THE THYMIC REPERTOIRE OF NEUROENDOCRINE SELF ANTIGENS AND THE CENTRAL IMMUNE TOLERANCE OF NEUROENDOCRINE FUNCTIONS

V. Geenen, N. Cormann, A. Benhida, H. Martens, I. Achour, M.P. Defresne and F. Robert

Key words:

thymus, cryptocrine signalling, immune tolerance, neuroendocrine self antigen, autoimmunity.

The thymus is now well recognized as the primary lymphoid organ responsible for T cell differentiation (1), and thymus research has provided significant insights into the molecular aspects of this complex process during the latter half of the eighties (2, 3). There is now ample evidence that T cell differentiation is not a pure automatic genetically-driven process, but is intimately regulated by thymic microenvironmental cells (4, 5, 6, 7). The physiological role of the thymus in T lymphocyte differentiation appears to be dual and apparently paradoxical.

In the first place, the thymus is required (8) for the induction of central immune tolerance by *negative selection* of potentially self-reactive T-cells emerging during the differentiative process after the random recombination of T cell receptor (TCR) genes (9, 10). From this point of view, the thymus may be regarded as a cemetery for ± 85% of thymocytes (pre-T cells) which will die locally by apoptosis (or programmed cell death) (11). This phenomenon was shown to result from a high affinity interaction between the association of a major histocompatibility complex (MHC) protein and a self peptide on one side, and a specific TCR offering the precise configuration for this association on the other side (12, 13, 14). Secondly, the thymic environment provides the various signals necessary for T cell *positive selection* and the development of the mature peripheral T cell repertoire (15). While the genetic programme as well as the successive stages of T cell differentiation are increasingly understood, it remains to firmly establish the molecular ways by which the thymic microenvironmental signals regulate the expression of T cell differentiation markers and the recombination of TCR-coding genes of developing T cells.

From: Institute of Pathology B-23, Laboratories of Radio-Immunology (VG, NC, HM, AB and FR), Experimental Diabetology (IA) and Pathological Anatomy (MPD), University Hospital of Liège-Sart Tilman, B-4000 Liège, Belgium.
Reprint address: Vincent Geenen, MD, PhD, Laboratory of Radio-Immunology B-23, Neuroendocrin-Immunology Unit, University Hospital of Liège, B-4000 Liège-Sart Tilman, Belgium
This work is supported by the Special Research Fund of the University of Liège, by FRSM grant n° 3.4562.90, by Télévie/FRSM grant n° 7.4611.91, by AFM, by cooperation agreement between CGRI (Communauté Française de Belgique) and INSERM, by the Ministère de la Region Wallonne (DGTR), and by ESF-Neuroimmunomodulation Network.
Submitted 2 March 1992; Accepted 25 March 1992.

On the basis of our original observations, we describe in this review a novel and highly effective model which transposes, from the tissular and cellular levels to the neuroendocrine self peptide repertoire, the dual physiological function of the thymus in negative and positive selection of T lymphocytes.

THYMIC CRYPTOCRINE CELL-TO-CELL SIGNALLING

In the investigation of the thymic neuropeptide repertoire, our group has previously shown that the central organ of T cell differentiation was the site of biosynthesis of neurohypophysial (NHP)- (16, 17) and tachykinin (TK)- related signals (18). This synthesis was shown to take place in the same epithelial cell subsets known to contain also ir interleukin-1 and thymulin (19, 20). However, the thorough examination of the cellular and molecular aspects underlying our initial observations has led us to conclude that the classical model of neurosecretion established in 1944 by Scharrer and Scharrer (21) for the hypothalamo-hypophysial axis could not be entirely applied to the intrathymic system of epithelial cell-to-T cell signalling.

Cryptocrine communication

The novel concept of cryptocrine communication was recently introduced by J.W. Funder (22) to describe the exchange of information in secluded microenvironments between specialized epithelial cells and migratory developing elements. At least two examples of cryptocrine signalling exist in the human body, at the levels of the *thymus*, between epithelial/"nurse" cells (TEC/TNC) and immature pre-T cells (thymocytes), as well as of the testis, between Sertoli cells and spermatids. The precise molecular mechanisms responsible for the thymic and testicular expression of NHP-related signals are under current investigation in our department and in other laboratories (23). The co-localization in TEC/TNC of a neurophysin (NP)-like protein domain together with oxytocin (OT) and vasopressin (VP)-like immunoreactivities suggests the existence of a hitherto unidentified gene coding for a new member of the NHP precursor family.

On the basis of immunocytochemical analyses, a plausible candidate encoded by this gene could be vasotocin (VT), the ancestral precursor of hypothalamic VP and OT neurohormones (24). VT is a hybrid NHP peptide formed by the 6 amino acid cyclic part

of OT and the 3 amino acid lateral linear sequence of VP. In the human species, the gene responsible for the intrathymic expression of NHP-related peptides would be predominantly functional during foetal development (25), the life period during which the human thymus exerts its principal regulatory activity upon T cell ontogeny.

