0163-769X/00/$03.00/0
Endocrine Reviews 21(4): 412–443

Printed in U.S.A.

Neuroendocrine Control of Thymus Physiology*

WILSON SAVINO AND MIREILLE DARDENNE

Laboratory on Thymus Research (W.S.), Department of Immunology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, 21045–900 Rio de Janeiro, Brazil; and CNRS UMR 8603 (M.D.), Université Paris V, Hôpital Necker, 75015 Paris, France

ABSTRACT

The thymus gland is a central lymphoid organ in which bone marrow-derived T cell precursors undergo differentiation, eventually leading to migration of positively selected thymocytes to the peripheral lymphoid organs. This differentiation occurs along with cell migration in the context of the thymic microenvironment, formed of epithelial cells, macrophages, dendritic cells, fibroblasts, and extracellular matrix components. Various interactions occurring between microenvironmental cells and differentiating thymocytes are under neuroendocrine control. In this review, we summarize data showing that thymus physiology is pleiotropically influenced by hormones and neuropeptides. These molecules modulate the expression of major histocompatibility complex gene products by microenvironmental cells and the extracellular matrix-mediated interactions, leading to enhanced thymocyte adhesion to thymic epithelial cells. Cytokine production and thymic endocrine function (herein exemplified by thymulin production) are also hormonally controlled, and, interestingly in this latter case, a bidirectional circuitry seems to exist since thymic-derived peptides also modulate hormonal production.

In addition to their role in thymic cell proliferation and apoptosis, hormones and neuropeptides also modulate intrathymic T cell differentiation, influencing the generation of the T cell repertoire.

Finally, neuroendocrine control of the thymus appears extremely complex, with possible influence of biological circuitry involving the intrathymic production of a variety of hormones and neuropeptides and the expression of their respective receptors by thymic cells. (*Endocrine Reviews* **21:** 412–443, 2000)

Address reprint requests to: Wilson Savino, Laboratory on Thymus Research, Department of Immunology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Avenue Brasil 4365, Manguinhos 21045-000, Rio de Janiero, Brazil. E-mail: savino@gene.dbbm.fiocruz.br

* This work was partially supported by grants from Brazilian Research Council (CNPq, Brazil) and Program for Excellency in Science (PRONEX/CNPq, Brazil), Centre National de la Recherche Scientifique (CNRS, France), Institut National de la Santé et de la Recherche Médicale (INSERM, France), Oswaldo Cruz Foundation (FIOCRUZ, Brazil) and Programme International de Cooperation Scientifique (PICS, France-Brazil).

I. Introduction

THE CROSS-TALK between the neuroendocrine and immune systems is now well demonstrated. These systems use similar ligands and receptors to establish a physiological intra- and intersystem communication circuitry that plays an important role in homeostasis. Increasing evidence has placed hormones and neuropeptides among potent immunomodulators, participating in various aspects of immune system function, in both health and disease (reviewed in Refs. 1–4). More particularly, the physiology of the thymus is modulated by a variety of biological circuits including those mediated by steroid and polypeptidic hormones, as well as neuropeptides. Herein, we focus on both the microenvironmental and the lymphoid aspects of neuroendocrine control of the thymus. We will first briefly comment on the structure of the thymic microenvironment and its role in the complex process of intrathymic T cell differentiation.

II. The Thymic Microenvironment and Its Role in T Cell Differentiation

The thymus gland is a central lymphoid organ in which bone marrow-derived T cell precursors undergo a complex process of maturation, eventually leading to migration of positively selected thymocytes to the T cell-dependent areas of peripheral lymphoid organs such as spleen, lymph nodes, Peyer's patches, and tonsils (5). Such a differentiation process involves sequential expression of various membrane markers and rearrangements of the T cell receptor genes.

Importantly, although thymocyte proliferation and differentiation persist throughout life, they diminish with aging. Older thymuses are significantly atrophied and have fewer thymocytes than younger ones. However, even in humans, the adult thymus is still active in terms of delivering mature T lymphocytes to the periphery of the immune system (6, 7).

A. Intrathymic T cell differentiation: general comments

From the entrance of T cell precursors into the thymus to the exit of mature cells from the organ, a vast body of interactions promotes the complex process of T cell differentiation. This differentiation involves regulation of the expression of various membrane proteins. A key membrane protein is the T cell receptor (TCR), which in the cell membrane is physiologically coupled with a molecular complex, termed CD3. Additional accessory molecules, including CD4 and CD8, as well as CD25 and the proteoglycan CD44, are useful to define stages of intrathymic T cell differentiation.

The TCR is a heterodimer formed by an $\alpha\beta$- or a $\gamma\delta$-chain configuration. Although $\gamma\delta^+$ thymocytes are the first to appear in the thymus with ontogeny of the organ, in the adult organ, around 99% of TCR^+ thymocytes express $TCR\alpha\beta$ and only 1% are $\gamma\delta$ T cells. A major point in intrathymic T cell differentiation is that, after gene rearrangements, any of the TCR peptide chains are generated, resulting in a large diversity of TCRs bearing distinct peptide specificities. This complex but well understood phenomenon is beyond the scope of this article but is reviewed in detail in recent publications (8–10).

The CD3 complex is an assembly of polypeptidic chains physically associated with the TCR. This association, together with the fact that CD3 bears cytoplasmic domains capable of phosphorylation, provides the intracellular signal transduction pathways necessary for TCR-driven T cell activation. Such activation follows ligation with a peptide presented by molecules of the major histocompatibility complex (MHC) expressed on the membranes of nonlymphoid cells. This ligation is favored by the accessory molecules CD4 and CD8, which are transmembrane glycoproteins that interact with class II and class I MHC molecules, respectively.

CD25 is the α-chain of the interleukin 2 (IL-2) receptor, and when it is expressed together with the β- and γ-chain, the receptor acquires high affinity for IL-2, thus favoring IL-2-driven thymocyte proliferation. The proteoglycan CD44 is a receptor for hyaluronic acid, and to a lesser extent for fibronectin and collagen. As seen below, it is associated with thymocyte migration events and is also considered a marker for T cell activation (11).

Cytofluorometric combined analysis of these markers proved to be useful in defining intrathymic T cell differentiation. The most immature thymocytes express neither the TCR/CD3 complex nor the accessory molecules CD4 or CD8 and thus are called double-negative thymocytes. Nevertheless, we can determine differentiation steps within the double-negative compartment by distinguishing the cells on the basis of their CD25 and CD44 expression. Thymocyte precursors that recently entered the thymus, in addition to being $TCR/CD3^-CD4^-CD8^-$, are also $CD44^+CD25^-$. As they differentiate, these immature cells acquire CD25 on the cell membrane, becoming $CD44^+CD25^+$, and then sequentially lose CD44 and CD25. The whole double-negative compartment represents about 5% of total thymocytes. Thymocyte maturation then progresses with the acquisition of both CD4 and CD8 markers, generating the so-called $CD4^+CD8^+$ double-positive thymocytes. These cells are the most common in the thymus, comprising 80% of total thymocytes. In the double-positive stage, TCR genes are rearranged. In differentiation of $TCR\alpha\beta$-bearing cells, the β-chain-related genes are rearranged first followed by the α-chain genes. At this stage, TCR is expressed in low density on the cell membrane. Thymocytes that do not undergo a productive TCR gene rearrangement (*i.e.*, that will not ultimately generate a peptide chain expressed on the cell membrane) die by default through apoptosis. By contrast, those expressing productive TCR will be able to react with peptides presented by molecules of the MHC, expressed on the membranes of nonlymphoid cells. This interaction will determine the positive and negative selection events, crucial for normal thymocyte differentiation. Positive selection allows the differentiation step whereby an immature, short-lived, $CD4^+CD8^+$ thymocyte

escapes from programmed cell death and becomes a mature, long-lived, $CD4^+$ or $CD8^+$ single positive cell. This is a highly stringent process, sparing only a small proportion of the $CD4^+CD8^+$ population. Positive selection also coincides with lineage commitment: the decision to become a $CD4^+$ or $CD8^+$ single positive thymocyte, as a function of the class of MHC molecule with which the TCR can interact. Negative selection in the thymus is the screen for establishing self-tolerance in the T cell repertoire, promoting deletion of T cells that might potentially be autoreactive to self-proteins. As illustrated in Fig. 1, positive selection events begin earlier in $CD3^+$ double-positive cells, whereas negative selection takes place in both double-positive and single-positive thymocytes (Reviewed in Ref. 8).

Positively selected thymocytes progress to the mature $CD4^+CD8^-$ or $CD4^-CD8^+$ single positive stage. These single positive cells comprise 15% of total thymocytes, with $CD4^+CD8^-$ cells being predominant over $CD4^-CD8^+$ cells. These mature thymocytes now express high densities of the TCR/CD3 complex and will ultimately leave the organ to form the large majority of the T cell repertoire in the periphery of the immune system (5, 12). Figure 1 is a simplified depiction of the sequential steps of thymocyte differentiation, with regard to the development of TCR$\alpha\beta$-bearing cells. For a basic description of intrathymic T lymphocyte differentiation, see Ref. 8, and to see this process in more detail, consult other recent reviews (9, 10).

It is noteworthy that thymocyte differentiation occurs as cells migrate within the thymic lobules. As seen in Fig. 2, *top panel*, most of the immature thymocytes, including those bearing the phenotypes $CD3^-CD4^-CD8^-$ and $CD3^+CD4^+CD8^+$ are cortically located, whereas mature $CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$ cells are found in the medulla, being those that will normally leave the organ to populate the T cell-dependent areas of peripheral lymphoid organs (5).

As briefly mentioned above, in parallel with migration and differentiation, thymocytes interact with various components of the thymic microenvironment (Fig. 2, *bottom panel*), a tridimensional network formed of epithelial cells, macrophages, dendritic cells (DC), fibroblasts, and extracellular matrix (ECM) components (5). Such interactions are necessarily transient, since most microenvironmental cells are sessile elements whereas thymocytes migrate within the organ while differentiating.

