 stands at a crucial place in the development of thymus epithelium that is an absolute requirement for T-cell differentiation. Moreover, the same study has provided strong evidence for a functional analogy between VLR assembly in these thymoids and TCR recombination in the thymus. This important discovery opens the questions about the potential existence of autoimmune-like responses in jawless vertebrates.

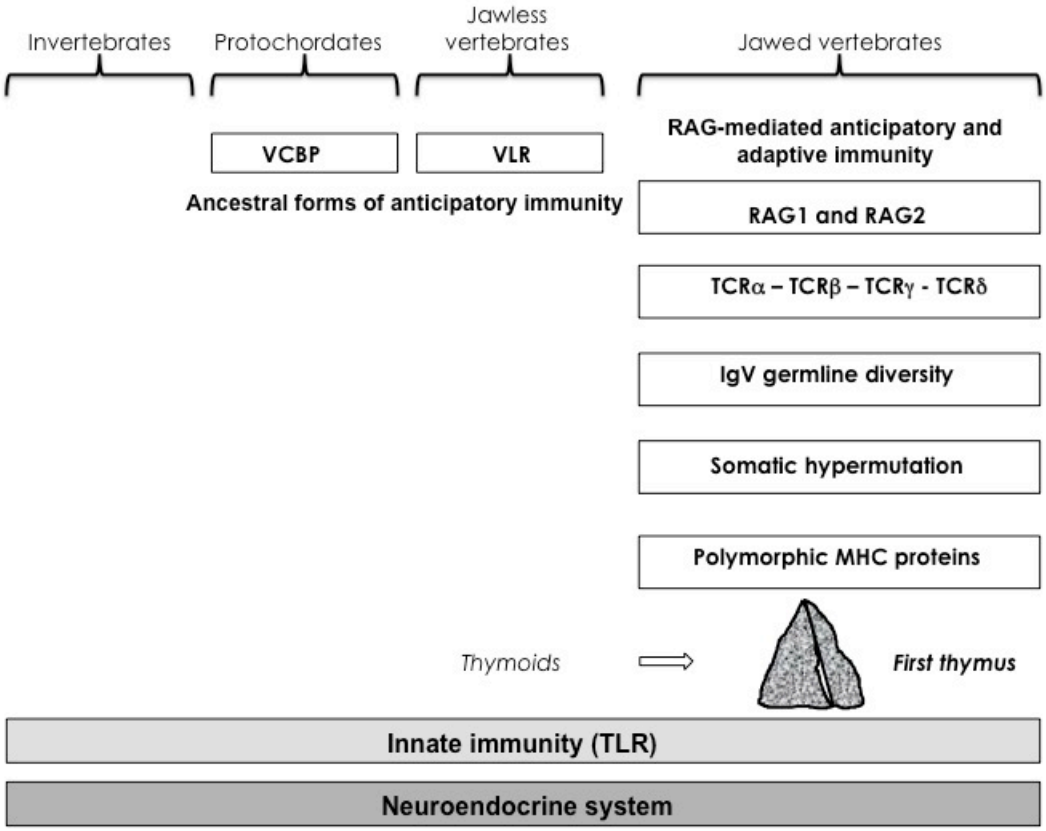

Figure 3 - Integrated evolution of the neuroendocrine system, innate and RAG-dependent immunity.
Essential components of the neuroendocrine system have been established long ago and did not display important variation during evolution besides gene duplication or differential RNA splicing. The appearance of RAG-dependent adaptive immunity in jawed vertebrates was associated with a high risk of autotoxicity directed against the neuroendocrine system. Of note, from ancestor lamprey thymoids, the first unique thymus emerged quite concomitantly in jawed fishes, and the intrathymic presentation of neuroendocrine-related genes may be viewed *a posteriori* as a very efficient and economic way for instructing the adaptive T-cell system to tolerate neuroendocrine antigens as early as during intrathymic T-cell development and differentiation.
VCBP, variable-region-containing chitin-binding protein; VLR, variable lymphocyte receptor; TCR, T-cell receptor.

Thymus-dependent immunological self-tolerance

Two essential and closely associated mechanisms are responsible for establishing the thymus-dependent central arm of self-tolerance: 1) Negative selection of self-reactive T cells that are stochastically generated by recombinase-dependent generation of TCR diversity in the thymus, and 2) Positive selection of self-specific natural regulatory T cells (nTreg), which are able to inactivate in periphery self-reactive T cells having escaped thymic negative selection. Today, the major unresolved question is to understand the precise mechanisms by which the same associations of self-antigens and thymic major histocompatibility complex (MHC) proteins are able to mediate both negative selection of self-reactive T cells and generation of self-specific nTreg [reviewed and discussed in 25].

