Role of the Thymus in the Development of Tolerance and Autoimmunity towards the Neuroendocrine System

VINCENT GEENEN AND FABIENNE BRILOT

Liege University Center of Immunology, Institute of Pathology CHU-B23, B-4000 Liege-Sart Tilman, Belgium

ABSTRACT: The thymus is the unique lymphoid organ inside which a confrontation occurs throughout life between neuroendocrine self-antigens and a recently evolved system with original recombination machinery driving random generation of immune response diversity. Through transcription of neuroendocrine genes in the thymus stromal network and expression of cognate receptors by immature T cells, the neuroendocrine system regulates early T cell differentiation. In addition and more specifically, intrathymic presentation of neuroendocrine self-antigens by, or in close association with, major histocompatibility complex (MHC) proteins is responsible for the establishment of central immune self-tolerance of neuroendocrine principles. All members of the insulin gene (*INS*) family are expressed in the thymus stroma according to a precise hierarchy and cell topography: *IGF2* (thymic epithelial cells) > *IGF1* (thymic macrophages) >> *INS* (thymic medullary epithelial cells and/or dendritic cells). Given this hierarchical pattern in gene expression, the protein IGF-2 is more tolerated than INS. *Igf2* transcription is defective in the thymus of bio-breeding (BB) rat, one animal model of type 1 diabetes (T1DM). This thymus-specific defect in *Igf2* expression may explain both the absence of central tolerance to INS-secreting ß cells and the lymphopenia (including lack of regulatory $RT6^+$ T cells) in diabetes-prone BB rats. INS B:9-23 and the homologous sequence of IGF-2 compete for binding to DQ8, an MHC class II allele conferring major susceptibility to T1DM. In young $DQ8^+$ T1DM patients, INS B:9-23 presentation by DQ8 elicits a dominant IFN-γ secretion by isolated PBMCs, whereas presentation of the IGF-2 self-antigen promotes a dominant regulatory interleukin-10 secretion. These data demonstrate that opposite immune responses are driven by MHC presentation of a self-antigen (here, IGF-2) and an autoantigen (INS, as "altered" self). The important tolerogenic properties of thymic self-antigens deserve now to be exploited for prevention and/or cure of devastating autoimmune diseases such as T1DM.

KEYWORDS: thymus; type 1 diabetes, insulin; IGF-2

Address for correspondence: Dr. Vincent Geenen, Liege University Center of Immunology, Institute of Pathology CHU-B23, B-4000 Liege-Sart Tilman, Belgium.

Ann. N.Y. Acad. Sci. 992: 186–195 (2003).

INTRODUCTION

The thymus is crucially located at the crossroad between the immune and the neuroendocrine systems, and among all lymphoid structures, only the thymus ensures the generation of a diverse repertoire of T cell receptors that are self-tolerant. This central self-tolerance is extremely powerful, because only 1–2% of T cell progenitors will leave the thymus as mature T cells that are self-tolerant but competent against non-self-antigens presented at the periphery. Molecular mechanisms involved in thymic clonal deletion of self-reactive T cells have been extensively described. However, little attention has been paid to the self-antigens that are effectively presented to thymocytes (pre-T cells) by the thymic major histocompatibility complex (MHC) machinery. Intrathymic expression of genes encoding peripheral tissue-specific autoantigens is more and more being documented.[1–11] Consequently, a defect in central self-tolerance as a crucial factor in pathogenesis of organ-specific autoimmune diseases has become a major topic of contemporary research in autoimmunity. Hierarchical patterns exist in intrathymic transcription of neuroendocrine-related genes so that one dominant self-antigen precursor for each family is expressed in the thymus stroma. Thymic neuroendocrine self-antigens are homologous, but not identical, to peripheral neuroendocrine autoantigens. Such biochemical difference led us to investigate that opposite immune responses might be driven by related homologous self-antigens and autoantigens. This hypothesis appeared to be correct after preliminary investigation of the cytokine profile elicited by the presentation of antigens derived from members of the insulin family.