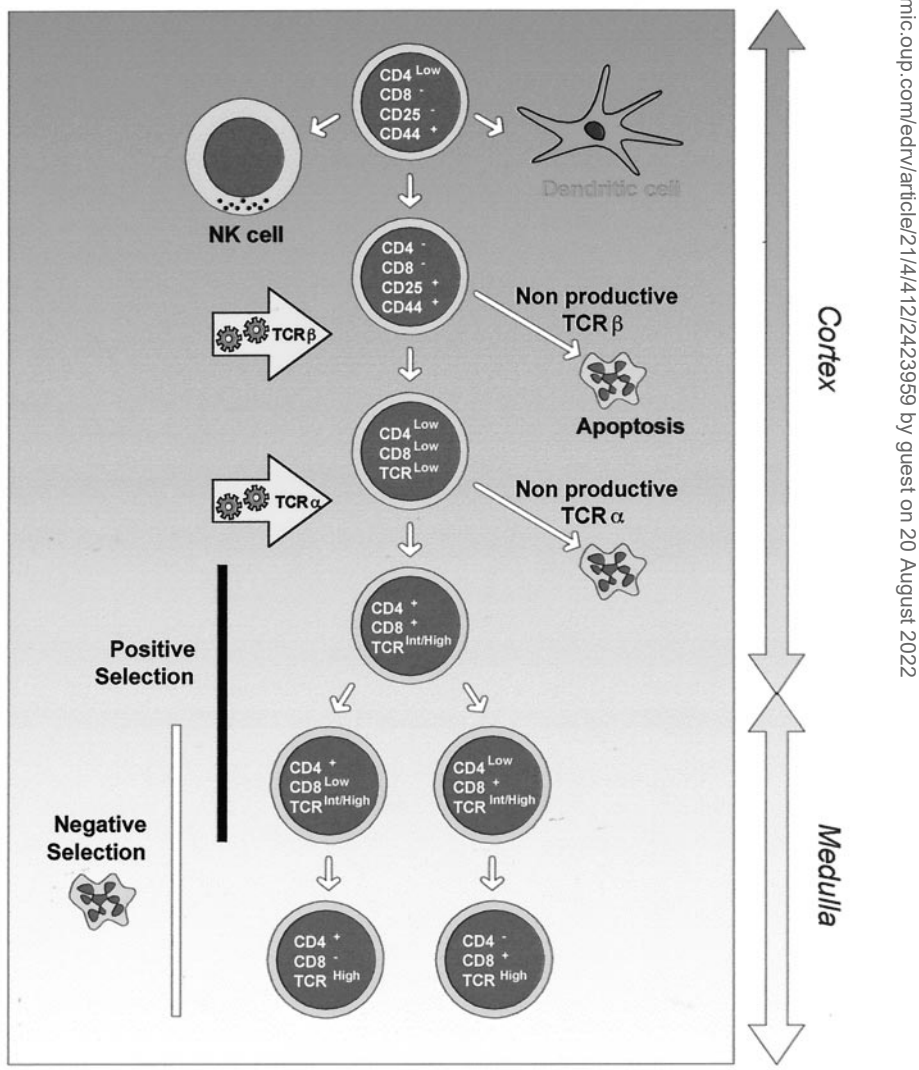

FIG. 1. Stages of thymocyte differentiation. This scheme depicts the differentiation pathway for TCR$\alpha\beta^+$ mouse thymocytes, as ascertained by the expression of various surface markers. When entering the thymus, T cell precursors bear the phenotype TCR/$CD3^-CD4^-CD8^-CD25^-CD44^+$. As they differentiate, immature cells acquire CD25, become $CD44^+CD25^+$, and then gradually lose CD44 followed by loss of CD25. Thymocyte maturation then progresses with the acquisition of CD4 and CD8 markers, generating the $CD4^+CD8^+$ thymocytes. In the double-positive stage, TCR genes are rearranged, for sequential expression of β- and α-chains, respectively (illustrated by the *intra-arrow wheels* in the figure). Productive TCRs, complexed with CD3, are initially expressed in low density on the cell membranes. These cells are then exposed to positive and negative selection events. Positively selected thymocytes progress to the mature $CD4^+CD8^-$ or $CD4^-CD8^+$ single positive stage. These mature thymocytes, which now express high densities of the TCR/CD3 complex, will ultimately leave the organ. Thymocytes that did not succeed in expressing TCR on the cell membrane, as well as those undergoing negative selection, will die by apoptosis. Localization of these various steps of thymocyte differentiation in the thymic lobule can be seen at the *right* side of the figure.

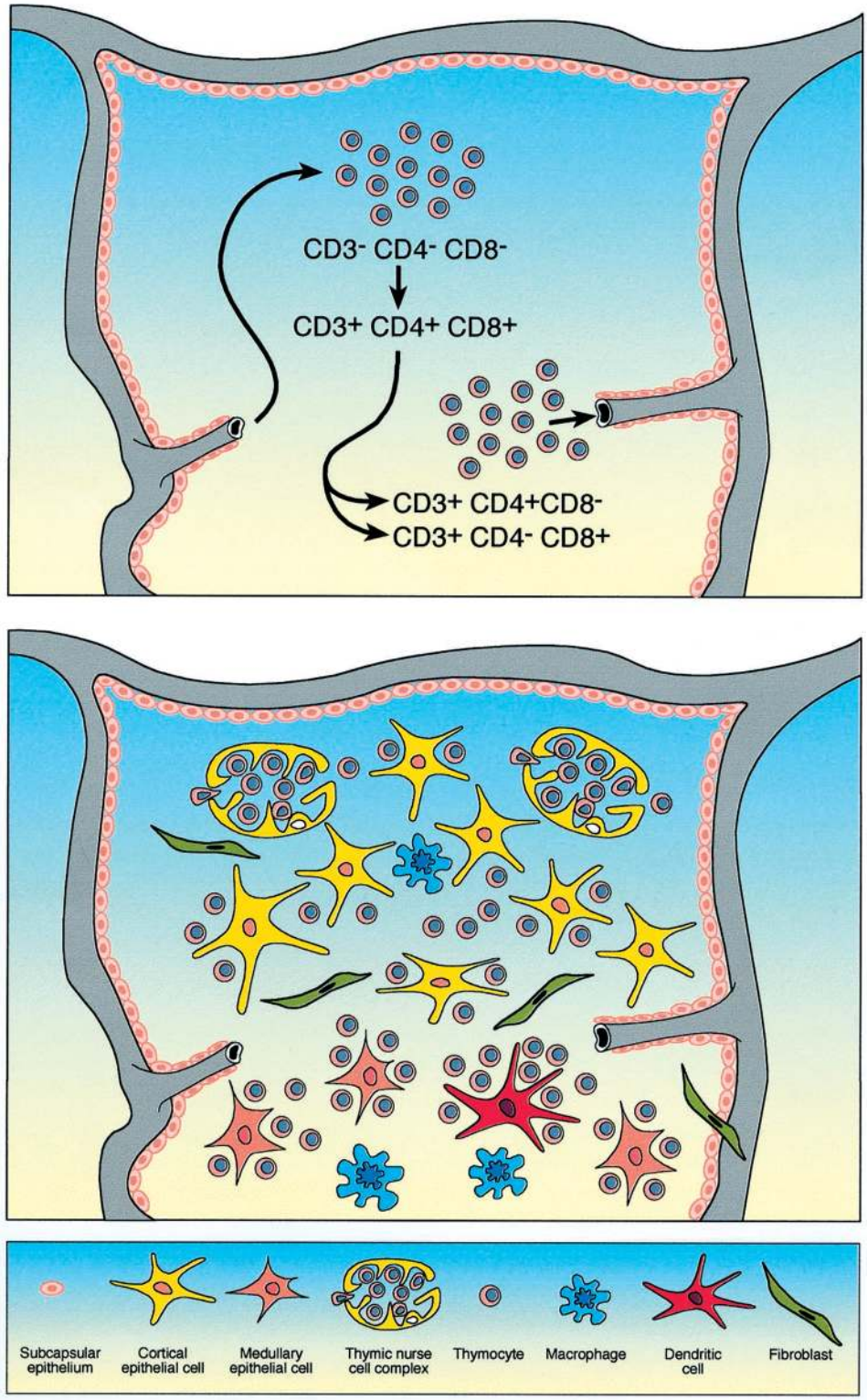

FIG. 2. The thymic microenvironment and intrathymic T cell differentiation. A simplified model of thymocyte migration can be seen in the *top panel*. This panel depicts the common entrance site of precursor cells into the organ through blood vessels. Having entered the thymus, thymocytes migrate during differentiation to ultimately leave the organ (also by blood vessels), bearing the mature phenotypes of $CD4^+CD8^-$ or $CD4^-CD8^+$ thymocytes. The *bottom panel* schematically depicts a thymic lobule, showing thymocytes intermingled with a heterogeneous cellular network representing the thymic microenvironment. This nonlymphoid compartment is composed of various cell types, including epithelial cells (*yellow and orange*), dendritic cells (*red*), macrophages (*blue*), and fibroblasts (*green*). Among TEC, morphological heterogeneity can be distinguished in subseptal/subcapsullary, cortical, and medullary regions. A particular cortically located lymphoepithelial complex, the thymic nurse cell, is seen.

B. Cellular interactions involving the thymic microenvironment

Several kinds of heterotypic interactions occur between differentiating thymocytes and microenvironmental cells. As mentioned above, one key cellular interaction involves the TCR/CD3 complex, expressed by differentiating thymocytes, with class I or class II MHC products on the microenvironmental cell membranes, complexed with a given endogenous peptide to be recognized, in the context of CD8 or CD4 molecules, respectively. The avidity of the resulting interaction is a determinant for positive *vs.* neg-

ative selection. Thymocytes with high avidity are negatively selected and are also deleted by apoptosis. This leads to the death of large numbers of potentially harmful autoreactive T cells. By contrast, a small percentage of thymocytes with intermediate avidity for recognition of MHC self-peptides appears to be rescued from death and is positively selected. Positive selection appears to be essentially conveyed by thymic epithelial cells (TEC) whereas negative selection can occur in the context of hematopoietic-derived DC, but also of TEC (5, 13).

In addition to the TCR/MHC-peptide interaction, the thymic microenvironment can influence the process of thymocyte migration/differentiation via other types of heterotypic membrane interactions. For example, TEC express classical membrane adhesion molecules such as ICAM-1 (intercellular adhesion molecule 1) and LFA-3, which respectively bind to LFA-1 and CD2 present on thymocytes (14–16). Moreover, TEC-thymocyte interactions can be mediated by ECM ligands such as fibronectin and laminin and their corresponding integrin receptors VLA-4/VLA-5 and VLA-6 (17–19). In fact, it is possible that ECM provides a complex macromolecular substrate onto which thymocytes migrate within the organ, following an ordered pattern as if on a conveyor belt (20). More recently, biochemical and functional evidence was provided that TEC communicate with each other by gap junctions, which are formed by proteins of the connexin family and allow direct passage of low molecular weight substances between adjacent cells (21). Microinjection of low molecular weight fluorochromes also revealed functional gap junction-mediated TEC-thymocyte interactions. Based on further findings that have appeared in the literature, we recently postulated that gap junctions may correspond to a novel route for cell-cell communication in the immune system (22).

Thymic microenvironmental cells can influence thymocyte differentiation and proliferation by means of soluble polypeptides. Both TEC and DC produce the cytokine IL-1, which stimulates thymocyte proliferation (23). Actually, various cytokines can be produced by thymic epithelium, including IL-3, IL-6, IL-7, IL-8, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, transforming growth factor-α, transforming growth factor-β (TGF-β), leukemia inhibitory factor, and stem cell factor (24, 25).

IL-7, in particular, has been proven to be crucial for thymocyte differentiation. For example, it was shown to promote rearrangement of the TCR genes by enhancing the production and activity of recombinases (26). In conjunction, IL-7$^{-/-}$ as well as IL-7 receptor-deficient mice display a severe reduction in lymphoid development, whereas the transgene incorporation of IL-7 in nude mice induces T cell development (27). In addition to IL-7, SCF (also termed c-Kit ligand) is necessary for early thymocyte differentiation. When fetal thymuses of spontaneously SCF-deficient mice are grafted into normal wild-type recipients, the number of CD3$^-$CD4$^-$CD8$^-$ is more than 10 times lower than in wild-type grafts (27).

In addition to classical cytokines, chemokines are also thymic microenvironment-derived secretory products important in thymus physiology. Chemokines correspond to a family of small polypeptidic molecules that control directional migration of leukocytes (reviewed in Ref. 28). Among others, one chemokine, the stromal cell-derived factor (SDF) is highly expressed in the thymus, being produced by stromal cells, particularly in the subcapsular region (29). In keeping with this topography, SDF preferentially attracts immature CD4$^-$CD8$^-$ and CD4$^+$CD8$^+$ thymocytes. Conversely, another chemokine, MIP3β, exerts chemoattraction for mature single positive thymocytes (30). This is in keeping with the differential expression of corresponding chemokine receptors in distinct CD4/CD8-defined stages of thymocyte differentiation.

This leads to the notion that several paracrine circuits involving TEC-derived factors are likely to have differentiating thymocytes as targets. In addition to producing cytokines, TEC secrete chemically defined thymic hormones, including thymosin-α1, thymopoietin, and thymulin (31–33), that can also act upon the general process of thymocyte maturation (reviewed in Refs. 34–36). For instance, thymulin, a nonapeptide whose biological activity depends on its coupling to zinc (37, 38), is able to enhance thymocyte proliferation and to induce several T cell markers and functions (reviewed in Refs. 34–36 and 39). The circulating levels of thymulin achieve maximal values early in postnatal life and decline with age (40). More recently, it has been shown that thymulin secretion follows a circadian rhythm, peaking during the night (41). The general characteristics of thymulin and its effects on the immune system are summarized in Table 1. For recent reviews on the various thymic hormones, see Refs. 34–36.