Another question has long concerned the nature of self that is presented in the thymus to differentiating T cells during fetal life. Since its formulation some 75 years ago, 'self' has been a seminal word coined in immunological language as a fecund metaphor with some equivocal correlations to philosophy and neurocognitive sciences. For unknown reasons, there was no serious attempt to elucidate the precise identity of self before a series of studies in the late 1980s and in the 1990s [7,26-31]. Our personal contribution in this field was to define the biochemical nature of the neuroendocrine self. First, thymic neuroendocrine self-antigens usually correspond to peptide sequences that have been mostly conserved throughout evolution of their related protein family. Second, a hierarchy characterizes their expression profile in the thymus as one dominant member synthesized in thymus epithelium represents its related neuroendocrine family during presentation to differentiating T lymphocytes (*i.e.* oxytocin [OT] for the neurohypophysial family, neurokinin A for tachykinins, neurotensin for neuromedins, corticostatin for somatostatins, and insulin-like growth factor 2 [IGF-2] for the insulin family). This hierarchical pattern is meaningful because the strength of immunological tolerance to a protein is proportional to its intrathymic concentration [32]. Third, and most importantly, following Aire-regulated gene transcription, thymic neuroendocrine precursors are not processed according to the classic model of neuroendocrine secretion but they undergo an antigen processing for membrane targeting and presentation by, or in association with, thymic MHC proteins [29]. Fourth, according to the cryptocrine model of cell-to-cell signaling [33], thymic T cells express functional neuroendocrine cognate receptors [34]. For example, binding of OT to its receptor expressed by thymic pre-T cells was shown to phosphorylate focal adhesion kinases [35], and this could promote the establishment of immunological 'synapses' between thymic epithelial cells and T cells. Finally, for some of them, their transcription in the thymus precedes their eutopic expression in neuroendocrine glands [34].

This hierarchy in the organization of the thymic repertoire of neuroendocrine self-antigens is also significant from an evolutionary point of view. Since many major physiological functions had been established before the emergence of the anticipatory adaptive immune response in jawless fishes, they had to be protected from the risk of autoimmunity inherent to this type of immune lottery. OT is a hypothalamic neuropeptide that is closely implicated at different steps of the reproductive process, starting from social affiliation and bonding to control of parturition and lactation. Thus, OT is fundamental for preservation of animal and human species. Through its dominant expression in thymus epithelium, OT is more tolerated than its hypothalamo-neurohypophysial homologue vasopressin, which essentially controls water homeostasis. Interestingly, rare cases of autoimmune hypothalamitis with vasopressin deficiency and diabetes insipidus have been repeatedly observed [36], whereas autoimmunity towards hypothalamic oxytocinergic neurons has never been reported. A similar reasoning may be applied to the members of the insulin family, IGF-2, IGF-1 and insulin itself. There is no report of autoimmunity against IGF-2, the dominant thymic self-peptide of the insulin family during fetal life, whereas insulin is the primary autoantigen of type 1 diabetes. Because of their close homology, thymic neuroendocrine self-antigens promote immunological cross-tolerance to their whole family, and tolerance to insulin was indeed shown to be weaker in *Igf2*$^{-/-}$ mice than in wild-type mice [37].

A thymus dysfunction as a primary event in the development of autoimmune endocrine diseases

As already theorized by Burnet, the pathogenesis of autoimmune diseases may first depend on failure of self-tolerance and the development of 'forbidden' self-reactive immune clones [38]. The progressive increase in immune complexity during evolution is associated with a higher incidence of self-tolerance failures, most of them occurring in the human species. There is more and more evidence that a dysfunction in the mechanisms responsible for thymus-dependent dominant and recessive self-tolerance is playing a primary role in the development of the autoimmune response toward many organs, in particular endocrine glands [39]. Thymus transplantation from non-obese diabetic (NOD) mice, an animal model of type 1 diabetes, was shown to induce diabetes in normal recipients [40]. *Igf2* transcription is deficient in the thymus of diabetes-prone Bio-Breeding (DPBB) rats, another animal model of type 1 diabetes, and such defect might contribute to both the absence of tolerance to β cells and the usual lymphopenia (including RT6+ Treg) observed in these animals [41]. Mice with thymus-restricted insulin defect develop strong proinsulin-specific T-cell reactivity [42]. Loss-of-function *Aire* single mutations are