Functional signalling

The existence of a functional thymic cryptocrine signalling through NHP-related peptides, is further demonstrated by the identification of NHP peptide receptors in rat thymus, on rat thymocytes (26), as well as on a murine immature lymphomatous pre-T cell line (RL12-NP) (27). This cell line is derived from a thymic lymphoma induced by fractionated X-irradiation in C57Bl/Ka mice. Using RL12-NP, various signals of the NHP peptide family were shown to be transduced according to the rules established for VP V1-type and OT receptors expressed by other cellular systems (28). In serum-free cultures, a phosphoinositide breakdown follows the binding of VP, OT or VT to their RL12-NP receptors, and this leads to an increase of cytoplasmic inositol-triphosphate (IP3). Preliminary results have also evidenced a stimulatory effect of VP, OT and VT on [3H]thymidine incorporation by thymocytes freshly isolated from human and murine thymuses, and cultured in serum-free conditions (29). This effect most probably reflects a mitogenic action since the hydrolysis of membrane phosphoinositides by receptors G-coupled to phosphoinositidase C generally appears to mediate mitogenic signals. Of high relevance is the fact that the stimulation of phosphatidyl-inositol breakdown is the principal pathway implicated in T cell activation (30). Altogether, even if some specific molecular points remain to be elucidated, these studies strongly support the intervention of thymic NHP-related peptides as accessory signals in the process of T cell differentiation/activation.

Mitogen response

NHP-related peptides have already been shown to intervene as early signals of the mitogenic response in different cell systems (31, 32). A disturbance of this molecular dialogue could be involved in oncogenesis, either at the level of the *signal* (paraneoplasic syndromes) or of the *receptor* (pathogeny of T cell lymphoma). At the first level, for example, it is well known that thymomas (tumours derived from the thymic epithelial compartment) are the third cause of Schwartz-Bartter syndrome (paraneoplasic VP secretion) (33, 34). At the second level, the implication of TNC in the pathogeny of lymphoproliferative disorders is now well demonstrated (35), whereas lymphoid malignancies may arise from aberrant differentiation (36).

Pharmacological manipulations

Further studies have also evidenced functional NHP peptide receptors on a murine differentiated cytotoxic T cell line (CTL-L2) (29). The specificity of the transduction of NHP-related signals by lymphocytes was demonstrated by the inhibition of IP3 production in presence of VP and OT antagonists (designed to the VP V1-type receptors and to the uterine OT receptor, respectively). The RL12-NP transduction of NHP signals was preferentially inhibited by a V1 antagonist (namely 1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(O-methyl)[tyrosine]arginine vasopressin, Peninsula), whereas an OT antagonist (1-deamino,-2DTyr(OEt)-4Thr-8-Orn oxytocin, Ferring AB Malmö, CAP 433) was more potent on CTL-L2 cells (29). These latter observations suggest a shift or some molecular maturation of the NHP peptide receptor expressed by T cells according to their stage of differentiation. They also argue for the pharmacological manipulation of the cryptocrine model, and open new potential immunomodulating strategies through the molecular design of immune-specific neuropeptide antagonists. Such OT antagonists could lead to selective immunotherapy in crucial life periods such as the post partum, during which an immune disequilibrium results from the increase of lactation-inducing hormones OT and prolactin, as well as from an increase in the oestrogen/progesterone ratio (37).

THYMIC NEUROHYPOPHYSIAL-RELATED SELF PEPTIDES

In the overall evolution of intercellular communication, cryptocrine cell-to-cell signalling is a rather primitive step located between the adhesion and paracrine stages (22, 38). The cryptocrine signalling evidenced in the thymus introduces an obligatory step in developmental biology: the recognition of the self molecular structure by the T cell system along its differentiation. Using various well-characterized polyclonal and monoclonal antibodies (mAbs) against NHP precursor-derived peptides, the immunodominant epitopes representative of the NHP family (NHP self epitopes) expressed in the thymus have been defined (39). They were found to be located within the 6 amino acid cyclic part of OT shared by different members of this family, as well as in the central domain of NP which is encoded by the second exon of NHP precursor genes and which exhibits a very high degree of conservation in phylogeny (23).

Central immune tolerance

If further demonstrated, the presentation by MHC molecules of these NHP self epitopes could logically induce the central immune tolerance of hypothalamo-neurohypophysial functions (40). This tolerogenic effect would result in this case from the clonal deletion of