Interestingly, TEC-thymocyte interactions mediated by soluble substances are bi-directional, so that thymocyte-derived secretory products can modulate TEC behavior. This is exemplified by the cytokine interferon-γ (IFN-γ), which can induce MHC class II expression by cultured TEC (42–44) as well as the expression of ECM ligands and receptors, with consequent modulation of TEC-thymocyte adhesion (44, 45). Figure 3, *left panel*, summarizes the various types of cellular interactions between thymocytes and TEC.

In addition to interacting with thymocytes, epithelial cells can interact with each other through surface molecules and soluble products (Fig. 3, *right panel*). That murine and human TEC establish functional gap junctions through connexin 43 (21) opens the possibility that the thymic epithelium may affect thymocyte behavior concertedly, with clusters of adjacent TEC behaving as functional syncitia, integrated via gap junctions.

Macrophages and DCs are hematopoietic-derived cell types and quantitatively are a minor component of the thymic microenvironment. Dendritic cells are preferentially located in the medulla and at the corticomedullary junction of the thymic lobules, whereas macrophages are distributed throughout the lobule (5); they both express MHC class II molecules and can interact with differentiating thymocytes via membrane proteins and cytokines, including IL-1.

TABLE 1. General characteristics and immunological effects of thymulin

Thymulin characteristics and immunomodulatory effects	References
Biochemistry and cell biology	
Chemically defined nonapeptide coupled to zinc	37, 343
Immunochemically defined zinc-dependent epitope and NMR-defined zinc-dependent structural conformation	38, 344
Zinc uptake from metallothionein produced by thymic epithelial cells	345
Secreted by thymic epithelial cells	31–33
Exocytosis mode of secretion	346
Calcium-dependent secretion	347
Zinc stimulation of secretion *in vitro*	348
Nyctohemeral pattern of secretion	41
Age-dependent decay of secretion	40
Specific receptors defined in T cell lines	349, 350
Immunomodulatory effects	
Enhancement of CD90 (Thy. 1) expression by mouse spleen T cells	351
Enhancement of CD3, CD4 and CD8 expression in immunodeficient children	352
Increase in avian PNA^+ thymocytes	353
Increase in IL-2 production by mouse thymocytes and nude mouse splenocytes	354
Increase in IgA and IgE synthesis in patients with ataxia telangectasia	355
Stimulatory effect on extrathymic maturation and activation of intraepithelial T lymphocytes	356
Increase of LPS-induced polyclonal B cell responses of CBA/N mice	357
Enhancement of T cell-dependent macrophage-mediated killing of *Salmonella*	358
Upregulation of NK activity in humans and mice	359–361
Retardation of skin graft rejection in normal mice	362
Increase in IL-1 and decrease in IL-6 and TNF-α production by peripheral blood mononuclear cells from normal volunteers	115
Decrease in IL-1, IL-2, and TNF-α production by peripheral blood mononuclear cells from systemic lupus erythematosus patients	115
Reduction in anti-DNA antibody production and glomerulonephritis in lupic [NZBxNZW]F1 mice	363
In vitro modulation of T cell markers in human rheumatoid arthritis and systemic lupus erythematosus patients	364
In vivo prevention of encephalomyocarditis virus-induced diabetes and myocarditis in mice	365
In vitro modulation of T cell markers and increase in mitogen- or antigen-induced T cell proliferation in bone marrow transplantation patients	366
In vitro induction of mature T cell markers in circulating immature T lymphocytes in malnourished children	367
Restorative effect on the stress-induced reduction of thymus-dependent antibody production in mice	368
Immunopotentiation in chickens vaccinated for various viral diseases	369
Decrease in hind paw swelling and anti-type II collagen antibody production in experimental arthritis in rats	370

NMR, Nuclear magnetic resonance; LPS, lipopolysaccharide; NK, natural killer.

Concerning the constitution of the intrathymically generated T cell repertoire, there is strong evidence that dendritic cells of the thymic microenvironment are involved in determining negative selection of the T cell repertoire by thymocyte deletion (13). In contrast, a role for macrophages in negative selection remains to be conclusively demonstrated.

C. Heterogeneity of the thymic epithelium: the thymic nurse cell complex

The thymic epithelial network is a rather heterogeneous tissue in terms of morphology and phenotype, and cells in different locations within the thymic lobules may be responsible for influencing specific steps in T cell maturation (46). One cortically located lymphoepithelial complex, the thymic nurse cell (TNC), has been isolated *in vitro*. TNCs are lymphoepithelial multicellular structures formed by one TEC, which in mice can harbor 20–200 thymocytes (47), and are located in the cortical region of thymic lobules (48, 49). Most intra-TNC lymphocytes bear the $CD4^+CD8^+$ double-positive phenotype (50), although immature double-negative as well as mature single-positive cells can also be found. Interestingly, TNCs may create special microenvironmental conditions for thymocyte differentiation and/or proliferation, and within this complex distinct interactions apparently occur, including those mediated by soluble products, gap junctions, ECM, and MHC/TCR (reviewed in Ref. 51). Self-antigens appear to be presented to thymocytes within TNC (52), and intra-TNC lymphocyte apoptosis has recently been reported (53, 54). Once settled in culture, TNCs spontaneously release thymocytes, and TNC-derived epithelial cells can reconstitute lymphoepithelial complexes after being cocultured with fetal thymocytes (55). Thus, TNCs constitute an *in vitro* model of thymocyte migration within the TEC context (18, 19, 45, 51, 56).

Other experimental models have been used to dissect the sequence of acquisition/loss of differentiation markers, as well as their respective roles in intrathymic T cell differentiation. A significant contribution was the generation of genetically engineered mice (57). The *in vitro* model of fetal thymus organ cultures (FTOC) is also used to study intrathymic T cell differentiation. By day 14 of gestation, only immature $CD4^-CD8^-$ thymocytes are seen, whereas after a 14-day culture of the thymic lobes, differentiation has progressed with the generation of $CD4^+$ or $CD8^+$ single-positive mature cells (58).

As detailed below, the various intrathymic cellular interactions as well as the *in vivo* and *in vitro* experimental models

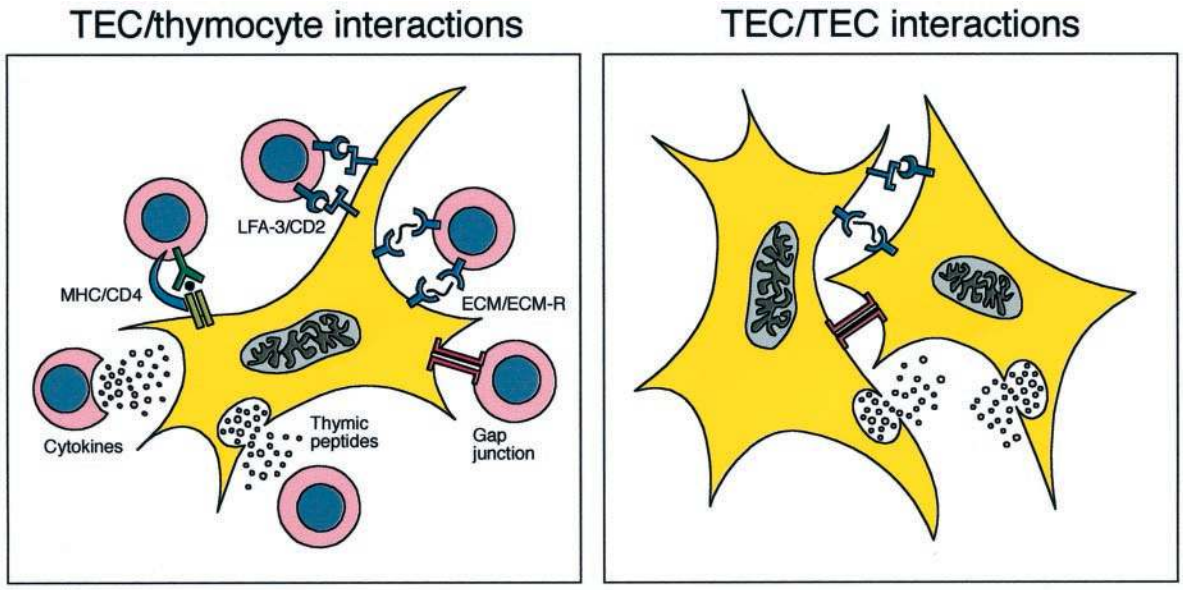

FIG. 3. Heterogeneity of interactions between TEC and thymocytes. *Left panel* shows types of TEC-thymocyte interactions. Among membrane interactions are those involving the MHC complex (in this case MHC class II molecules) expressed by TEC, binding endogenous peptide presented to the TCR/CD3 complex on thymocytes, in the context of accessory molecules (exemplified herein by the CD4, labeled in *blue*). Additional cell-cell interactions, comprising receptors and coreceptors, are represented by adhesion molecules, namely LFA-3/CD2 and ICAM-1/LFA-1. Moreover, TEC can interact with thymocytes via ECM ligands and receptors (ECM-R), or gap junctions. In addition to membrane interactions, bidirectional TEC-thymocyte exchange of biological signals can be conveyed by secretory products such as cytokines and thymic hormones. *Right panel* illustrates that homotypic TEC-TEC interactions can also take place, involving adjacent cell membranes as well as soluble molecules.

summarized above can be regarded as potential targets for control by hormones and neuropeptides.

III. Neuroendocrine Control of Membrane Interactions Between Thymocytes and Microenvironmental Cells

A. Is there a hormonal effect upon MHC expression by microenvironmental cells?

Few data are so far available to determine whether hormones or neuropeptides alter the expression of MHC gene products by thymic microenvironmental cells. Recent findings suggest that this may be the case. When human peripheral blood leukocytes were subjected to a mixed lymphocyte culture, the levels of cell proliferation and cytotoxic activity were significantly higher in the presence of recombinant GH, respectively 4- and 7-fold compared with controls (59). This is in keeping with previous evidence that GH treatment in children after renal transplantation worsened allograft function (60). In addition, a rise in IFN-γ secretion and in the number of cells expressing the corresponding mRNA was observed *in vitro* (6- to 10-fold more cells in GH-treated patients than in controls). In the same study, it was demonstrated that such effects require the presence of autologous antigen-presenting cells. Given the enhancing effect of GH upon IFN-γ production, together with the cytokine-induced up-regulation of MHC class I and class II expression (42–44) and the role played by adhesion molecules of thymic microenvironmental cells in MHC-TCR interactions (45, 55), we suggest that the MHC-mediated influence on thymocyte differentiation may be modulated by fluctuations in GH. This suggestion remains a working hypothesis and should be tested in specifically designed experiments.

More direct evidence, recently reported by Sacedon and co-workers (61–63), showed an effect of glucocorticoid hormones. Thymic dendritic cells treated *in vitro* with dexamethasone exhibited a slight, yet consistent, increase in the membrane expression of MHC class I molecules. This seems to be specific to class I, since no effect was observed on the expression of MHC class II gene products.

The same research group showed earlier expression of MHC class I and II molecules during thymus ontogeny in rat fetuses whose mothers had been previously adrenalectomized (52). It should be noted that before full development of the hypothalamus-pituitary-adrenal axis, circulating glucocorticoids in the fetus are strictly of maternal origin. Thus, what these experiments tell us is that absence of circulating glucocorticoids in early fetal life accelerates intrathymic MHC expression. Although in this particular work double labeling for simultaneous detection of MHC and cytokeratin was not reported, the micrographs, showing MHC labeling in the whole microenvironmental network, led us to think that the thymic epithelial network may include MHC expression. This idea is supported by the authors' finding of earlier detection of other known markers for TEC differentiation in fetal thymuses derived from adrenalectomized mothers.