DUAL ROLE OF THYMIC NEUROENDOCRINE SELF-ANTIGEN PRECURSORS

The intrathymic synthesis of the neurohypophysial peptide oxytocin (OT) was reported in 1986.[12] Subsequent studies to elucidate the physiological role of thymic OT have indicated that thymic OT does not behave as a secreted neurohormone, but as the self-antigen of the neurohypophysial peptide family.[13,14] OT presentation to immature T cells involves a 55-kDa protein that is expressed in thymic epithelial cell (TEC) plasma membrane and that is labeled by both antibody to neurophysin (OT-binding 10-kDa protein) and an antibody directed to the 45-kDa heavy chain of MHC class I proteins.[15] The precise biochemical mechanisms responsible for the synthesis of this chimeric protein remain to be elucidated, but a similar neurophysin-MHC class I protein has been identified in cell membranes of small cell lung carcinoma.[16] Neurotensin (NT) and somatostatin were the first neuropeptides detected by immunocytochemistry in the thymic parenchyme.[17] Further studies conclusively demonstrated that NT (and NT-derived peptides) are presented by MHC class I proteins purified from TEC plasma membrane.[18]

Genes encoding specific neurohypophysial receptors are transcribed by thymic T cell subsets: OT receptor (OTR) is expressed by CD4−8−, CD4+8+, CD4+, and CD8+ thymic T cells, whereas V3R expression is restricted to CD4+8+ and CD8+ thymic T cells. Neither V1R nor V2R expression can be detected in the thymus.[19] Thymocyte OTR and V3R can transduce neurohypophysial peptides, in particular OT, according to the rules established for those receptors in other cellular systems

(increase of phosphoinositide turnover coupled to mitogenic action).[20] In addition, OT markedly increases the phosphorylation of kinases implicated in focal adhesion ($p125^{FAK}$, $p130^{Cas}$).[21] Such an early event in the process of T cell differentiation could be crucial for the establishment of immunological synapses between immature T cells and thymic antigen-presenting cells (APCs), namely, TECs, dendritic cells (DCs), and macrophages. During ontogeny in Balb/c mice, *OT* transcripts are detected on E13 in both brain and thymus, whereas *VP* transcription starts on E14 in the brain and is clearly detected in the thymus only on E15 (unpublished data). *OT* precocious transcription in the thymus further concords with the role of thymic OT in the induction of central self-tolerance of the neurohypophysial peptides before their expression by hypothalamic neurons. Based on these two roles of thymic OT acting both as a self-antigen and as a ligand for neurohypophysial receptors expressed by pre-T cells, a model was elaborated transposing at the peptide level the dual role of the thymus in T cell differentiation.[22] Such model applies to other neuroendocrine-related self-antigen precursors expressed in the thymic stroma, and a definition of neuroendocrine self was proposed as follows: (1) neuroendocrine self-antigens correspond to peptide sequences highly conserved throughout evolution of one given family; (2) a hierarchy characterizes their expression pattern in the thymus; (3) the intrathymic processing of neuroendocrine self-antigen precursors is not coupled to the classic model of (neuro)secretion but to pathways of antigen presentation by MHC proteins; and (4) some differences exist in the processing between thymic APCs and dedicated peripheral APCs. For some neuroendocrine self-antigens (OT and NT), those differences imply that their presentation by thymic APCs is not restricted by MHC alleles.[23]

THYMIC EXPRESSION OF INSULIN-RELATED GENES

While searching for a precursor able to represent the whole insulin peptide family in front of T cells during their differentiation in the thymus, insulin-like growth factor 2 (IGF-2) was found to be the dominant insulin-related peptide expressed by TECs from different species.[24] Immunoreactive (Ir) IGF-1 was also detected in thymic cells with a macrophage-like morphology and topography, whereas Ir insulin could not be clearly identified within human thymic lobules. Again, IGF-2 secretion was not evidenced in human TEC primary cultures, although Ir IGF-2 could be detected by confocal microscopy at the outer surface of TEC plasma membranes (data not published). By reverse transcription-polymerase chain reaction (RT-PCR) and *in situ* hybridization (ISH), *IGF2* transcription by human TECs was further demonstrated and found to be under the control of the same promoters as in extrahepatic fetal and adult tissues.[25] *IGF1R* was found to be expressed in Jurkat T cells, and *IGF1* transcripts were also identified by ISH in thymic macrophages. Functional significance of the thymic IGF axis was evaluated by using murine fetal thymic organ cultures (FTOCs). FTOC treatment with an anti-IGF-2 monoclonal antibody induced an inhibition of the transition from CD4−8− T cells to CD4+8+ T cells as well as an increase in CD8+ cells. A similar inhibition of early T cell differentiation was observed when FTOCs were treated with anti-IGF-1R monoclonal antibody or anti-IGF-2R polyclonal antibody. In addition, anti-IGF-1R and anti-IGF-2R induced an 81% and a 34% decrease in FTOC total T cell number, respectively. In the same

model, treatment with a specific antibody directed to (pro)insulin did not exert any significant effect on either T cell cellularity or T cell differentiation.[26]