responsible for a very rare autosomal recessive disease named autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED) or autoimmune polyglandular syndrome type 1 (APS-1). Depending on their genetic background, *Aire*$^{-/-}$ mice exhibit several signs of peripheral autoimmunity, which are associated with a significant decrease in the level of intrathymic neuroendocrine gene transcription, including those encoding oxytocin, insulin and IGF-2 [14, 43]. Of note with regard to autoimmune thyroiditis, which is the most frequent autoimmune disease, all major thyroid-related antigens (thyroperoxydase, thyroglobulin and thyrotropin receptor [TSHR]) are also transcribed by thymic epithelium in normal conditions [30,44]. Thymic hyperplasia is commonly observed in Graves' disease [1,45], and it was recently shown that homozygotes for an SNP allele predisposing to Graves' disease have significantly lower intrathymic *TSHR* transcripts than carriers of the protective allele [46]. Another recent credit to a defective central tolerance in organ-specific autoimmune disease was provided by a very elegant study showing the central role played by a defect of intrathymic α-myosin expression in autoimmune myocarditis in mice and humans [47]. Our current in-depth knowledge in thymus physiology and physiopathology will translate very soon into the design of innovative tolerogenic and regulatory strategies (such as the concept of negative 'self-vaccination' [48,49]) aimed at restoring central self-tolerance that is defective in autoimmunity, the heavy price paid by so many patients for preserving human self against non-self.

Thymus involution and immunosenescence

Aging of the immune system (immunosenescence) is characterized by a higher susceptibility to infections, an increase in the incidence of cancer, as well as a decrease in response to vaccines. Although thymopoiesis (generation of naïve T cells) is maintained until late in life, thymus adipose involution has been long considered as 'the' hallmark of immunosenescence. Thymic involution is associated with a marked decrease in the generation of diverse T cells (in particular naïve CD4+ T cells), an expansion of memory CD8+ T cells, and a diminished influence of thymus-dependent central self-tolerance. Involution of the thymus after hypophysectomy was one of the first evidences for the control of the immune system by a neuroendocrine gland [50]. Numerous studies have unambiguously demonstrated that growth hormone (GH) is able to reverse the age-dependent involution of the thymus [51-53]. The intrathymic proliferation of T-cell precursors and thymic output of naïve T cells are significantly decreased in adults with GH deficiency, and GH replacement restores these two parameters [54]. Today, the restoration of thymus function appears as an important objective in the elderly, as well as in patients suffering with AIDS or several hematological diseases [55,56]. It can now be anticipated that GH, IGF-1,

GH secretagogues (such as ghrelin), GH and ghrelin receptor agonists, as well as other thymus-specific growth factors will be used in the near future for regenerating thymopoiesis and thymus tolerogenic function as well as, secondarily, several immune functions including responses to vaccines in aged and other immunocompromised patients.

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Vincent Geenen is research director at the FRS, professor of Developmental biology and History of biomedical research at the University of Liege, and Clinical Head at the division of Endocrinology at the University Hospital of Liege (Belgium).