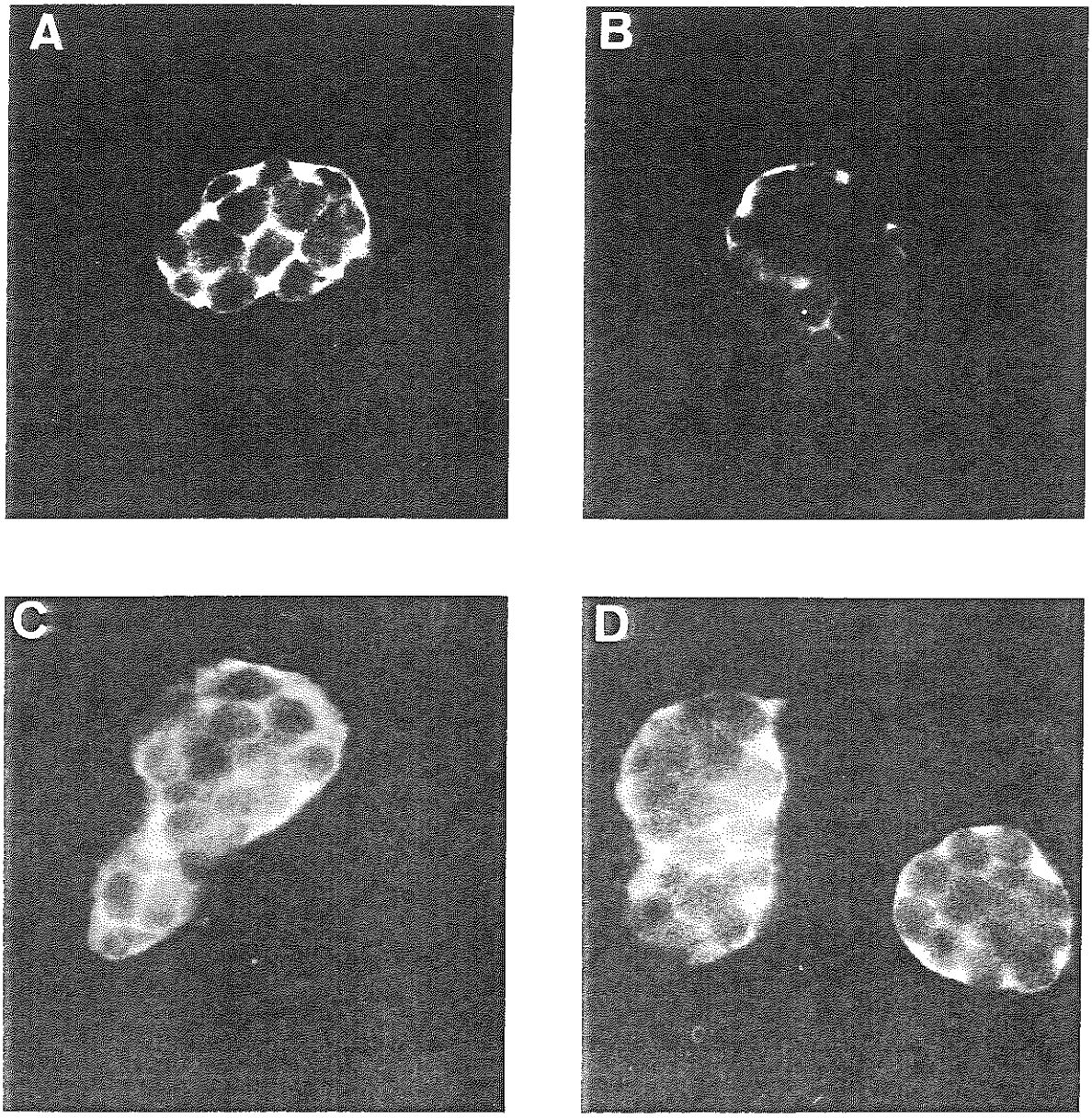

Figure 1: Immunofluorescence staining of TNCs isolated from C57Bl/Ka mice with a panel of antibodies: (A) mAb O33 directed against the cyclic immunodominant epitope of NHP family (first NHP self epitope) (39); (B) polyclonal antiserum against the central domain of NP (second NHP self epitope) (24); (C) mAb to a complex ganglioside (A2B5) expressed by various components of the diffuse neuroendocrine system (79); (D) mAb to murine MHC class II molecules (Ia). The external limits and the cytoplasm of the epithelial part of TNCs are immunostained. The shaded areas correspond to TNC caveoles containing pre-T cells (41).

highly reactive T cells harbouring a randomly rearranged TCR offering the precise configuration for the MHC/self NHP epitope(s) association(s). A first argument supporting that view is the co-expression by isolated murine TNC of MHC class II (Ia) molecules, of neuroendocrine markers (A2B5, NSE), and of the immunodominant epitopes of the NHP peptide family (O33 epitope, NP) (Fig. 1). As demonstrated in our original report (41), TNC represent an original, if not unique, example of an extremely intimate association between one component of the diffuse neuroendocrine system (42) and developing T cells, the TNC-engulfed thymocytes. It has also been shown that TNC are able to present self antigens (43). Given the importance of OT-related peptides in the control of reproductive functions (parturition, lactation and gonadal functions), it seems logical that they are strongly "protected" against a potential autoaggression by the immune system.

Table I. Interactions between the NHP peptide family and the immune system.