From 1994, studies have repeatedly reported that the insulin gene (*INS*) is also transcribed in the thymus.[27] A debate recently raised about the precise nature of the thymic cell type responsible for *INS* transcription, that is, medullary TECs[28] or DCs.[29] Compared to IGF-2 (± 100 ng/g wet weight), the thymic content in INS protein is very low, ranging from 98–1,200 fmol/g wet weight in one study to 0.437 ± 0.218 pmol/mg protein in another.[30,31] Those studies did not investigate the functional effect of thymic INS on T cell differentiation, but evidenced a correlation between thymic *INS* mRNA levels and the presence of alleles conferring genetic susceptibility to type 1 diabetes (T1DM).

THYMIC INSULIN-RELATED PEPTIDES, CENTRAL TOLERANCE OF ISLET β CELLS, AND TYPE 1 DIABETES

As just discussed, a precise hierarchy and cell topography exist in the intrathymic expression of *INS*-related genes: *IGF2* (TEC) > *IGF1* (macrophages) ≫ *INS* (thymic DCs and/or medullary TECs). Such hierarchy is important since it is known that immune tolerance of a protein or a protein family primarily involves dominant epitopes of this protein or this family.[32,33] Theoretically according to this observation, IGF-2 should be more tolerated than IGF-1, and much more than INS. This assumption is indirectly verified by immunization, that is, active experimental breakdown of immune tolerance, with INS-related peptides. Following immunization, the titers and frequency of antibodies to INS are higher than those against IGF-1 and much higher than those against IGF-2. Also, the high prevalence of autoantibodies against INS (±40%) in the population could be related to the very low expression of *INS* in the thymus.[34] Finally, a number of studies recently failed to indicate any significant tolerogenic activities of INS administered either orally or subcutaneously.[35–37]

To gain further insight into the central tolerance mediated by thymic INS-related proteins, we advanced the hypothesis that thymus dysfunction could be involved in T1DM pathophysiology. This hypothesis was investigated through analysis of *Ins*, *Igf1,* and *Igf2* expression in the thymus of biobreeding (BB) rats, an animal model of human T1DM. *Ins*, *Igf1*, and *Igf2* transcripts were detected in the thymus of all diabetes-resistant BB rats (BBDR). By contrast, while *Ins* and *Igf1* transcripts were detected in the thymus of all diabetes-prone BB rats (BBDP), a defect in *Igf2* transcription was observed in 11 of 15 thymuses from BBDP rats, in close accordance with the diabetes incidence in this strain (86%).[38] This defect was thymus-specific since *Igf2* transcription was detected in the brain and liver of BBDP rats. The correlation between percentage of *Igf2*-deficient thymuses and incidence of autoimmune diabetes in BBDP rats, on the one hand, and the functional properties of IGF-2-mediated signaling on T cell proliferation/differentiation, on the other hand, may both contribute to the absence of central self-tolerance of islet β cells and to lymphopenia in BBDP rats.

A number of genetic loci conferring susceptibility or resistance to type 1 diabetes have been identified.[39] Recent studies support the hypothesis that genetically determined thymic INS levels play a critical role in INS-specific autoreactive T cell selection. Using an elegant model of graded *INS* transcription in the thymus, Chen-

toufi and Polychronakos[40] found that specific T cell reactivity to INS was inversely correlated with *INS* intrathymic expression. Expression of the transcription factor autoimmune regulator *Aire* is maximal in murine medullary TECs and is absent in the thymus epithelium of diabetic NOD mice.[41,42] The use of *Aire*$^{-/-}$ mice helped in demonstrating that the Aire protein promotes thymic transcription of tissue-specific genes,[43] in particular genes that are known to be expressed in the thymus and to intervene in central self-tolerance such as *OT*, *IGF2*, *INS,* and *NPY*.[44] These findings are important in view of the fact that thymic expression of autoantigens correlates with a higher level of self-tolerance and resistance to autoimmune diseases.[4,45] Finally, a defect in central self-tolerance of NOD mice has been demonstrated since Fas-dependent and independent apoptosis pathways are defective in the thymus of these mice.[46]