Thus, the findings discussed above argue in favor of hormonal regulation of MHC expression by the thymic microenvironment. Yet, formal demonstration of a hormonal regulation of MHC expression by TEC is still lacking. Nor has the possible influence of hormones and neuropeptides on

intrathymic MHC expression been approached in terms of its consequences on MHC-TCR interactions.

B. Extracellular matrix-mediated TEC-thymocyte interactions are hormonally modulated

Initial studies revealed that the intrathymic production of ECM proteins, including fibronectin, laminin, and type IV collagen, was enhanced *in vivo* in mice injected with hydrocortisone; thickened ECM-containing fibrils were observed in both cortical and medullary regions of the thymic lobules as early as 24 h posttreatment. In the protocol of a single dose injection, this effect was transient, being progressively reversed in parallel with thymocyte expansion. Additionally, augmented amounts of such ECM components were detected in mouse TEC cultures treated with glucocorticoid hormone (64), indicating that the effect of hydrocortisone enhancing ECM in the thymus represents direct activity on the thymic epithelium. Similar results were obtained with sex steroids (64). At variance with these data, however, it was reported that the levels of fibronectin [measured by enzyme-linked immunosorbent assay (ELISA)] in human TEC culture supernatants were not altered after hydrocortisone treatment (65). Considering that this steroid hormone was also shown to enhance ECM receptor on the TEC membranes (18), it is possible that the lack of modulation in the supernatant derives from the augmented levels of the ECM bound on the TEC surface stimulated by hydrocortisone, which would then mask the levels in the culture supernatants.

In a further study, long-term treatment (30 days) with T_3 in mice also yielded changes in the intrathymic distribution profile of ECM proteins, with an increase in thin ECM fibrils (thus differing from the thick fiber pattern seen after glucocorticoid injection), particularly in the cortical region of thymic lobules. Again, such an effect seems to be direct upon TEC, since enhanced ECM production was also seen when T_3 was added to cultures of a murine TEC line and to TNC-derived TEC preparations (66). More recently, similar results were obtained *in vitro* when various TEC cultures were subjected to PRL, GH, or insulin-like growth factor I (IGF-I) (56). It should be pointed out that, regarding the *in vitro* models mentioned above, not only were the amounts of fibronectin and laminin enhanced by various hormone treatments, but also was the expression of their corresponding receptors, VLA-5 and VLA-6 (56, 66).

Since thymocyte-TEC adhesion is at least partially mediated by ECM ligands and receptors, we also tested the various hormones cited above, corticosteroid, thyroid, and pituitary hormones, for their ability to modulate such heterotypic cellular interaction. All enhanced the degree of thymocyte adhesion to cultured TEC. Furthermore, regarding pituitary hormones (56), the hormone-induced enhancement of TEC-thymocyte adhesion was abrogated by monoclonal antibodies specific for each hormone or its corresponding receptor, and also by various anti-ECM or anti-ECM receptor antibodies (Fig. 4).

Together, these data clearly indicate that ECM-mediated TEC-thymocyte interactions are under endocrine control. Whether neuropeptides also modulate these heterotypic interactions remains to be determined.

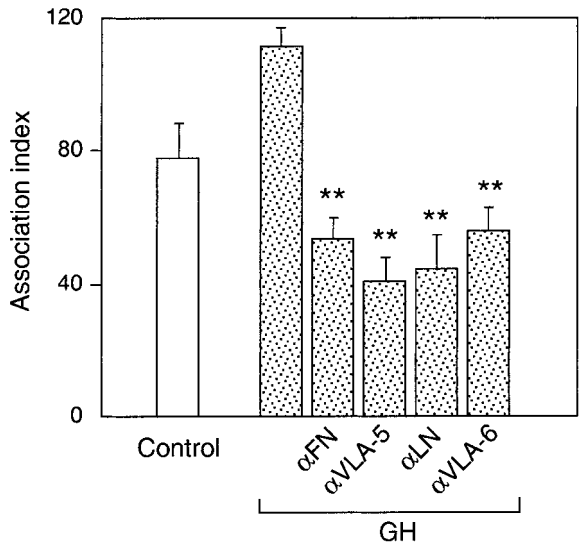

FIG. 4. ECM-mediated, GH-induced enhancement of thymocyte adhesion to TEC. Freshly isolated mouse thymocytes are left to adhere onto cultures of a mouse TEC line in the absence or presence of GH. Additionally, some GH-treated cultures are incubated with antifibronectin (α-FN), anti-VLA5 (α-VLA5), antilaminin (α-LN), or anti-VLA6 (α-VLA-6) monoclonal antibodies before GH treatment. The GH-induced enhancement of thymocyte-TEC adhesion is clearly abrogated by anti-ECM or anti-ECM receptor antibodies. **, $P < 0.01$. [Adapted with permission from V. de Mello-Coelho *et al.: J Neuroimmunol* 76:39–49, 1997 (56) © Elsevier Science.]

C. Are inter-TEC gap junctions under neuroendocrine control?

Very recent data indicate that gap junctions mediating communication between adjacent TEC can also be under hormonal control. Decreased cell coupling between adjacent TEC (ascertained by diffusion of intracellularly injected low molecular weight fluorochrome) was seen when cultures were treated by the sex steroids testosterone, progesterone, and estrogen, as well as by the pituitary hormones ACTH and GH, and the neuropeptide calcitonin gene-related peptide (CGRP) and substance Y (67, 68). Conversely, results from our laboratory indicate that vasointestinal peptide (VIP), a neuropeptide that increases intracellular cAMP, enhances inter-TEC cell coupling (69). Similar enhancement was seen when cultures were treated with glucocorticoid hormones, an effect that was significantly abrogated by the use of the glucocorticoid receptor antagonist RU486 (70). Nevertheless, a systematic survey to determine which hormones and neuropeptides modulate gap junction opening between adjacent TEC and between TEC and thymocytes has not yet been made. Nor are data so far available concerning putative neuroendocrine control of connexin expression by TEC.

IV. Thymic Endocrine Function and Cytokine Secretion by Microenvironmental Cells Are Controlled by Hormones and Neuropeptides: The Paradigm of Thymulin

A. Steroid and peptidic hormones influence in vitro cytokine secretion

In addition to acting upon membrane interactions, hormones and neuropeptides can modulate the production of cytokines and hormones by thymic microenvironmental cells. Exogenous IL-1β enhances IL-6 and IL-8 production by cultured human TEC and induces granulocyte macrophage colony stimulating factor (GM-CSF) as well as leukemia inhibitory factor (LIF) production by these cells. Interestingly, hydrocortisone is able to selectively block the latter effect on GM-CSF and LIF, as ascertained by ELISA assessment of cytokines in TEC culture supernatants (65). In a mouse TEC line, retinoic acid promoted enhanced expression of IL-1α, IL-6, and IL-7 (71). The production of IL-1α and IL-1β by bovine nonepithelial thymic microenvironmental cells *in vitro* was increased by exogenous GH and by PRL (72). It was further shown that secretion of IL-6 is also up-regulated by GH or by PRL treatment, an effect that could be abrogated by the use of the IL-1 receptor antagonist. This indicates that hormonal effects on microenvironmental cell-derived IL-6 secretion are at least partially exerted through the IL-1 production pathway. Autocrine/paracrine control of cytokine production by the thymic microenvironment appears to involve TEC-derived neuropeptides as well. Martens and colleagues (73) demonstrated that the constitutive production of both IL-6 and LIF (but not IL-1β) by primary cultures of human TEC was enhanced when monoclonal antibodies to oxytocin were added to the cultures. This strongly suggests that the secretion of these TEC-derived cytokines is partially under the control of oxytocin.

Together, the findings discussed above strongly favor the notion that cytokine production by the thymic microenvironment is under neuroendocrine control. Nonetheless, such a notion should be cautiously viewed since corresponding *in vivo* data are still lacking. In this respect, evaluation of cytokine production by the microenvironmental cells in mice genetically engineered for hyperproduction or lack of hormones or neuropeptides will be certainly worthwhile. The same is true for more precise analysis of IL-7 and of SCF production by thymic microenvironmental cells under various hormonal fluctuations.

B. Thyroid and pituitary hormone status modulates thymulin secretion

The concept of neuroendocrine control of thymic secretory substances has been particularly developed with regard to the production of the thymic hormone thymulin. In a first series of studies we demonstrated that *in vivo* treatment with T_3 enhanced thymulin secretion by mouse TEC, and that an opposite effect was seen if the animals were treated with propiothiouracil, an inhibitor of thyroid hormone synthesis (74). These results were confirmed by others (75). In aging mice, injection of T_4 increased thymulin serum levels to values found in young individuals (75). In humans, it was shown that patients with hyperthyroidism exhibit higher levels of circulating thymulin, whereas in hypothyroidism the opposite was observed. In both situations, adequate therapy brought thymulin serum levels within normal range (76).

Experiments using TEC cultures showed that the stimulatory effect of thyroid hormones upon thymulin secretion was due to a direct action of the hormone on the epithelial cells (66) and depended on *de novo* synthesis of thymulin, since it could be abrogated by cycloheximide (77).

Pituitary hormones were also shown to be potent up-regulators of thymulin secretion. For instance, experimental hyperprolactinemia induced by repeated PRL injections increased thymulin levels in both young and old animals. Conversely, administration of bromocriptine, an agonist of the dopamine receptor, which inhibits PRL biosynthesis, promoted a consistent, dose-dependent decrease in thymulin production (78). These results, obtained in mice, are in keeping with data derived from hyperprolactinemic patients bearing pituitary adenomas, who present abnormally high thymulin serum levels (79).

Fluctuations in GH levels also modulate thymulin secretion. Initial studies revealed that dwarf mice exhibit a precocious decay in thymulin levels (80). This is in keeping with more recent data showing that hypophysectomy in rats yielded a profound, although transient, decrease in thymulin serum levels (81). Similarly, low thymulin levels accompanied deficient GH production in children, whereas GH treatment consistently restored this thymic endocrine function, as early as 24 h after injection (82, 83). Moreover, GH treatment induced an increase in thymulin serum levels that correlated with the amounts of circulating IGF-I. In acromegalic patients, high thymulin serum titers also correlated with high IGF-I serum levels (84).

It is noteworthy that the enhancing effects of PRL and GH on thymulin secretion were directly obtained by treating murine or human TEC cultures and were abrogated by corresponding antihormone antibodies (78, 84). *In vitro* GH effects were abrogated by anti-IGF-I or anti-IGF-I receptor antibodies, thus incriminating TEC-derived IGF-I as a mediator of the GH effects upon TEC (84). This was further supported by findings that both anti-IGF-I and anti-IGF-I receptor antibodies were also able to block GH-dependent enhancement of TEC-thymocyte adhesion (56).

In addition to the effects observed with classical peptidic hormones, we found that endogenous opioids, namely β-endorphin and Leu-enkephalin, can up-regulate thymulin secretion by cultured TEC (85). I*n vivo* experiments to confirm this notion are still lacking, however. Nor are data available to define whether other neuropeptides influence thymulin secretion.