Positive selection (cryptocrine signalling)	Negative selection (NHP self epitope presentation)
Physiology :	
Accessory signals in T cell differentiation/activation	Tolerance of hypothalamo-neurohypophysial functions
Implications :	
3rd cause of paraneoplasic VP secretion = thymomas (pathology of NHP signal)	Autoimmune hypothalamitis ("idiopathic" diabetes insipidus)
Oncogenesis of T cell lymphomas (pathology of NHP receptor)	Breakdown of tolerance by active immunization against VP
Immunomodulation by OT/VP receptor agonists or antagonists	Frequence and titers of Abs against NHP-related peptides (VP > OT > VT)

Since the water metabolism-regulating neurohormone VP differs from OT by only one amino acid in the immunodominant cyclic part, it may be less "tolerated" by the immune system. Interestingly, some authors have described cases of idiopathic diabetes insipidus that were secondary to autoimmune processes against hypothalamic NHP-producing neurones (44). To our knowledge, such pathogenetic processes have never been described against hypothalamic or gonadal granulosa (45, 46) OT-producing cells. Experimental breakdown of immune tolerance by active immunization against VP was also shown to induce inflammatory lesions in the hypothalamo-neurohypophysial axis (47). Finally, another indirect argument for a stronger immune tolerance of OT-related peptides may be deduced from the frequence and the titers of antibodies resulting from active immunization against NHP peptides (VP > OT > VT) (48).

Therefore, by concentrating our interest towards the impact of thymic NHP-related peptides upon the process of T cell differentiation, the model of neurosecretion has progressively shifted to the novel concept of cryptocrine cell-to-cell signalling. In parallel, at the molecular level, the classical "neuropeptide" model was replaced by the more effective working concept of NHP "self peptide(s)" (49, 50). At the genetic level, these evolutionary aspects of cellular communication appear to have their counterpart in the molecular evolution of signal-coding genes (38). The Table I summarizes the actual state of our knowledge about the physiological and pathological implications of the interactions between the NHP peptide family and the immune system. As outlined above, thymic NHP self antigens may exert a dual role in T cell differentiation according to the molecular type of intercellular dialogue considered (cryptocrine signalling or NHP self epitope presentation by MHC molecules).

Table II The thymic repertoire of neuroendocrine self antigens.

Hormone members of neuroendocrine gene families	Thymic peptide repertoire (tissue members of neuroendocrine gene families)
Insulin	Insulin-like growth factors (IGF-1, IGF-2) (54, 55)
Parathormone	Parathormone-related peptide (PTH-rP) (80)
Calcitonin	Calcitonin gene-related peptide (CGRP) (81)
Neurohypophysial hormones (NHP)	VT ? (17, 24, 38) Other OT/VP-related peptide ?
Tachykinins (TK)	Neurokinin A (NKA) (18)

THE THYMIC REPERTOIRE OF NEUROENDOCRINE SELF ANTIGENS

The model described for NHP-related self peptides can also be applied to other neural-related or endocrine molecules (Table II). In collaboration with the Laboratory of Molecular Neurobiology of the Karolinska Institute, we have previously reported the intrathymic expression of neuropeptide Y and preprotachykinin-A (PPT-A) mRNAs in the rat thymus (18). At the peptide level, neurokinin A (NKA) was the product of PPT-A gene which was detected in thymic A2B5+ epithelium. The other peptide encoded by the PPT-A gene, substance P (SP), could be extracted from rat and human thymuses; however, using immunocytochemistry, it could only be detected in sensory nerve fibers of the thymus and, most probably, derived from the expression of PPT-A gene in ganglionic nerve cell bodies, outside the thymus (18). This observation is in perfect accordance with the reported mitogenic effects of NKA (but not of SP) upon thymocytes (51). Since specific SP-receptors are found on cells of the thymic bed vasculature (52), the SP-mediated sensory innervation seems to be involved in the control of thymic blood flow, rather than in the regulation of T cell differentiation. At the level of the primary amino acid sequence, NKA and SP are highly homologous TK-related peptides which share a common 4 amino acid C-terminal immunodominant epitope (53). Therefore, besides the accessory activation properties of NKA in T cell positive selection, it is logical to see the 10 amino acid sequence of NKA as the tolerogenic TK self peptide in front of the differentiating T cell system.

Thymic insulin-like growth factors

With regard to the insulin hormone superfamily, insulin-like growth factors were found to be expressed in the thymic stroma, both at the peptide and mRNA

levels (54, 55). The cellular localization of thymic ir IGFs, as well as their precise immunochemical nature, remain however to be further defined. Given the high homology (± 60% identity) between the primary amino acid sequences of insulin and IGFs, classical immunochemical methods failed for a long time to distinguish the precise molecular identities of these polypeptides. Again, the presentation by MHC molecules of immunodominant epitopes representative of the insulin family (common insulin/IGF self epitopes) could induce the central immune tolerance of pancreatic endocrine function. The central immune tolerance would result in this case from the negative selection of potential insulin/IGF self epitope-reactive T cells emerging during the random recombination of TCR genes. A recent study has further provided another indirect argument supporting the involvement of insulin-IGF2 region on chromosome 11p in HLA-DR4-dependent diabetes susceptibility (56). This tolerogenic effect of insulin/IGFs self epitope(s) does not exclude the implications of thymic IGFs in T cell positive selection; this latter action was attested by the implication of IGF-mediated signalling in the development of T cell lymphomas (57). More significantly, the identification of the precise insulin/IGF self epitope presented in the thymus to the maturing T cell system could lead to the identification of the primary self antigen involved in the autoimmune cascade leading finally to overt type I insulin-dependent diabetes (58).