Thus, increasingly more studies indicate that a defect in thymus central self-tolerance could be involved as a crucial factor in the development and pathophysiology of autoimmune T1DM. Through such thymus dysfunction, self-reactive T cells will continuously leave the thymus, and the peripheral T cell pool will gradually be enriched with T cells equipped with a TCR directed towards epitopes specific for the

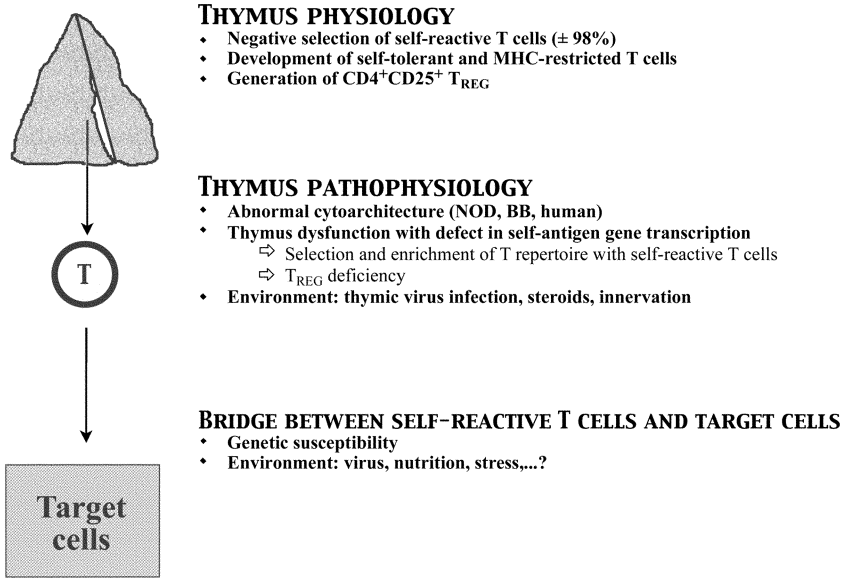

FIGURE 1. Thymus dysfunction in the pathophysiology of organ-specific autoimmune diseases. A defect in central self-tolerance results from a failure or a marked decrease in thymic transcription of genes encoding self-antigen precursors. This defect is responsible for a permanent thymic output and progressive enrichment of the peripheral T cell repertoire with self-reactive T cells. The impact of thymic self-antigens on the generation of specific T_{REG} lymphocytes is plausible but has not been demonstrated. The development of self-reactive T cells is not sufficient to induce autoimmune disease, and a molecular bridge must be established between self-reactive T cells and tissue autoantigens.

INS family. The hypothesis that thymic dysfunction could follow an infection by the diabetogenic strain B4 of coxsackie (CVB4) is currently being explored in our laboratory. From this perspective, a persistent and productive infection by CVB4 of cultured human TECs has been demonstrated.[47] The immunological effects induced by CVB4 thymus infection are currently investigated in depth. A thymus dysfunction resulting from CVB4 infection could intervene in intimate conjunction with the bystander effect evidenced by CVB4 infection of islet β cells.[48]

The presence of self-reactive lymphocytes is not a sufficient condition to develop T1DM. A bridge must be installed between self-reactive T cells and target β cell autoantigens depending on the influence of some external factors and a genetic background determined by the balance between susceptible and protective alleles (FIG. 1).

THYMUS SELF-ANTIGENS AS A WAY FOR THE DEVELOPMENT OF A NEW TYPE OF VACCINE

Increasingly more studies are documenting the highly immunogenic properties of INS, in particular the sequence INS B:9-23, which is the dominant epitope of the whole INS protein. Moreover, INS B:9-23 can be presented and has been co-crystallized with DQ8, an MHC class II allele conferring major genetic susceptibility to T1DM.[49] A cellular response to INS B:9-23 occurs in patients with T1DM. This CD4 response is MHC class II restricted and exhibits a proinflammatory profile with a high induction of IFN-γ in response to the presentation of INS B:9-23.[50] Following the "altered peptide ligand" strategy, the sequence B:9-23 of INS was modified by alanine substitutions introduced at different places. The peptide with alanine substitutions in positions 16 and 19 (NBI-6024) induced Th2 T cell responses in NBI-6024-derived T cell lines from NOD mice. Also, after administration to female NOD mice, NBI-6024 significantly delays but does not abolish the onset of autoimmune diabetes.[51] However, this strategy is based on INS B:9-23, and two recent studies have indicated that administration of INS-derived peptides to NOD mice can prime the autoimmune diabetogenic process or promote a fatal anaphylactic reaction.[52,53]