C. Effects of adrenalectomy and gonadectomy on thymulin levels

The effects of adrenal and gonadal steroids on thymulin secretion appear to be rather more complex. One of the experimental strategies to approach this issue is ablation of the adrenals and/or gonads. When a single surgery (adrenalectomy or gonadectomy) was performed, we observed in both male and female mice a transient fall in thymulin serum

levels that lasted 1 month, peaking 1 week postsurgery, and then progressively returning to normal. More impressive, adrenalectomy + castration resulted in a long-term decrease in the levels of circulating thymulin that persisted until 3 months postsurgery, being followed by gradual restoration to normal levels by 6 months after surgery (86). Interestingly, in both single and double surgical procedures, an increase in the intrathymic content of thymulin was seen. Concomitant to the sustained low levels of the thymic hormone in these experimental conditions, an endogenous low molecular weight thymulin inhibitor was transiently detected in the mouse sera, with concentrations peaking when thymulin levels were lowest. Although the biochemical nature of such a thymulin inhibitor was not defined, its appearance was thymus dependent, since it was not found in mice undergoing thymectomy before adrenalectomy + gonadectomy (86). This series of experiments, although not conclusive, pointed to a rather complex mechanism involved in *in vivo* steroid hormone influence upon thymic hormone production, possibly comprising other biological circuits, including the hypothalamus-pituitary axis.

This possibility led us to study the influence of steroid hormones on murine and human TEC cultures, and we observed that physiological concentrations of glucocorticoid hormones, estradiol, progesterone, or testosterone, enhanced thymulin release into the culture supernatants. This effect was abrogated when TEC were simultaneously incubated with a given steroid hormone plus the specific antagonist of the corresponding hormone receptor (87).

In view of these findings, it is possible that the transient *in vivo* increase in the intrathymic contents of thymulin observed after adrenalectomy and/or gonadectomy corresponds to a TEC response to the fall in circulating levels of biologically active thymulin, secondary to the appearance of its natural inhibitor. Such a feedback circuit with increase in thymulin production had been previously shown in mice treated with antithymulin monoclonal antibodies (88).

D. Is there an autocrine/paracrine circuitry controlling thymulin secretion?

That *in vivo* treatment of mice with antithymulin monoclonal antibodies resulted in an increase in the intrathymic content of the hormone suggested that the level of circulating thymulin could influence its rate of secretion. Similarly, in keeping with these findings, we noted that incubation of cultured TEC with antithymulin monoclonal antibodies resulted in increased numbers of thymulin-containing cells (89). Conversely, thymulin release into TEC culture supernatants can be down-regulated by exogenous addition of the hormone itself.

Interestingly, IL-1, which is also produced *in vivo* and *in vitro* by TEC, is able to stimulate *in vivo* zinc uptake by the thymus (likely to be due to the increase in metallothionein biosynthesis by the epithelial cells), thus up-regulating thymulin secretion *in vitro* (90, 91).

Altogether, the data discussed above clearly indicate that the general control of thymulin secretion may be very complex, involving distinct biological circuits whose overall balance will dictate the amounts of thymulin to be secreted at a given moment. Due to this apparent complexity, it is predictable that compensatory loops may be triggered when one or more thymulin-controlling axes are disturbed. This would explain why in some experimental situations, fluctuations in thymulin serum levels are transient.

E. Thymic hormones modulate endocrine glands and neuroendocrine circuits

The concept of bidirectionality between the neuroendocrine and immune systems can also be applied to analysis of thymic hormones, since these substances modulate the production of hormones and neuropeptides of the hypothalamus-pituitary axis and some of their target endocrine glands.

Initial experiments revealed that neonatal thymectomy promotes developmental atrophy of female sexual organs (92). One might argue that such an effect could reflect an autoimmune process rather than a direct action of thymic hormones on the neuroendocrine system. This assumption is based on the fact that perinatal thymectomy in BALB/c mice induces autoimmune disease (93). Nevertheless, in the experiments supporting this view, thymectomy was performed on day 3 postnatally, when the thymus has already released a significant amount of thymocytes (initiated on the day of birth). By contrast, in the former experiments, thymectomy was carried out at birth, thus before colonization of peripheral lymphoid organs by T cells. Moreover, as detailed below, it has been shown that production of sex steroids is enhanced *in vivo* and *in vitro* by a single thymic hormone, thymulin.

In addition to the action on sexual organs, thymectomy at birth promoted a decrease in the number of secretory granules in acidophilic cells of the adenopituitary (94). This is in keeping with data showing that athymic *nude* mice exhibit significantly low levels of various pituitary hormones, including PRL, GH, LH, and FSH (95).

Regarding the effects of thymic peptides, it was shown that thymosin-β4, when perfused intraventricularly, stimulates *in vivo* LH and its hypothalamic-releasing hormone LHRH (96). A similar stimulation of LH release was obtained with thymulin in perifused or fragmented pituitary preparations (97, 98). Another thymosin component, the MB-35 peptide, also enhanced PRL and GH production (99). *In vivo* studies in children showed that administration of thymopoietin increases GH and cortisol serum levels (100). Moreover, thymopentin (the synthetic biologically active peptide of thymopoietin) enhanced *in vitro* the production of POMC derivatives such as ACTH, β-endorphin, and β-lipotropin (101). Thymulin exhibited a similar *in vitro* stimulatory effect on perifused rat pituitaries, enhancing the release of GH, PRL, and, to a lesser extent, TSH (102). With regard to its effect on GH release, it has been shown to be age-dependent, being less efficient in pituitary cell cultures derived from senescent animals (103). The same study showed that this effect of thymulin is mediated by calcium influx, as well as cAMP and inositol phosphate (103). However, contrasting results were reported using short-term cultures of pituitary fragments: a consistent increase in ACTH secretion after *in vitro* thymulin treatment, with no changes in GH levels but a significant inhibition of PRL release (98).

Thymosin-α1 was apparently able to down-regulate TSH,

ACTH, and PRL secretion *in vivo*, although effects on GH levels were not detected (104). Interestingly, these inhibitory effects seem to occur through hypothalamic pathways, since production of corresponding releasing hormones by hypothalamic neurons was also decreased after *in vitro* treatment of medial basal hypothalamic fragments with thymosin-α1 (105).

In addition to affecting the hypothalamus-pituitary axis, thymic hormones may act directly on its target endocrine glands (Fig. 5). *In vitro* experiments showed that thymulin can modulate gonadal tissues. Proliferation of oogonia from fetal rat ovaries, as well as gonocytes from fetal rat testicles, was consistently increased in the presence of thymulin (106–108). At least regarding the expansion of male germ cells in the same culture system, thymulin-inducing proliferative effects were largely prevented by TGF-β1 (109).

In addition to the effect of thymic hormone on germinal cells, it was shown that TEC-derived culture supernatants could stimulate *in vitro* progesterone and estradiol secretion by granulosa cells of rat ovary (110). However, this effect was not seen with thymosin-α1 or thymulin, thus suggesting the involvement of other TEC-derived hormone(s) or cytokine(s), which have not yet been identified. At variance with these results are the recent data showing that *in vivo* injection of thymulin in mice enhanced circulating progesterone levels, which is likely to account for the delay in the vaginal opening seen in thymulin-treated animals (111). In keeping with this finding, studies conducted in boars showed that thymulin increases testosterone levels *in vitro* and *in vivo*, enhancing the secretion of testosterone in short-term cultures of testicular minces. Additionally, testosterone-circulating levels were enhanced 2–3 h postinjection (112) only in those animals previously selected for having spontaneous high levels of circulating LH. The authors suggested that the effects of thymulin upon testosterone secretion occur via the action of LH on Leydig cells. Although further studies are obviously necessary to better dissect the role of thymulin in reproductive physiology, the data discussed above generally favor this hypothesis.

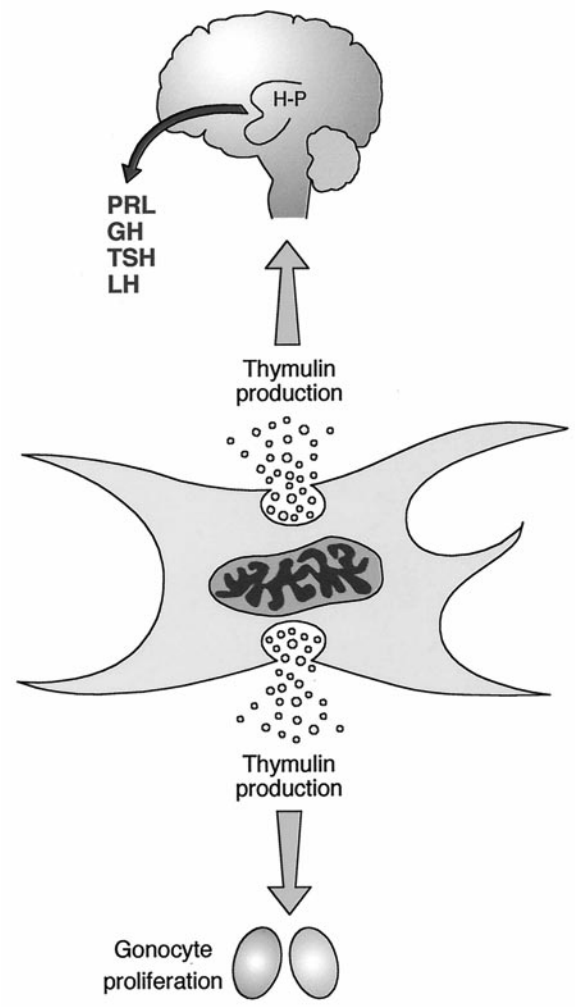

FIG. 5. Effects of thymulin on endocrine glands, as exemplified by the stimulatory effects on PRL, GH, TSH, and LH secretion by pituitary cells, as well as gonocyte proliferation.

It should be pointed out that the existence of direct effects of thymic hormones upon other endocrine glands that are physiological targets of the hypothalamus-pituitary axis, such as thyroid and adrenals, has not yet been studied.

Recent work has shown that thymulin can also modulate some peripheral nervous sensory functions, such as those related to pain sensitivity. *In vivo* injections of thymulin at high doses significantly reduced the hyperalgesia (related to both mechanical and thermal nociceptors) induced by intraplantar injection of endotoxin in rats and mice (113). Interestingly, when applied at much lower doses, this peptide instead generated hyperalgesia, an effect paralleled by a significant enhancement in the intrahepatic production of IL-1β (114). Such paradoxical effects are in keeping with previous data showing that low doses of thymulin enhance IL-1β secretion by peripheral blood cells, whereas, at high concentrations, thymulin suppresses its release as well as that of IL-2, IL-6, and TNF-α (115). More recently, the cellular and molecular mechanisms involved in the thymulin-induced hyperalgesic effect have been further investigated. In the peripheral nervous system, the involvement of capsaicin-sensitive primary afferent neurons has been revealed. Intraperitoneal injection of capsaicin (known to destroy afferent nervous fibers) significantly abrogated the stimulatory thymulin effect on pain (116). Additionally, spinal cord neurons appear to be involved, since thymulin induces sustained expression of c-*fos* (a marker of spinal cord neuron activation) in those neurons known to be involved in nociception (117). It should be noted, however, that, in spite of these convincing data, the molecular basis for thymulin action on neurons is not complete, since thymulin receptors in neurons have not yet been determined.

Some data also indicate that other thymic hormones may exert a modulatory role in the central nervous tissue, including an effect on behavioral functions. *In vivo* injection of a thymopentin analog in rats was shown to counteract the stress response to experimentally induced social defeat (118), as measured in the elevated plus-maze apparatus, a recognized animal model of anxiety (119). Although the mechanism(s) involved in this thymopentin-mediated event were not characterized, direct action of the thymic hormone analog on the cholinergic innervation of the hypothalamus with

consequent inhibition of CRF release was suggested (118). Such a putative direct anxiolytic effect of the thymopentin analog is further supported by previous data showing that injections of thymopentin normalize the numbers of benzodiazepine and γ-aminoisobutyric acid receptors in the hippocampus after stress (120). It is also noteworthy that neonatal thymectomy modulates the densities of nicotinic cholinergic receptors in skeletal muscle and brain (121).

For further details on the relationship between thymic hormones and the neuroendocrine system, including behavioral adaptive responses, see Refs. 122 and 123.