Other thymic neuroendocrine-related polypeptides

Although analogous comparisons have to be made very cautiously, the same model can also be easily applied to the immune tolerance of calciotropic hormones, parathormone and calcitonin, through the tissular members of the corresponding families. These latter derived either from an ancestral distinct gene (*e.g.* PTH-rP) (59, 60), or from an alternative splicing of the hormonal gene (*e.g.* CGRP) (61). At the amino acid level, these tissue factors also share immunodominant epitopes with the corresponding hormone, a biochemical characteristic which rendered their identification impossible through classical immunochemical methods.

Other recent studies have also described the intrathymic expression of several other neuropeptides, sometimes at the mRNA level (62, 63, 64, 65). However, these studies do not permit a precise evaluation of the role exerted by these polypeptides in T cell differentiation.

The dual role of the thymus in T lymphocyte differentiation

The dual role of the thymic self peptide repertoire in T cell development is schematized in Figure 2. The coherence and the validity of our model is further supported by previous reports which have proven the implication of self peptides in positive selection (66, 67). The key point in the validation of this model will be of course to demonstrate that thymic MHC molecules are able to "present" neuroendocrine precursor-derived peptidic self sequences, in a similar way as the presentation of nonself peptides by peripheral MHC (68, 69, 70). This point is presently under active investigation in our laboratory. In close relation with this research objective, it has been recently shown that naturally processed peptides fragments eluted from MHC class II molecules were peptides of 13-17 amino acids in length (71). In the actual stage of its development, our model nevertheless presents significant advantages by comparison with other previous hypotheses. Those models were in fact established on what appears a major paradox in immunology, *i.e.* that both positive and negative selection could be mediated by the same set of molecules (TCR and MHC + self X complexes). Consequently, a difference in the affinity of interactions had to be advanced to explain a negative (following a high affinity interaction TCR/MHC + self X) or a positive (resulting from a moderate or low affinity interaction between the same molecules) selection of T lymphocytes (72, 73). As outlined in this review, we propose another explanation that is based on two separate types of interactions with distinct sets of molecules. In this model (24), the positive selection is mediated by cryptocrine signalling and implicates an interaction of moderate affinity (around 10-10 M) (29) between a signal peptide X and a specific X receptor (R "X") expressed by the target T cell. The negative selection follows the presentation by a thymic MHC molecule of the self X epitope and a high affinity interaction with a TCR offering the precise configuration for this association MHC/self X epitope.

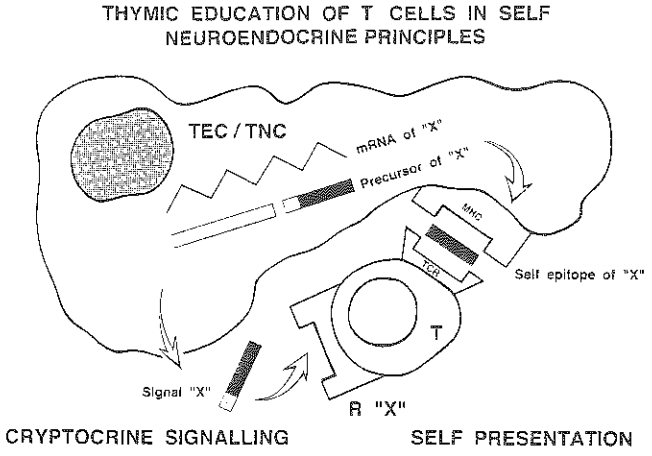

Figure 2: Schematic representation of the two types of interactions occurring in the thymus between TEC/TNC and pre-T cells. This molecular model transposes at the peptide level the dual physiological role of the thymus in both positive and negative selection of T lymphocytes. See the text for a full explanation of the figure.

A novel model

In the present stage of our research about the neuroendocrine self-peptide repertoire expressed in the thymus, this organ appears progressively as a highly specialized school for the education of T cells in self neuroendocrine principles. In the human species, this school is working essentially during foetal development, and the only reward scheduled for a perfect educational degree is the programmed death or the apoptosis of the T cell graduate (74)!

Another important consequence resulting from the above model is the fact that the thymus is not the site for the presentation of all self peptides, but that molecular evolution has selected primitive tissular members of hormone gene families which are effectively expressed within that organ. Finally, it must be recalled that the thymic clonal deletion of self-reactive T cells is not a perfect mechanism since the presence of autoreactive T cells is a frequent observation in normal individuals (75). This is an important argument supporting the intervention in the pathogenesis of autoimmune disorders of several different factors, among which one may include the emergence of self-reactive T cells during their differentiation in the thymus.

As discussed before, new specific immunomodulating approaches may be designed on the pharmacological manipulation of the cryptocrine signalling (76). The precise nature of the thymic neuroendocrine self antigens may also lead to the design of early medical detection procedures and of preventive therapeutics for various autoimmune endocrine disorders (77, 78).