On the basis that IGF-2 is the dominant thymic peptide of the INS family and that IGF-2 is much more tolerated than INS, a novel type of vaccine could be developed for prevention and/or cure of T1DM. The immunogenic response in classic vaccination induces activated/memory T cells that are specific for antigen(s) shared by infectious agents. Analogously in T1DM, the presentation in the periphery of INS-derived autoantigen(s) activates specific CD4/CD8 T cells as well as memory T cells directed against these autoantigenic epitopes. In the novel type of tolerogenic vaccination, administration of a self-peptide derived of IGF-2 could anergize or even delete self-reactive T cells that have escaped thymus censorship because of thymic dysfunction in the establishment of β cell central self-tolerance. We are currently performing such studies with IGF-2 peptides that exhibit the same affinity and compete with INS B:9-23 for binding to DQ8. Preliminary data in DQ8+ T1DM adolescents show this hypothesis to be correct and that DQ8 presentation of INS B:9-23 and an IGF-2 derived peptide drives opposite immune responses with a dominant IL-10 response after DQ8 presentation of an IGF-2 self-antigen. Two mechanisms may be proposed to explain those findings. On the one hand, the peripheral T cell pool of

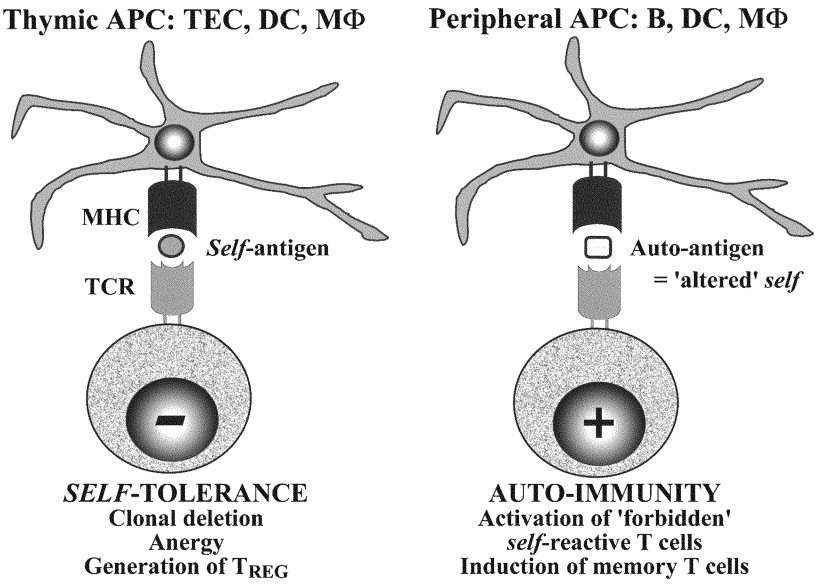

FIGURE 2. MHC presentation of self-antigen and autoantigen drives opposite immune responses. This novel concept proved to be valid for an IGF-2–derived self-antigen and the INS B:9-23 dominant immunogenic autoantigen. The tolerogenic properties of thymic self-antigens should lead to the development of a new generation of vaccines against organ-specific autoimmune diseases.

diabetic patients could contain only CD4 directed against INS-derived epitopes and the IGF-2 self-antigen would be recognized by those cells as a natural altered peptide ligand, eliciting a different response because of different transduction after binding to TCR specific for the DQ8-INS B:9-23 complex. On the other hand, the IGF-2 self-antigen could stimulate IGF-2–specific CD4+25+ T_{REG} cells. These preliminary data show that IGF-2 is a potent inducer of the secretion of IL-10, a major regulatory cytokine with potent immunosuppressive and anti-inflammatory properties.[54,55] They also document the potent tolerogenic properties of an IGF-2–derived peptide and may explain why it is so difficult to obtain antibodies to IGF-2 at high titers after active immunization with this peptide.