V. Proliferation of Thymic Cells Is Hormonally Influenced

A. In vitro effects of pituitary hormones on TEC growth

One potentially relevant factor for better understanding thymus ontogeny is whether the expansion of TEC can be under neuroendocrine control. Pituitary hormones, such as PRL and GH, as well as IGF-I (78, 84), consistently enhance TEC proliferation *in vitro*. Similar results were obtained by such various methodological strategies as direct cell counting, [^{3}H]thymidine incorporation, and immunofluorescence analysis of cultured TEC after bromodeoxyuridine uptake (Fig. 5, *top panel*). The proliferative effects of GH and IGF-I were recently confirmed using TEC lines derived from normal and from thymomatous rat thymus (124). Interestingly, the GH releasing inhibitor somatostatin, as well as its analog octreotide, significantly blocks human cultured TEC proliferation, as ascertained by [^{3}H]thymidine incorporation (125).

A recent work suggested that TEC growth might also be under the control of thymic hormones. It was shown that thymopentin induces DNA synthesis in human TEC lines, either alone or in conjunction with FCS (126). Further studies using the complete thymopoietin molecule, in conjunction with similar assays using other thymic hormones, are necessary, however, to establish the concept of thymic hormone control of TEC growth.

Data are also scarce concerning the effects of steroid hormones on TEC proliferation. An apparent decrease in the proliferation rate was seen in a rat TEC line after treatment with progesterone, estrogens, or androgens, an effect probably mediated by protein kinase C (127). A similar inhibitory effect was seen with cortisol (128).

Much less is known about the *in vivo* effect of these hormones on TEC growth. However, it was shown that injections of metaclopramide, which promotes hyperprolactinemia, increased the number of solid epithelial islands in adult rat thymuses (129).

In vivo, Scheiff and co-workers (130) provided morphometric evidence that thyroid hormones were also able to induce TEC proliferation. Nevertheless, we and others observed no significant *in vitro* growth effect of T_3 using the model of a murine TEC line (66, 131). The reason for these apparently contrasting results is likely to lie in the distinct evaluation methodologies as well as the *in vivo vs. in vitro* situations.

B. Modulation of thymocyte proliferation by hormones and neuropeptides

In vitro thymocyte proliferation can be stimulated by supernatants of TEC cultures, whereas supernatants derived from fibroblast cultures have no effect. This thymocyte proliferative activity of TEC-derived supernatants was almost completely abolished by the presence of antithymulin monoclonal antibodies, but was enhanced when TEC were treated with T_3. By contrast, in the same study, T_3 did not directly influence thymocyte proliferation, as ascertained by short-term thymidine incorporation (132).

More recently, similar proliferative effects were seen using supernatants from PRL- or GH- treated TEC cultures (133, 134). Additionally, as depicted in Fig. 6, *bottom panel*, GH itself synergized with anti-CD3 in its stimulatory effect on thymocyte proliferation (135). This is in keeping with recent data showing that transgenic mice that overexpress GH or GH releasing hormone exhibit overgrowth of the thymus (136).

As detailed below, it was reported that thymocyte-derived GH-induced proliferation in thymocyte suspensions was apparently mediated by IGF-I (137); another work demonstrated that IGF-I *per se* is able to increase total thymocyte numbers (138). Interestingly, in the latter study it was also shown that infusion of this growth factor in dexamethasone-treated rats accelerated the recovery of $CD4^+CD8^+$ cells (the main targets for glucocorticoid hormones) in the thymus. Lastly, the notion that a GH/IGF-I circuit enhances thymic cell proliferation is further supported by a clinical case of an acromegalic patient, bearing high circulating levels of GH and IGF-I, who displayed thymic hyperplasia (139). To date, no study has been made in a large number of patients and so far, a cause-effect relationship between high levels of GH and thymic cell proliferation has not been shown in the human.

Further hormonal mediators are involved in the general control of thymocyte proliferation. For example, mitogen-induced human thymocyte expansion was blocked in the presence of anti-LH-RH serum, suggesting that intrathymically produced LH-RH may act as a costimulatory factor for thymocyte growth (140). At least in some experimental conditions, PRL also appears to be effective in directly stimulating thymocyte proliferation by enhancing IL-2 production and IL-2 receptor expression (141). This is keeping with data suggesting that PRL may play the role of a T cell growth factor, since it induces gene expression of cyclins D2 and D3 in the rat thymic lymphoma cell line Nb2 (142).

Proliferation of cultured human thymocytes was also directly stimulated *in vitro* by met-enkephalin, whereas VIP promoted an inhibitory effect, as compared with control untreated cultures, in both conditions of spontaneous and of phytohemagglutinin-induced mitogenesis (143). Similarly, somatostatin prevented concanavalin A-induced rat thymocyte proliferation (144). Another neuropeptide, CGRP, also inhibited mitogen-induced thymocyte proliferation, an effect that was abrogated in the presence of its antagonist CGRP8–37 (145).

Using the model of fetal thymus organ cultures, we recently demonstrated that expansion of thymocytes could be

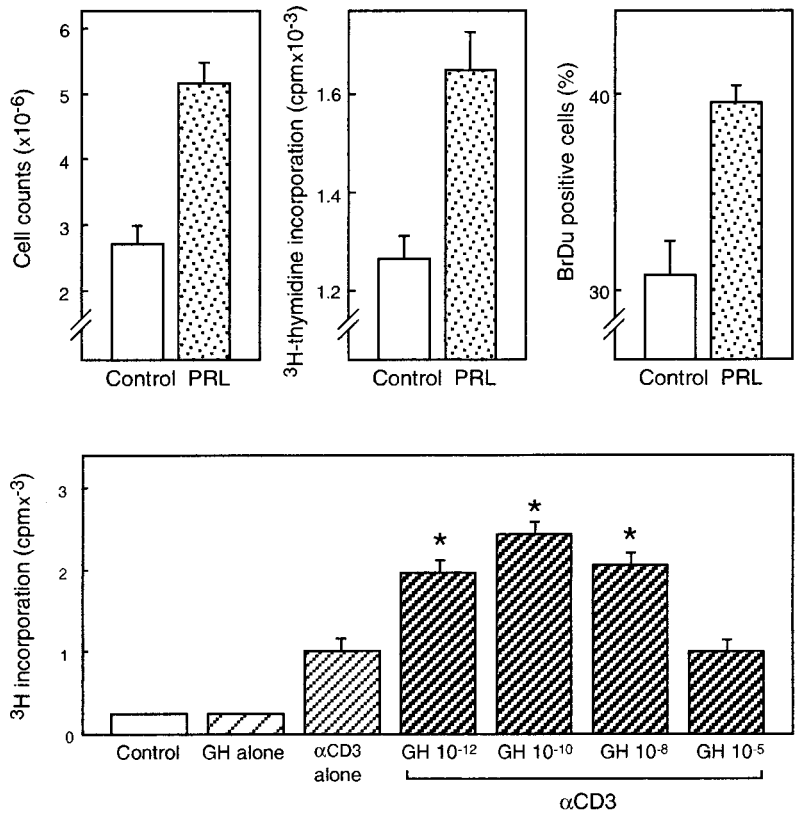

FIG. 6. *In vitro* modulation of TEC and thymocyte proliferation by hormones. In the *top panel*, the stimulatory effect of PRL on human TEC growth is ascertained by direct cell counting, [^{3}H]thymidine incorporation, and bromodeoxyuridine uptake, revealed by immunocytochemistry. The comitogenic effect of GH on thymocyte proliferation is seen in the *bottom panel*. In this experiment, mitogenic response was induced with anti-CD3 monoclonal antibody, and GH was added at various molar concentrations. *Asterisks* indicate statistically higher thymocyte expansion induced by anti-CD3 in the presence of GH. [Derived from Refs. 78 and 135.]

stimulated by insulin (146). Yet, since this is a complex heterocellular model, it remains to be determined whether the proliferative insulin effects are direct on the thymocytes or are mediated through microenvironmental cells. Tables 2 and 3 summarize the vast series of *in vitro* experiments concerning modulation of TEC or thymocyte proliferation by hormones and neuropeptides.

Some *in vivo* data also clearly suggest that various hormones and neuropeptides can induce thymocyte proliferation. Injections of GH3 (pituitary adenoma cells able to produce GH and PRL) to old rats reversed age-dependent thymic atrophy with a consequent increase in thymocyte numbers (147). Similar results were obtained with injection of IGF-I (148). Additionally, a transgene for IGF-II, once expressed in dwarf mice, promoted a progressive increase in thymus growth and thymocyte numbers, as compared with age-matched animals not carrying the transgene (149–151).

Injection of T_3 also promoted a consistent increase in thymocyte numbers with enhancement of spontaneous *ex vivo* [H^3]thymidine incorporation (66).

Administration of synthetic TRH was also shown to enhance bromodeoxyuridine uptake by thymic cell suspensions (152). More recently, the same group showed in the rat model that such an effect could be seen *in vivo*, provided TRH was injected in the morning. In addition to confirming the previous data, these results reflect a time of day dependency for a physiological effect of TRH on thymocyte proliferation (153). Female rats continuously treated with leuprolide, an LH-RH agonist, exhibited a consistent increase in thymus weight (154), while *in vivo* injections of met-enkephalin in mice also enhanced thymus weight and cellularity (155). The latter finding is in keeping with the above *in vitro* stimulatory effect of this opioid on thymocyte proliferation.

Finally, intrathymic implants of the pineal gland derived from young mice, into age-matched recipients, led to a remarkable long-term maintenance of thymic size and the cortico-medullary architecture in the latter, thus preventing physiological age-related thymus atrophy (156). This suggested an effect of melatonin, either favoring proliferation and/or partially preventing apoptosis. In keeping with these findings, melatonin injection partially prevented stress-induced thymic atrophy (157). More recently, the mechanism

TABLE 2. Expression of steroids and their receptors by thymic cells; effects on proliferation and apoptosis

Hormone	Site of production	Receptor expression		Proliferation		Apoptosis	References
		Thymocytes	TEC	Thymocytes	TEC	Thymocytes	
Glucocorticoids	TEC	+	+	↓	↑ or ↓	↑ or ↓	128, 225–234, 279, 280
Estradiol	ND	+	+	↓	↑ or ↓	↑	127, 159, 160, 193–197, 236, 246–250
Progesterone	ND	−	+	↓	↑	↑	127, 159, 199, 240–242, 245, 246
Androgens	ND	+	+	↓	↑	↑	127, 159, 161, 200, 201, 237–239, 243, 244, 246

↑, Increase; ↓, Decrease; ND, not determined.

TABLE 3. Control of thymocyte or TEC proliferation by peptidic hormones and neuropeptides

Hormone	Thymocytes	TEC	References
Thyroid Hormones			
T_3/T_4	↑	↑ or −	66, 130, 131, 132
Hypothalamo/pituitary hormones			
PRL	↑	↑	78, 133, 141
GH	↑	↑	84, 124, 137–139
IGF-I	↑	↑	84, 124, 137, 138, 139
ACTH	ND	↑	67, 68
GH-RH	↑	ND	136
LH-RH	↑	ND	140, 154
TRH	↑	ND	152, 153
Somatostatin	↓	↓	125, 144
Other hormones and neuropeptides			
Insulin	↑	ND	146
Enkephalins	↑	ND	143, 155
VIP	↓	ND	143
PACAP	↓	ND	166
CGRP	↓	ND	145

↑, increase; ↓, decrease; −, no effect; ↑ or −, conflicting results; ND, not determined. PACAP, Pituitary adenylate cyclase-activating peptides.

by which melatonin could exert such an antiapoptotic role was revealed, with the demonstration that melatonin downregulates the expression of glucocorticoid receptor (158).