ACKNOWLEDGMENTS

V. Geenen and M.P. Defresne are Research Associate and Senior Research Associate of the National Fund of Scientific Research (Belgium), respectively. F. Robert is Chargé de Recherches 1ère Classe of INSERM (France). Our gratitude is due to Dr. Pierre Bonnet who has drawn Figure 2.

This work has been awarded the Smithkline-Beecham Prize for Scientific Research (1989-1991) selected by the Royal Academy of Medicine of Belgium.

REFERENCE

1. Miller JFAP, Osoba D. Current concepts of the immunological function of the thymus. *Physiol Rev* 1967; **47**:437-520.
2. Ritter MA, Crispe IN. The Thymus. In Focus Series, IRL Press, Oxford, 1992.
3. Ritter MA, Rozing J, Schuurman HJ. The true function of the thymus? *Immunol Today* 1988; **9**:189-93.
4. van Ewijk W. Cell surface topography of thymic microenvironments. *Lab Invest* 1988; **59**:579-90.
5. Kendall MD. Functional anatomy of the thymic microenvironment. *J Anat* 1991; **177**:1-29.
6. Haynes BF. The human thymus microenvironment *Adv Immunol* 1984; **36**:87-142.
7. von Gaudecker B. Functional histology of the human thymus. *Anat Embryol* 1991; **183**:1-15.
8. Fry AM, Jones LA, Kruisbeek AM, Matis LA. Thymic requirement for clonal deletion during T cell development. *Science* 1989; **246**:1044-6.
9. von Boehmer H, Kisielow P. Self-nonself discrimination by T cells. *Science* 1990; **248**:1369-73.
10. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature* 1988; **334**:395-402.
11. Smith CAA, Williams GT, Kingston R, Jenkinson EJ, Owen JT. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature* 1989; **337**:181-4.
12. Blackman MA, Kappler JW, Marrack P. T-cell specificity and repertoire *Immunol Rev* 1988; **101**:5-19.
13. Garrett TPJ, Saper MA, Bjorkman PJ, Strominger JL, Wiley DC. Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature* 1989; **342**:692-6.
14. von Boehmer H. Self recognition by the immune system. Eur *J Biochem* 1990; **194**:693-8.
15. Boyd RL, Hugo P. Towards an integrated view of thymopoiesis. *Immunol Today* 1991; **12**:71-9.
16. 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.
17. Geenen V, Robert F, Martens H, *et al.* At the Cutting Edge. Biosynthesis and paracrine/cryptocrine actions of self neurohypophysial-related peptides in the thymus. *Mol Cell Endocrinol* 1990; **76**:C27-31.
18. Ericsson A, Geenen V, Robert F, *et al.* Expression of preprotachykinin-A and neuropeptide-Y messenger RNA in the thymus. *Mol Endocrinol* 1990; **4**:1211-8.
19. Robert F, Geenen V, Schoenen J, *et al.* Colocalization of immunoreactive oxytocin, vasopressin and interleukin-1 in human thymic epithelial neuroendocrine cells. *Brain Behav Immun* 1991; **5**:102-14.
20. Savino W, Dardenne M, Papiernik M, Bach JF. Thymic hormone-containing cells. Characterization and localization of serum thymic factor in young mouse thymus studied by monoclonal antibodies. *J Exp Med* 1982; **156**:628-33.
21. Scharrer B, Scharrer E. VI. A comparison between the intercerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. *Biol Bull* 1944; **87**:242-51.
22. Funder JW. At the Cutting Edge. Paracrine, cryptocrine, acrocrine. *Mol Cell Endocrinol* 1990; **70**:C21-4.
23. Ivell R, Burbach JPH. The molecular biology of vasopressin and oxytocin genes. *J Neuroendocrinol* 1991; 3:583-5.
24. Robert F, Martens H, Cormann N, Benhida A, Schoenen J, Geenen V. The recognition of hypothalamo-neurohypophysial functions by developing T cells. *Dev Immunol* 1992; in press.
25. Geenen V, Robert F, Martens H. *et al.* Neuroendocrinology of the thymus. In: Horizons in Endocrinology vol II. Maggi M, Geenen V (eds), Raven Press, New York, 1991: 121-34.
26. Elands J, Resink A, De Kloet ER. Neurohypophyseal hormone receptors in the rat thymus, spleen, and lymphocytes. *Endocrinology* 1990; **126**:2703-10.
27. Geenen V, Robert F, Fatemi M, *et al.* Vasopressin and oxytocin: thymic signals and receptors in T cell ontogeny. In: Recent Progress in Posterior Pituitary Hormones 1988, Yoshida S, Share L (eds), Elsevier, New York, 1989; 303-10.
28. Kirk CJ, Guillon G, Balestre MN, Jard S. Stimulation by vasopressin and other agonists of inositol-lipid breakdown and inositol phosphate accumulation in WRK 1 cells. *Biochem J* 1986; **240**:197-204.
29. Martens H, Robert F, Legros JJ, Geenen V, Franchimont P. Expression of functional neurohypophysial peptide receptors by murine immature and cytotoxic T cell lines. *Prog Neuro Endocrin Immunol* 1992; in press.