References

1. Hammar JA. The new views as to the morphology of the thymus gland and their bearing on the problem of the function of the thymus. *Endocrinology* 1921; 5: 543-73.
2. Burnet FM. Role of the thymus and related organs in immunity. *Br Med J* 1962; 2: 807-11.
3. Miller JFAP. Role of the thymus in murine leukaemia. *Nature* 1959; 183: 1069.
4. Miller JFAP. Immunological function of the thymus. *Lancet* 1961; 2: 748-9.
5. Kisielow P, Blüthmann H, Staerz UD, Steinmetz M, von Boehmer H. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+CD8+ thymocytes. *Nature* 1988; 333: 156-9.
6. Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. *Cell* 1988; 49: 273-80.
7. Geenen V, Legros JJ, Franchimont P, Baudrihaye M, Defresne MP, Boniver J. The neuroendocrine thymus: Coexistence of oxytocin and neurophysin in the human thymus. *Science* 1986; 232: 508-11.
8. Geenen V, Robert F, Legros JJ *et al.* Neuroendocrine-Immunology: from systemic interactions to the immune tolerance of self neuroendocrine functions. *Acta Clin Belg* 1991; 46: 135-41.
9. Geenen V, Goxe B, Martens H *et al.* Cryptocrine signaling in the thymus network and T cell education to neuroendocrine self-antigens. *J Mol Med* 1995; 73: 449-55.
10. Martens H, Goxe B, Geenen V. The thymic repertoire of neuroendocrine-related self-antigens: Physiological implications in T-cell life and death. *Immunol Today* 1996; 17: 312-7.
11. Kyewski B, Klein L. A central role for central tolerance. *Annu Rev Immunol* 2006; 24: 571-606.
12. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S *et al.* Positional cloning of the APECED gene. *Nat Genet* 1997; 17: 393-8.
13. Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genetics* 1997; 17: 399-403.
14. Anderson MS, Venanzi ES, Klein L *et al.* Projection of an immunological self shadow in the thymus by the Aire protein. *Science* 2002; 298: 1395-401.
15. Sakagushi S, Sakagushi N, Asano N, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; 155: 1151-64.
16. Fontenot JD, Gavin MA, Rudensky A. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4: 330-6.
17. Heddon B, Mason D. The third function of the thymus. *Trends Immunol* 2000; 21: 95-9.
18. Bennett AR, Farley A, Blair NF, Gordon J, Sharp L, Blackburn CC. Identification and characterization of thymic epithelial progenitor cells. *Immunity* 2002; 16: 803-14.
19. Rossi S, Jenkinson W, Anderson G, Jenkinson EJ. Clonal analysis reveals a common progenitor for cortical and medullary epithelium. *Nature* 2006; 441: 988-91.
20. Anderson G, Lane PJ, Jenkinson EJ. Generating intrathymic microenvironments to establish T-cell tolerance. *Nat Rev Immunol* 2007; 7: 954-63.
21. Agrawal A, Eastman QM, Schatz DG. Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. *Nature* 1998; 394: 744-51.
22. Boehm T, Bleul CC. The evolutionary history of lymphoid organs. *Nat Immunol* 2007; 8: 131-5.

23. Hirano M, Das S, Guo P, Cooper MD. The evolution of adaptive immunity in vertebrates. *Adv Immunol* 2011; 109: 125-57.
24. Bajoghli B, Guo P, Aghaallaei N *et al.* A thymus candidate in lampreys. *Nature* 2011; 470: 90-5.
25. Klein L, Hinterberger M, Wirnsberger G, Kyewski B. Antigen presentation in the thymus for positive selection and central tolerance induction. *Nat Rev Immunol* 2009; 9: 833-44.
26. Ericsson A, Geenen V, Robert F *et al.* Expression of preprotachykinin-A and neuropeptide-Y in mRNA of the thymus. *Mol Endocrinol* 1990; 4: 1211-18.
27. Geenen V, Achour I, Robert F, *et al.* Evidence that insulin-like growth factor 2 (IGF-2) is the dominant thymic member of the insulin superfamily. *Thymus* 1993; 21: 115-27.
28. Jolicœur C, Hanahan D, Smith KM. T-cell tolerance toward a transgenic beta-cell antigen and transcription of endogenous pancreatic genes in thymus. *Proc Natl Acad Sci USA* 1994; 91: 6707-11.
29. Vanneste Y, Ntodou Thome A, Vandersmissen E *et al.* Identification of neurotensin-related peptides in human thymic epithelial cell membranes and relationship with major histocompatibility complex class I molecules. *J Neuroimmunol* 1997; 76: 161-6.
30. Sospedra M, Ferrer-Francesch X, Dominguez O, Juan M, Foz-Sala M, Pujol-Borrell R. Transcription of a broad range of self-antigens in human thymus suggests a role for central mechanisms in tolerance toward peripheral antigens. *J Immunol* 1998; 161: 5918-29.
31. Derbinski J, Schulte A, Kyewski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat Immunol* 2001; 2: 1032-39.
32. Ashton-Rickardt PG, Bandeira A, Delaney JR *et al.* Evidence for a differential avidity model of T cell selection in the thymus. *Cell* 1994; 74: 651-63.
33. Funder JW. Paracrine, cryptocrine, acrocrine. *Mol Cell Endocrinol* 1990; 70: C21-4.
34. Hansenne I, Rasier G, Pequeux C *et al.* Ontogenesis and functional aspects of oxytocin and vasopressin gene expression in the thymus network. *J Neuroimmunol* 2005; 158: 67-75.
35. Martens H, Kecha O, Charlet-Renard C, Defresne MP, Geenen V. Neurohypophysial peptides stimulate the phosphorylation of pre-T cell focal adhesion kinases. *Neuroendocrinology* 1998; 67:282-9.
36. Imura H, Nakao K, Shimatsu A *et al.* Lymphocytic infundibuloneurohypophysitis as a cause of central diabetes insipidus. *N Engl J Med* 1993; 239:683-9.
37. Hansenne I, Renard-Charlet C, Greimers R, Geenen V. Dendritic cell differentiation and tolerance to insulin-related peptides in *Igf2*-deficient mice. *J Immunol* 2006; 176: 4651-57.
38. Burnet FM. A reassessment of the forbidden clone hypothesis of autoimmune diseases. *Aust J Exp Biol Med* 1973; 50: 1-9.
39. Geenen V, Chrousos GP. *Immunoendocrinology in Health and Disease*. Marcel Dekker Ltd, New York, USA, 2004.
40. Georgiou HM, Mandel TE. Induction of insulitis in athymic (nude) mice. The effect of NOD thymus and pancreas transplantation. *Diabetes* 1995; 44: 49-59.
41. Kecha-Kamoun O, Achour I, Martens H *et al.* Thymic expression of insulin-related genes in an animal model of type 1 diabetes. *Diab Metab Rev* 2001; 17: 146-52.
42. Chentoufi A, Polychronakos C. Insulin expression levels in the thymus modulate insulin-specific autoreactive T-cell tolerance: the mechanism by which the *IDDM2* locus may predispose to diabetes. *Diabetes* 2002; 41: 1383-90.