Most probably it is now time to distinguish an autoantigen and a self-antigen (FIG. 2). Although they are highly homologous and belong to the same family, they are not identical and this biochemical difference drives opposite immune responses (i.e., immunogenic vs tolerogenic, respectively). The powerful physiologic role of the thymus in self-tolerance induction should be further exploited to prevent and/or cure severe autoimmune diseases (such as T1DM) that constitute the tribute, paid mainly by mankind, for the complexity and efficiency of the immune system.

ACKNOWLEDGMENTS

This work was supported by Liège University, Fondation Leon Fredericq, Belgian NFSR and Belgian Federation against Cancer, Fondation Vaugrenier for Tolerance, European Association for the Study of Diabetes, European Union, and Juvenile Diabetes Research Federation. Our gratitude is due to Kai Wücherpfennig (Dana Farber Cancer Institute, Harvard University) who compared binding of INS B:9-23 and IGF-2 B:11-25 to human DQ8 MHC-II protein.

REFERENCES

1. PASCHKE, R. & V. GEENEN. 1995. Messenger RNA expression for a TSH receptor variant in the thymus of a two-year-old child. J. Mol. Med. **73:** 577–580.
2. MURAKAMI, M., Y. HOSOI, T. NEGISHI, *et al.* 1996. Thymic hyperplasia in patients with Graves' disease. Identification of thyrotropin receptors in human thymus. J. Clin. Invest. **98:** 2228–2234.
3. SMITH, K.M., D.C. OLSON, R. HIROSE & D. HANAHAN. 1997. Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance. Intern. Immunol. **9:** 1355–1365.
4. EGWUAGU, C.E., P. CHARUKAMNOETKANOK & I. GERY. 1997. Thymic expression of autoantigens correlates with resistance to autoimmune disease. J. Immunol. **159:** 3109–3112.
5. FARR, A.G. & A. RUDENSKY. 1998. Medullary thymic epithelium: a mosaic of epithelial "self"? J. Exp. Med. **188:** 1–4.
6. HEATH, V.L., N.N. MOORE, S.M. PARNELL & D.W. MASON. 1998. Intrathymic expression of genes involved in organ specific autoimmune diseases. J. Autoimmunol. **11:** 309–318.
7. WERDELIN, O., U. CORDES & T. JENSEN. 1998. Aberrant expression of tissue specific proteins in the thymus: a hypothesis for the development of central tolerance. Scand. J. Immunol. **47:** 95–100.
8. SALMON, A.M., C. BRUAND, A. CARDONA, *et al.* 1998. An acetylcholine receptor alpha subunit promoter confers intrathymic expression in transgenic mice. Implications for tolerance of a transgenic self-antigen and autoreactivity in myasthenia gravis. J. Clin. Invest. **101:** 2340–2350.
9. SOSPEDRA, M., X. FERRER-FRANCESCH, O. DOMINGUEZ, *et al.* 1999. Transcription of a broad-range of self-antigens in human thymus suggests a role for central mechanisms in tolerance toward peripheral antigens. J. Immunol. **161:** 5918–5929.
10. KLEIN, L., M. KLUGMANN, K.-A. NAVE, *et al.* 2000. Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. Nat. Med. **6:** 56–61.
11. KYEWSKI, B., J. DERBINSKI, J. GOTTER & L. KLEIN. 2002. Promiscuous gene expression and central T-cell tolerance: more than meets the eye. Trends Immunol. **23:** 364–371.
12. GEENEN, V., J.J. LEGROS, P. FRANCHIMONT, *et al.* 1986. The neuroendocrine thymus: coexistence of oxytocin and neurophysin in the human thymus. Science **232:** 508–511.
13. ROBERT, F.R., H. MARTENS, N. CORMANN, *et al.* 1992. The recognition of hypothalamo-neurohypophysial functions by developing T cells. Dev. Immunol. **2:** 131–140.
14. MARTENS, H., B. MAGRANGE, F. ROBERT, *et al.* 1996. Cytokine production by human thymic epithelial cells: control by the immune recognition of the neurohypophysial self-antigen. Regul. Pept. **67:** 39–45.
15. GEENEN, V., E. VANDERSMISSEN, N. CORMANN-GOFFIN, *et al.* 1993. Membrane translocation and relationship with MHC class I of a human thymic neurophysin-like protein. Thymus **22:** 55–66.
16. NORTH, W.G. 2000. Gene regulation of vasopressin and vasopressin receptors in cancer. Exp. Physiol. **85:** 27s–40s.