By contrast, hormones such as sex steroids appear to provide an inhibitory tonus on thymocyte proliferation (reviewed in Ref. 159). Castration in young adult male mice promoted a rapid wave of thymocyte proliferation *in vivo*, particularly in cortically located cells bearing the immature phenotypes $CD4^-CD8^-$ and $CD4^+CD8^+$ (160). This is in keeping with previous findings that castration in old rats led to enhancement of thymus weight, an effect that was abrogated by androgen treatment of castrated animals (161).

Taken together, the data summarized above clearly indicate that distinct hormones and neuropeptides can convey positive and negative signals for thymocyte proliferation.

VI. Hormonal Modulation of Intrathymic T Cell Differentiation

A. Thymocyte-derived cytokine profile

One thymocyte function influenced by neuroendocrine circuits is the cytokine profile produced by these cells. For instance, concanavalin-A mitogenic response and IL-6 production were enhanced in thymocytes from GH-treated aging animals (162), and PRL induced IL-2 production by thymocytes (141). Vasopressin can replace IFN-γ in its role of inducing IL-2 production (163), and substance P stimulated IL-2 synthesis by the mouse thymic lymphoma cell line EL-4 (164). By contrast, VIP, PACAP27, and PACAP38 exerted an *in vitro* inhibitory action on the production of some thymocyte-derived cytokines, including IL-2, IL-4, and IL-10 (165, 166). Altogether, these findings provide consistent evidence for a hormone/neuropeptide balance in the control of cytokine production by thymocytes.

B. Changes in the T cell differentiation markers and TCR Vβ repertoire

As detailed above, hormones and neuropeptides can affect thymic functions related to thymocyte differentiation. Thus, such differentiation could also be a target for neuroendocrine control. Implants of GH3 pituitary cells in aging rats increased total thymocyte numbers and the percentage of CD3-bearing cells, with a parallel decrease in the $CD4^-CD8^-$ double-negative thymocytes, which normally accumulate in the aging rat thymus (147, 167). The role of GH in thymus development was further supported by findings in GH-deficient dwarf mice. In addition to the precocious decline in thymulin serum values (80), there was progressive thymic hypoplasia with decreased numbers of $CD4^+CD8^+$ double-positive thymocytes. Such defects could be restored by prolonged treatment with GH (168).

Injections of T_3 promoted an increase, both in relative and absolute numbers of thymocytes bearing the CD44 marker (66), which is an ECM receptor of the proteoglycan family, with specificities for hyaluronate and to a lesser extent fibronectin and collagen. By contrast, administration of high doses of glucocorticoid hormones yielded a profound decrease in the percentages of $CD4^+CD8^+$ thymocytes, with a relative increase in $CD4^-CD8^-$ as well as $CD4^+CD8^-$ and $CD4^-CD8^+$ cells (169, 170). *In vivo* treatment of mice with estradiol also promoted a depletion in the absolute numbers of $CD4^+CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^+$ thymocytes, with a decrease in the proportion of double-positive cells and an increase in the percentage of double-negative as well as single-positive mature cells (171). A striking loss of the very immature $CD3^-CD4^-CD8^-$ cells was further demonstrated (172). Interestingly, the estrogen-induced thymic involution appeared to occur independently of glucocorticoids, since it was seen in adrenalectomized animals (170). In this same study, the proportions of $CD4^+CD8^+$ thymocytes in adrenalectomized estrogen-treated mice, although significantly lower than in control animals, were higher than those in hormone-treated mice not subjected to adrenalectomy.

Although considerable work is available concerning the neuroendocrine control of T cell differentiation markers, the influence of hormones and neuropeptides on shaping the intrathymically generated T cell repertoire remains poorly studied. However, the few data available point to such an influence. Mice treated with estradiol exhibited a selective increase in the percentages of $CD4^-CD8^-$ TCR^+ thymocytes expressing Vβ6, Vβ8, or Vβ11 but not Vβ3 gene products (173), thus promoting an imbalance in the generation of the TCR repertoire of the double-negative TCR^+ cell lineage. Interestingly, an enhancement of IL-1α mRNA was seen in parallel with the increase in $V\beta8^+$ cells, in keeping with previous data showing that this cytokine exerts a mitogenic effect on $V\beta8^+$ thymocytes (173). More recently, release of autoreactive T cells bearing the Vβ3 or Vβ11 phenotypes, with autoreactivity to hepatocytes, was seen in mice injected with a single dose of estradiol (174).

In rat lymphoma-derived Nb2 cells, PRL induced *in vitro* gene expression of the TCR γ chain, whereas the TCR β chain gene was suppressed (175), suggesting that the intrathymic PRL content may drive T-cell differentiation pathways. In

keeping with this hypothesis, it has been demonstrated, not only in the Nb2 cell line but also in human thymocytes, that in the absence of TCR ligation PRL induces rapid phosphorylation of multiple TCR/CD3 complex proteins, including the CD3 ϵ-chain and the ZAP-70 tyrosine kinase, both essential for TCR function (176). However, recent data argue against the above hypothesis, since PRL receptor knockout mice apparently develop a normal thymocyte differentiation pathway, at least in terms of CD3, CD4, and CD8 markers (177, 178).

As detailed below, very elegant data strongly suggest that intrathymically produced glucocorticoids influence the generation of the T cell repertoire in the thymus by modulating positive and negative selection of thymocytes.

C. Hormone-mediated apoptosis in thymocytes

One of the best studied effects concerning the hormonal control of intrathymic cell death is that mediated through glucocorticoid hormones. When applied in relatively high doses, these substances trigger an apoptotic cell machinery in differentiating thymocytes, particularly cells bearing the immature $CD4^+CD8^+$ phenotype. The molecular basis of such a glucocorticoid hormone-induced biological response is now known in detail and is used as a paradigm for studies of apoptosis in various cell types (reviewed in Ref. 179). Briefly, glucocorticoids activate calcium-dependent endonucleases that eventually cleave DNA, with the formation of oligo-nucleosomes. A series of findings suggest that such events are potentiated by cAMP (180) and depend on the recently isolated transcription factor SRG3 (181). They do not require p53 tumor suppressor gene expression, which has been shown to be necessary for triggering apoptosis in thymocytes by contact with TEC or after radiation (182, 183). Oligonucleosomal DNA fragmentation is preceded by the enzymatic degradation of lamin B1, a protein involved in the nuclear lamina architecture (184). Interestingly, glucocorticoid-induced thymocyte apoptosis also modulates cytoplasmic processes, including thiol oxidation in mitochondria (185), activation of the protease calpain (186), and decrease in the intracellular K^+ concentration (187). The need for glucocorticoids to bind to the corresponding receptor to induce apoptosis in thymocytes has been definitely established by the demonstration that thymocytes from glucocorticoid receptor null mice do not undergo apoptosis after the respective hormonal treatment (188). In a recent study, it was further shown that glucocorticoid-dependent apoptosis in thymocytes is mediated by transactivation of the glucocorticoid receptor (189). This was approached by constructing a point mutation in the glucocorticoid receptor gene, able to abolish DNA binding-dependent transactivation, and further generating mice expressing this mutant gene. Thymocytes from these animals were resistant to dexamethasone-induced apoptosis, thus showing that DNA binding of the glucocorticoid receptor is a prerequisite for glucocorticoid-induced apoptosis.

Although the mechanism of glucocorticoid-induced apoptosis in thymocytes is relatively well known at the molecular level, a paradox remains to be understood: low doses of glucocorticoid hormones antagonized TCR-mediated apoptosis in thymocytes (190, 191) and partially rescued thymocytes from apoptosis induced by *in vitro* treatment of fetal thymus organ cultures with anti-CD3 antibodies (192). As further discussed below, this may be relevant for a physiological role of these substances in positive selection of the T cell repertoire. Thus, understanding the molecular control of these balancing effects demands further investigation.

Although less studied, sex steroids also appear to affect thymocyte apoptosis. For example, injections of estradiol in rats promoted cortical thymocyte depletion (193), with an increase in apoptosis (ascertained by caryopyknosis) and a decrease in mitotic indexes (194–197) (see Table 2).

A natural condition for studying the influence of sex steroids on thymocyte apoptosis is pregnancy, during which the thymus undergoes progressive and extensive involution with loss of $CD4^+CD8^+$ cells, partially due to apoptosis (198). Interestingly, this progressive thymic atrophy of pregnancy inversely correlates with the rise in circulating progesterone (199). Other hormones such as pituitary hormones and glucocorticoids could also be involved in this process.

Androgens, such as testosterone, also induce thymocyte death *in vivo*, by a mechanism independent of the glucocorticoid and estrogen receptors (200), but which requires specific androgen receptors since the effect is prevented by treatment with the antiandrogen drug flutamide. Since the levels of androgen receptor mRNA are 6-fold higher in TEC than in thymocytes, it was postulated that the induction of thymocyte apoptosis by androgens is mediated by TEC-derived products (200). The acceleration of thymocyte apoptosis by androgens was recently confirmed *in vitro* using thymic organ culture (201).

Studies in mice revealed that estrogen treatment also causes a dramatic reduction in thymic size and cellularity. All CD4/CD8-defined T cell subsets were reduced, particularly the $CD4^+CD8^+$ double positive cells. Examination of the $CD3^-CD4^-$ $CD8^-$ subset revealed a striking loss of developmental progression of the early precursor cells, since in treated animals, this compartment was composed almost entirely of the earliest population, $CD44^+CD25^-$, with depletion of cells in the remaining maturational stages, $CD44^+CD25^+$, $CD44^-CD25^+$, and $CD44^-CD25^-$ (202). It should be recalled that in these studies, apoptosis was not specifically checked with any of the present available tools. Intriguingly, in the same work, the authors showed that estrogen deprivation by oophorectomy did not enhance T cell development. Such a paradox is further apparent in recent findings that estrogen receptor α knockout mice exhibit lower thymic cellularity compared with normal or heterozygous littermates (203). In these animals, despite the reduced cell numbers, thymocyte maturation stages were apparently normal, as revealed by CD3, CD4, CD8, CD25, and CD44 labeling. Nevertheless, their sensitivity to *in vivo* estradiol treatment was distinct from wild or heterozygous age-matched counterparts. While estrogen receptor null mice α underwent thymic atrophy with estradiol treatment (thus similar to control animals), the phenotypic differences in thymocyte subpopulations such as loss of $CD4^+CD8^+$ cells, seen in usual conditions of estradiol treatment, were not detected in mutant mice. These findings point to a dichotomy of estradiol in terms of influence on thymocyte numbers and

differentiation. In fact, in the same work, chimera experiments elegantly demonstrated that the sensitivity to estradiol in terms of thymic atrophy was dependent on the expression of the null mutation in the thymic microenvironment rather than in thymocytes.

Protection from apoptosis may also be under neuroendocrine control. Dihydroepiandrosterone, for example, is apparently able to counteract at least *in vivo* thymocyte apoptosis induced by pharmacological doses of glucocorticoid hormones (204, 205).

Very few data are so far available concerning the role of pituitary hormones in modulating thymocyte apoptosis. Using the rat lymphoma Nb2 cell line, it was demonstrated that the apoptotic effect of dexamethasone was inhibited in a dose-dependent manner by PRL or GH (206), raising the hypothesis that a similar effect may occur with normal thymocytes. In fact, as regards PRL, this hypothesis fits with the data discussed above, showing that this hormone can trigger phosphorylation of TCR-related proteins (207).

The VIP also appears to play a role in protecting thymocytes from apoptosis induced by dexamethasone treatment *in vitro*. It inhibits the typical DNA fragmentation induced by glucocorticoids and increases cell survival, both effects being mediated through the VIP receptor (208).

The pineal neurohormone melatonin also appears to partially prevent apoptosis of rat thymocytes *in vivo* and *in vitro*, as defined by morphological criteria and DNA fragmentation seen in agarose gel electrophoresis (209). Similar findings were obtained in aging mice, where thymus atrophy was partially prevented by long-term melatonin treatment or pineal grafting (210). As mentioned above, this antiapoptotic effect of melatonin is likely to be, at least in part, due to down-regulation of the glucocorticoid receptor (158).