43. Ramsey C, Winqvist O, Puhakka M *et al.* Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum Mol Genet* 2002; 11: 397-409.
44. Paschke R, Geenen V. Messenger RNA expression for a TSH receptor variant in the thymus of a two-year-old child. *J Mol Med* 1995; 73: 577-80.
45. Murakami M; Hosoi Y, Negishi T *et al.* Thymic hyperplasia in patients with Graves' disease. Identification of thyrotropin receptors in human thymus. *J Clin Invest* 1996; 98: 2228-34.
46. Colobran R, del Pilar Armengol M, Faner R *et al.* Association of an SNP with intrathymic transcription of TSHR and Graves' disease: a role for defective thymic tolerance. *Hum Mol Genet* 2011; doi:10.1093/hmg/ddr247
47. Lv H, Havari E, Pinto S *et al.* Impaired thymic tolerance to a-myosin directs autoimmunity to the heart in mice and humans. *J Clin Invest* 2011; 21: 1561-73.
48. Geenen V, Mottet M, Dardenne O *et al.* Thymic self-antigens for the design of a negative/tolerogenic self-vaccination against type 1 diabetes. *Curr Opin Pharmacol* 2010; 10: 461-72.
49. Chentoufi AA, Geenen V. Thymic self-antigen expression for the design of a negative/tolerogenic self-vaccination against type 1 diabetes. *Clin Dev Immunol* 2011; doi:10.1155/2011/349368
50. Smith P. The effect of hypophysectomy upon the involution of the thymus in the rat. *Anat Rec* 1930; 47: 119-43.
51. Kelley KW, Weigent DA, Kooijman R. Protein hormones and immunity. *Brain Behav Immun* 2007; 21: 384-92.
52. Savino W, Dardenne M. Neuroendocrine control of thymus physiology. *Endocr Rev* 2000; 21: 412-43.
53. Taub DD, Murphy WJ, Longo DL. Rejuvenation of the aging thymus: growth hormone-mediated and ghrelin-mediated signaling pathways. *Curr Opin Pharmacol* 2010; 10: 408-24.
54. Morrhaye G, Kermani H, Legros JJ *et al.* Impact of growth hormone (GH) deficiency and GH replacement upon thymus function in adult patients. *PLoS ONE* 2009; 4: e5668.
55. Napolitano LA, Schmidt D, Gotway MB *et al.* Growth hormone enhances thymic function in HIV-1-infected patients. *J Clin Invest* 2008; 118: 1085-98.
56. Castermans E, Hannon M, Dutrieux J *et al.* Thymic recovery after allogeneic hematopoietic cell transplantation with non-myeloablative conditioning is limited to patients younger than 60 years of age. *Haematologica* 2011; 96: 298-306.