30. Desai DM, Newton ME, Kadlecek T, Weiss A. Stimulation of the phosphatidyl-inositol pathway can induce T-cell activation. *Nature* 1990; **348**:66-9.
31. Rozengurt E. Early signals of the mitogenic response. *Science* 1986; **234**:161-6.
32. Taylor AH, Ang VTY, Jenkins JS, Silverlight JJ, Coombes RC, Luqmani YA. Interaction of vasopressin and oxytocin with human breast carcinoma cells. *Cancer Res* 1990; **50**:7882-6.
33. Suster S, Rosai J. Thymic carcinoma. A clinicopathologic study of 60 cases. *Cancer* 1990; **67**:1025-32.
34. Giraud F, Fabien N, Auger C, Girod C, Loire R, Monier JC. Human epithelial thymic tumors: heterogeneity in immunostaining of epithelial cell markers and thymic hormones. *Thymus* 1990; **15**:15-29.
35. Defresne MP, Rongy AM, Boniver J. Cellular aspects of radiation leukemogenesis in C57Bl/Ka mice. II. Thymic microenvironment and nurse cells. *Leukemia Res* 1986; **10**:783-9.
36. Greaves MF. Differentiation-linked leukemogenesis in lymphocytes. *Science* 1986; **234**:697-704.
37. 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.
38. Geenen V, Martens H, Robert F, *et al.* Thymic cryptocrine signalling and the immune recognition of self neuroendocrine functions. *Prog Neuro Endocrin Immunol* 1991; **4**:135-42.
39. Burgeon E, Chapleur M, Schoenen J, *et al.* Monoclonal antibodies to oxytocin: production and characterization. *J Neuroimmunol* 1991 **31**:235-44.
40. Cibotti R, Kanellopoulos JM, Cabaniols JP, *et al.* Tolerance to self-protein involves its immunodominant but does not involve its subdominant determinants. *Proc Natl Acad Sci* USA 1992; **89**:416-20.
41. Geenen V, Defresne MP, Robert F, Legros JJ, Franchimont P, Boniver J. The neurohormonal thymic microenvironment: immunocytochemical evidence that thymic nurse cells are neuroendocrine cells. *Neuroendocrinology* 1988; **47**:365-8.
42. Polak JM, Bloom SR. Immunocytochemistry of the diffuse neuroendocrine system. In: Immunocytochemistry. Modern Methods and Applications. Polak JM, Van Noorden SM (eds), Wright, Bristol, 1986; 328-48.
43. Marrack P, Mc Cormack J, Kappler J. Presentation of antigen, foreign major histocompatibility complex proteins and self by thymus cortical epithelium. *Nature* 1989; **338**:503-5.
44. Scherbaum WA, Bottazzo GF, Czernichow P, Wass JAH, Doniach D. Role of autoimmunity in central diabetes insipidus. *Front Horm Res* 1985; **13**:232-9.
45. Wathes DC, Swann RW. Is oxytocin an ovarian hormone? *Nature* 1982; *297*:225-7.
46. Geenen V, Legros JJ, Hazée-Hagelstein MT, *et al.* Release of immunoreactive oxytocin and neurophysin I by cultured luteinizing bovine granulosa cells. *Acta Endocrinol* 1985; **110**:263-70.
47. Cau P, Rougon-Rapuzzi G. Autoimmune alterations in the neurohypophysis of rabbits immunized against vasopressin. *Brain Res* 1979; *177*:265-71.
48. Amico JA. The radioimmunoassay of oxytocin: new developments. In: Oxytocin. Clinical and Laboratory Studies. Amico JA, Robinson AG (eds), Elsevier, Amsterdam, 1985; 3-15.
49. Kourilsky P, Chaouat G, Rabourdin-Combe C, Claverie JM. Working principles in the immune system implied by the "peptidic self" model. *Proc Natl Acad Sci* USA 1987; **84**:3400-4.
50. Geenen V, Robert F, Ericsson A, Persson H. Thymus gland, Neuroendocrinology. In: Neuroscience Year, Supplement to the Encyclopedia of Neuroscience, Smith B, Adelman G. (eds), Birkhauser, Boston, 1992; 149-50.
51. Söder O, Hellström P. The tachykinins neurokinin A and physaelemin stimulate murine thymocyte proliferation. *Int Arch Appl Immunol* 1989; **90**:91-6.
52. Shigematsu K, Saavedra JM, Kurihara M. Specific substance P binding sites in rat thymus and speen: in vitro autoradiographic study. *Regul Pept* 1986; **16**:147-56.
53. Brodin E, Lindefors N, Dalsgaard CJ, Theodorsson-Norheim E, Rosell S. Tachykinin multiplicity in rat central nervous system as studied using antisera raised against sustance P and neurokinin *A*. *Regul Pept* 1986; **13**:253-72.
54. Han VKM, D'Ercole AJ, Lund PK. Cellular Localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science* 1987; **286**:193-7.
55. Han VKM, Hill DJ, Strain AJ, *et al.* Identification of somatomedin/insulin-like growth factor immunoreactive cells in the human fetus. *Pediatr Res* 1987; **22**:245-9.
56. Julier C, Hyer RN, Davies J, *et al.* Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. *Nature* 1991; **354**:155-9.
57. Gjerset RA, Yeargin J, Vokman SK, Vila V, Arya J, Haas M. Insulin-like growth factor-1 supports proliferation of autocrine thymic lymphoma cells with a pre-T cell phenotype. *J Immunol* 1990 **145**:3497-501.
58. Castano L, Eisenbarth GS. Type I-diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annu Rev Immunol* 1990; **8**:647-79.
59. Broadus AE, Mangin M, Ikeda K, *et al.* Humoral hypercalcemia of cancer. Identification of a novel parathyroid hormone-like peptide. *N Engl J Med* 1988; **319**:556-63.
60. Martin TJ, Allan EH, Caple IW, *et al.* Parathyroid hormone-related protein: isolation, molecular cloning, and mechanism of action. *Recent Prog Horm Res* 1989; **45**:467-506.
61. Rosenfeld MG, Mermod JJ, Amara SG, *et al.* Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature* 1983; **304**:129-33.
62. Fuller PJ, Verity K. Somatostatin gene expression in the thymus gland. *J Immunol* 1989; **143**:1015-7.
63. Vollmar AM, Schulz R. Atrial natriuretic peptide is synthesized in the human thymus. *Endocrinology* 1990; **126**:2277-80.
64. Fukayama M, Hayashi Y, Shiozawa Y, Maeda Y, Koike M. Human chorionic gonadotrophin in the thymus. *Amer J Pathol* 1990; **136**:123-9.
65. Lauriola L, Maggiano N, Larocca LM, *et al.* Cells immunoreactive for neuropeptide in human thymomas. *J Clin Pathol* 1990; **43**:829-32.
66. Berg LJ, Frank GD, Davis MM. The effects of MHC gene dosage and allelic variation in T cell receptor selection. *Cell* 1990; **60**:1043-53.
67. Sha WC, Nelson CA, Newberry RD, *et al.* Positive selection of transgenic receptor-bearing thymocytes by Kb antigen is altered by Kb mutations that involve peptide binding. *Proc Natl Acad Sci* USA 1990; **87**:6186-90.
68. Unanue ER. Macrophages, antigen-presenting cells, and the phenomena of antigen handling and presentation. In: Fundamental Immunology, Paul WE (ed), Raven Press, New York, 1989; 95-115.
69. Falk K, Rotzschke O, Rammensee HG. Cellular peptide composition governed by major histocompatibility complex class I molecules. *Nature* 1991; **348**:248-51.
70. Townsend AR, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 1986; **44**:959-68.
71. Rudensky AY, Preston-Hulburt P, Hong SC, Barlow A, Janeway CAJr. Sequence analysis of peptides bound to MHC class II molecules. *Nature* 1991; **353**:622-7.
72. Blackman M, Kappler J, Marrack P. The role of the T cell receptor in positive and negative selection of developing T cells. *Science* 1990; **248**:1335-41.