17. SUNDLER, F., R.E. CARRAWAY, R. HAKANSON, *et al.* 1978. Immunoreactive neurotensin and somatostatin in the chicken thymus. Cell Tiss. Res. **194:** 367–376.
18. VANNESTE, Y., A. NTODOU THOME, E. VANDERSMISSEN, *et al.* 1997. Identification of neurotensin-related peptides in human thymic epithelial cell membranes and relationship with major histocompatibility complex class I molecules. J. Neuroimmunol. **76:** 161–166.
19. HANSENNE, I., G. RASIER, M.P. DEFRESNE, *et al.* 2003. Neurohypophysial receptor gene expression by thymic T cell subsets and thymic T cell lymphoma cell lines. Dev. Immunol. In press.
20. MARTENS, H., F. ROBERT, J.J. LEGROS, *et al.* 1992. Expression of functional neurohypophysial peptide receptors by immature and cytotoxic T cell lines. Prog. NeuroEndocrinImmunol. **5:** 31–39.
21. MARTENS, H., O. KECHA, C. CHARLET-RENARD, *et al.* 1998. Neurohypophysial peptides stimulate the phosphorylation of pre-T cell focal adhesion kinases. Neuroendocrinology **67:** 282–289.
22. MARTENS, H., B. GOXE & V. GEENEN. 1996. The thymic repertoire of neuroendocrine-related self-antigens: physiological implications in T-cell life and death. Immunol. Today **17:** 312–317.
23. GEENEN, V., F. BRILOT, I. HANSENNE, *et al.* 2003. Thymus and T cells. *In* Encyclopedia of Neuroscience, 3rd Ed. G. Adelman & B.H. Smith, Eds. Elsevier. New York. In press.
24. GEENEN, V., I. ACHOUR, F. ROBERT, *et al.* 1993. Evidence that insulin-like growth factor-2 (IGF-2) is the dominant thymic peptide of the insulin superfamily. Thymus **21:** 115–127.
25. KECHA, O., H. MARTENS, N. FRANCHIMONT, *et al.* Characterization of the insulin-like growth factor axis in the human thymus. J. Neuroendocrinol. **11:** 435–440.
26. KECHA, O., F. BRILOT, H. MARTENS, *et al.* 2000. Involvement of insulin-like growth factors in early T cell development: a study using fetal thymic organ cultures. Endocrinology **141:** 1209–1217.
27. JOLICŒUR, C., D. HANAHAN & K.M. SMITH. 1994. T-cell tolerance toward a transgenic beta-cell antigen and transcription of endogenous pancreatic genes in the thymus. Proc. Natl. Acad. Sci. USA **91:** 6707–6711.
28. DERBINSKI, J., A. SCHULTE, B. KYEWSKI & L. KLEIN. 2001. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat. Immunol. **2:** 1032–1039.
29. PUGLIESE, A., D. BROWN, D. GARZA, *et al.* 2001. Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. J. Clin. Invest. **107:** 555–564.
30. VAFIADIS, P., S.T. BENNETT, J.A. TODD, *et al.* 1997. Insulin expression is modulated by *INS* VNTR alleles at the *IDDM2* locus. Nat. Genet. **15:** 289–292.
31. PUGLIESE, A., M. ZELLER, A. FERNANDEZ, JR., *et al.* 1997. The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the *INS* VNTR-*IDDM2* susceptibility locus for type 1 diabetes. Nat. Genet. **15:** 293–297.
32. ADORINI, L., E. APPELLA, G. DORIA & Z.A. NAGY. 1988. Mechanisms influencing the immunodominance of T cell determinants. J. Exp. Med. **168:** 2091–2104.
33. CABANIOLS, J.P., R. CIBOTTI, P. KOURILSKY, *et al.* 1994. Dose-dependent T cell tolerance to an immunodominant self-peptide. Eur. J. Immunol. **24:** 1743–1749.
34. NAQUET, P., J. ELLIS, D. TIBENSKY, *et al.* 1988. T cell autoreactivity to insulin in diabetic and related non-diabetic individuals. J. Immunol. **140:** 2569–2578.
35. CHAILLOUS, L., H. LEFEVRE, C. THIVOLET, *et al.* 2000. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. Lancet **356:** 545–549.
36. POZZILLI, P., D. PITOCCO, N. VISALLI, *et al.* 2000. No effect of oral insulin on residual beta-cell function in recent-onset type 1 diabetes (the IMDIAB VII). Diabetologia **43:** 1000–1004.
37. DIABETES PREVENTION TRIAL-TYPE 1 DIABETES STUDY GROUP. 2002. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N. Engl. J. Med. **346:** 1685–1691.