73. Sprent J, Lo D, Gao EK, Ron Y. T cell selection in the thymus. *Immunol Rev* 1988; **101**:5-19.
74. Ellis RE, Yuan J, Horvitz R. Mechanisms and functions of cell death. *Annu Rev Cell Biol* 1991 7:663-98.
75. Naquet P, Ellis J, Tibensky D, *et al.* T cell autoreactivity to insulin in diabetic and related non-diabetic individuals. *J Immunol* 1988; **140**:2568-78.
76. Geenen V, Martens H, Robert F, *et al.* Immunomodulatory properties of cyclic hexapeptide oxytocin antagonists. 74th Annual Meeting of the Endocrine Society, San Antonio, 1992; in press.
77. Smilek DE, Lock CB, McDevitt HO. Antigen recognition and peptide-mediated immunotherapy in autoimmune disease. *Immunol Rev* 1990; **118**:37-71.
78. Wraith DC. The use of MHC-binding peptides in the immunotherapy of autoimmune diseases: a commentary. *Res Immunol* 1991; **142**:505-8.
79. Eisenbarth GS, Walsh FS, Nirenberg M. Monoclonal antibody to a plasma antigen of neurons. *Proc Natl Acad Sci* USA 1979; **76**:4913-7.
80. Kramer S, Reynolds FHJr, Castillo M, Valenzuela DM, Thorikay M, Sorvillo JM. Immunological identification and distribution of parathyroid Hormone-like protein polypeptide in normal and malignant tissues. *Endocrinology* 1991; **128**:1927-37.
81. Bulloch K, Radjocic T, Yu R, Hausman J, Lenhard L, Baird S. The distribution and function of calcitonin gene-related peptide in the mouse thymus and spleen. *Prog Neuro Endocrin Immunol* 1991; **4**:186-94.