38. KECHA-KAMOUN, O., I. ACHOUR, H. MARTENS, *et al.* 2001. Thymic expression of insulin-related genes in an animal model of autoimmune type 1 diabetes. Diabetes/ Metab. Res. Rev. **17:** 146–152.
39. TODD, J.A. 1995 Genetic analysis of type 1 diabetes using whole genome approaches. Proc. Natl. Acad. Sci. USA **92:** 8560–8565.
40. CHENTOUFI, A.A. & C. POLYCHRONAKOS. 2002. 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 **51:** 1383–1390.
41. HEINO, M., P. PETERSON, N. SILLANPAA, *et al.* 2000. RNA and protein expression of the murine autoimmune regulator gene (Aire) in normal, RelB deficient and in NOD mice. Eur. J. Immunol. **30:** 1884–1893.
42. ZUKLYS, S., G. BALCIUNAITE, A. AGARWAL, *et al.* 2000. Normal thymic architecture and negative selection are associated with Aire expression, the gene defective in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). J. Immunol. **165:** 1976–1983.
43. ANDERSON, M.S., E.S. VENANZI, L. KLEIN, *et al.* 2002. Projection of an immunological self-shadow within the thymus by the Aire protein. Science **298:** 1395–1401.
44. ERICSSON, A., V. GEENEN, F. ROBERT, *et al.* 1990. Expression of preprotachykinin A and neuropeptide Y mRNA in the thymus. Mol. Endocrinol. **4:** 1211–1218.
45. SEBZDA, E., V.A. WALLACE, J. MAYER, *et al.* 1994. Positive and negative thymocyte selection induced by different concentrations of a single peptide. Science **263:** 1615–1618.
46. KISHIMOTO, H. & J. SPRENT. A defect in central tolerance in NOD mice. Nat. Immunol. **2:** 1025–1031.
47. BRILOT, F., W. CHEHADEH, C. CHARLET-RENARD, *et al.* 2002. Persistent infection of human thymic epithelial cells by coxsackievirus B4. J. Virol. **76:** 5260–5265.
48. HORWITZ, M.S. & N. SARVETNICK. 1999. Viruses, host responses and autoimmunity. Immunol. Rev. **169:** 241–253.
49. LEE, K.H., K. WUCHERPFENNIG & D.C. WILEY. 2001. Structure of a human insulin peptide-HLA-DQ8 complex and susceptibility to type 1 diabetes. Nat. Immunol. **2:** 501–507.
50. ALLEVA, D.G., P.D. CROWE, L. JIN, *et al.* 2001. A disease-associated cellular immune response in type 1 diabetics to an immunodominant epitope of insulin. J. Clin. Invest. **107:** 173–180.
51. ALLEVA, D.G., A. GAUR, L. JIN, *et al.* 2002. Immunological characterization and therapeutic activity of an altered-peptide ligand, NBI-6024, based on the immunodominant type 1 diabetes autoantigen insulin B-chain (9-23) peptide. Diabetes **51:** 2126–2134.
52. LIU, E., H. MORIYAMA, N. ABIRU, *et al.* 2002. Anti-peptide autoantibodies and fatal anaphylaxis in NOD mice in response to insulin self-peptides B:9-23 and B:13-33. J. Clin. Invest. **110:** 1021–1027.
53. KARGES, W., K. PECHHOLD, S. AL DAHOUK, *et al.* 2002. Induction of autoimmune diabetes through insulin (but not GAD65) vaccination in non obese diabetic and in RIP-37.1 mice. Diabetes **51:** 3237–3244.
54. COOKE, A., J.M. PHILLIPS & N.M. PARISH. 2001. Tolerogenic strategies to halt or prevent type 1 diabetes. Nat. Immunol. **2:** 810–815.
55. YANG, Z., M. CHEN, R. WU, *et al.* 2002. Suppression of autoimmune diabetes by viral IL-10 gene transfer. J. Immunol. **168:** 6479–6485.