These data indicate that neuroendocrine-mediated regulation of thymocyte apoptosis does exist, facilitating or preventing the cells to enter a programmed pathway of cell death. They favor the notion that negative *vs.* positive selection of the T cell repertoire within the thymus may be under the control of a neuroendocrine homeostatic axis involving various hormones and neuropeptides. Consequently, it should be important to define to what extent such neuroendocrine circuits influence shaping of the intrathymically generated T cell repertoire.

VII. Is Thymocyte Traffic Under Neuroendocrine Control?

A. Effects on the entrance of cell precursors into the thymus

Very few studies have been conducted so far to determine whether the entrance of T cell precursors into the thymus is under neuroendocrine control. It was demonstrated that recombinant human GH increases human T cell engraftment into the thymus of SCID mice (211). These investigators suggested that such influence is mediated by adhesion molecules and ECM, since the entrance of T cells into the thymus can be abrogated with anti-β1 or anti-β2 integrin antibodies, and GH-treated cells exhibit an increase in adhesion to ICAM-1, VCAM-1, and fibronectin (211). In keeping with this idea, it was recently demonstrated that administration of IGF-I together with bone marrow cells into syngeneic old recipients resulted in an increase in thymus cellularity, compared with transfer of bone marrow cells alone (212). In the same study, it was demonstrated that IGF-I potentiates the colonization of fetal thymus organ cultures with T cell precursors, clearly indicating that IGF-I enhances the entrance of cell precursors into the thymus.

A second example is dexamethasone and hydrocortisone enhancement of the *in vitro* migration of bone marrow-derived progenitor cells into the thymus (213). Additionally, *in vivo* treatment of rats with estradiol enhanced permeability of cortical blood vessels (214), which may facilitate the entry of pro-thymocytes. However, this issue has not yet been experimentally approached.

It has been reported that the lack of maternal glucocorticoids in the progeny of adrenalectomized pregnant rats accelerates early colonization of the thymic primordium by lymphoid progenitors (62, 63). As previously discussed herein, this is probably related to the earlier maturation of the thymic microenvironment seen in this particular experimental condition.

B. Modulation of thymocyte traffic in TNCs

Intrathymic lymphocyte traffic also appears to be under neuroendocrine control. Spontaneous as well as phorbol ester-induced *in vitro* mobility of thymocytes was shown to be inhibited by VIP and the two pituitary adenylcyclase-activating polypeptides (PACAP27 and PACAP38), an effect paralleled by a rise in cAMP concentration (215). Whether these neuropeptides change the expression of membrane receptors related to cell migration remains to be determined.

Considering that ECM plays a role in intrathymic cell migration (17), and since various hormones could up-regulate the expression of ECM ligands and receptors (56, 66), we investigated whether thyroid and pituitary hormones could be involved in TEC-thymocyte interactions related to cell migration. One key interaction is the heterotypic adhesion of thymocytes to epithelial cells. As discussed above, we demonstrated that these hormones up-regulate TEC-thymocyte adhesion, as ascertained by direct counting under the optical microscope or by ELISA using CD90 (the Thy.1 antigen) as a membrane marker for thymocytes (56, 66). With regard to PRL and GH, the hormonal specificity of this effect was confirmed since respective antihormone antibodies could block it, totally or partially. We also noted that the GH-enhancing effects were prevented by anti-IGF-I or anti-IGF-I receptor antibodies. The involvement of ECM-mediated interactions was further demonstrated since the hormone-enhancing effects on TEC-thymocyte adhesion were also abrogated by antibodies with specificities for fibronectin, laminin, and their respective receptors, VLA-5 and VLA-6 (56, 216).

We further attempted to modulate a direct cell migration process, namely the entrance of thymocytes into and their exit from TNCs. In a first set of experiments (Fig. 7), we showed that thymocyte release from TNC complexes was accelerated if these complexes were treated *in vitro* with T_3. Most importantly, when TNCs were harvested from T_3-treated mice, thymocyte release was also faster than in con-

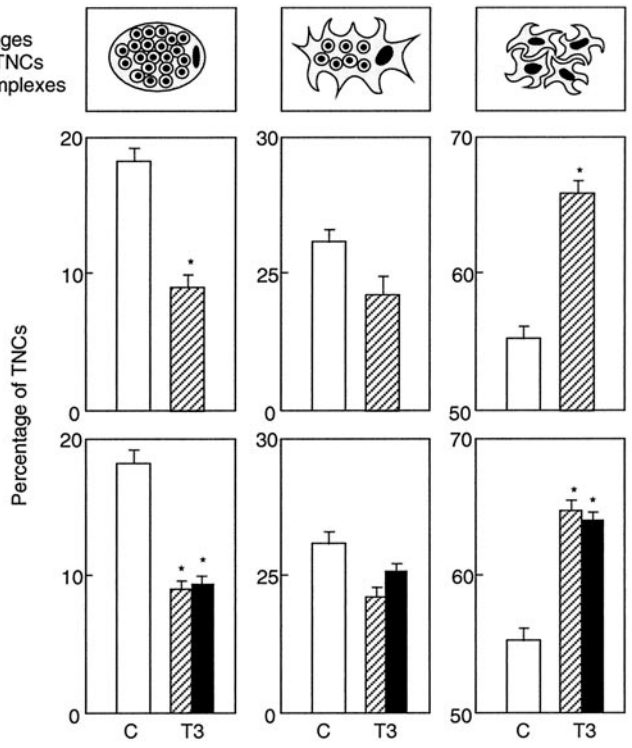

FIG. 7. Enhancement of thymocyte release from mouse TNC complexes by T_3. The *upper panels* show schematically the three stages of TNC complexes: round TNCs that did not release thymocytes (*left*), spread epithelial cells that partially released thymocytes (*middle*), and thymocyte-free epithelial cells (*right*). The *middle panels* depict thymocyte release from TNCs derived from control (□) or T3-injected mice (▨) and the *bottom panels*, TNCs recovered from normal mice and treated *in vitro* with 10^{-8} M (▨) or 10^{-10} M (■) of T_3. After both *in vivo* and *in vitro* treatment, thymocyte release was significantly faster ($*P < 0.01$) than in corresponding controls. [Derived from Ref. 66.]

trols (66). More recently, we observed that when TNC-derived epithelial cells were treated with the thyroid hormones and cocultured with fetal thymocytes, the ratio of reconstitution of lymphoepithelial complexes was enhanced (our unpublished results), indicating that the entrance of thymocytes into TNC is also hormonally regulated. Further *in vitro* studies revealed that both thymocyte release from TNC and TNC reconstitution were consistently increased if cultures were subjected to PRL, GH, or IGF-I (56).

C. Control of thymocyte exit from the thymus

Although the study of hormonal control of intrathymic cell migration has so far been mostly restricted to *in vitro* models, the influence of the neuroendocrine system on the exit of thymocytes has been approached *in vivo*. It was recently shown that in the progeny of adrenalectomized rats, emigration of TCR$\alpha\beta$ to the spleen occurred earlier than in control fetuses (63), suggesting that release of mature thymocytes is controlled, at least in fetal life, by glucocorticoids.

One direct strategy to evaluate thymocyte exit in adult animals is analysis of the so-called recent thymic emigrants. It is well established that intrathymic injection of fluorescein isothiocyanate (FITC) randomly labels the cell membranes of many thymocytes. This allows recovery of the $FITC^+$ cells that have recently exited the thymus (217, 218). We observed, 16 h following a single intrathymic injection of T_3, a consistent increase in the numbers of $FITC^+$ cells in lymph nodes (219). Additionally, using a similar protocol, we noted that GH is also able to modulate the homing of recent thymic emigrants, enhancing the numbers of $FITC^+$ cells in the lymph nodes and diminishing them in the spleen (220).

An *in vivo* model in which immature $CD4^+/CD8^+$ T cells were detected in peripheral lymphoid organs (thus reflecting an abnormal thymocyte exit) is that of transgenic mice with impaired corticosterone receptor function by partial knockout of the glucocorticoid receptor, secondary to endogenous expression of the corresponding antisense RNA (221). These animals showed disruption of the hypothalamus-pituitary-adrenal axis, bearing abnormally high circulating levels of ACTH and corticosterone (222). The intrathymic levels of glucocorticoid receptor mRNA were 50% lower, and the ability of thymus extracts to bind hydrocortisone was reduced to one third of that in normal mice (221). In this respect, it is interesting to note that intrathymic expression of the antisense was restricted to thymocytes since the construct applied used the promoter from the thymocyte-specific thyrosine kinase *lck*.

Abnormal leakage of $CD4^+CD8^+$ cells was also detected in lymph nodes of the Snell Bagg dwarf mouse (168). This phenomenon progressed with age and paralleled the cortical thymocyte depletion found in dwarf mice. In those animals with dramatic loss of $CD4^+CD8^+$ thymocytes, the percentages of $CD3^+CD4^+CD8^+$ cells in the lymph nodes reached 50%. Importantly, both $CD4^+CD8^+$ thymocyte depletion and the abnormal presence of $CD4^+CD8^+$ cells in the periphery were restored after daily GH injection (168).

Lastly, it is noteworthy that release of immature $CD4^+CD8^+$ thymocytes may also be under the influence of sex hormones. Adult rats treated with estradiol benzoate exhibited $CD4^+CD8^+$ in the spleen, which was related to increased vascular permeability of cortical blood vessels (223).

Taken together, the three models summarized above indicate that a disturbance in distinct neuroendocrine compartments may result in a partial imbalance of the process of thymocyte exit, allowing immature cells to emigrate from the organ. Considering the autoreactive potential of $CD4^+/CD8^+$ cells found in peripheral lymphoid organs from neonates of certain mouse strains (224), it is conceivable that a neuroendocrine-driven imbalance resulting in abnormal traffic of immature thymocytes toward peripheral lymphoid organs may favor the development of some autoimmune diseases.

It is worth emphasizing that the question of whether the neuroendocrine control of thymocyte migration occurs partially through the modulation of thymic microenvironment-derived chemokines has not been tested in any model and certainly represents a promising field for original investigation.

VIII. Expression of Receptors for Hormones and Neuropeptides by Thymic Cells

The above discussion of the variety of effects of distinct hormones and neuropeptides on thymus physiology obviously implies that distinct thymic cell types should express receptors for such molecules. As detailed below, the expression of specific receptors for adrenal and sex steroids, thyroid and pituitary hormones, as well as neuropeptides has been detected by several groups, using various methodological approaches, not only in thymocytes but also in TEC (Tables 2 and 4).

A. The steroid/thyroid hormone receptor family

The expression of glucocorticoid receptors by thymocytes has been well established by ligand binding, immunocytochemistry, immunoblotting, and molecular biology. These receptors have been located in both the cytoplasm and nucleus of thymocytes (225–229). In the human thymus, immature thymocytes exhibited a higher receptor density, although the affinity, kinetic characteristics, and ability to translocate from cytoplasm to nucleus were similar in the various thymocyte subsets (230). In the rat, glucocorticoid receptor mRNA was detected in the embryonic thymus as early as day 13 of gestation (231), and receptor concentration (measured by binding assay) apparently decreased with aging (232). Glucocorticoid receptors were also detected in cultures of the thymic epithelium (233), and the recently described β-isoform of this receptor was found *in situ*, in medullary TEC (234). Interestingly, in transgenic mice with impaired corticosterone receptor function due to partial knockout of the glucocorticoid receptor secondary to endogenous expression of the corresponding antisense mRNA (221), the thymic microenvironment was altered as of early ontogeny of the thymus, and large TEC-free areas were seen in adult individuals (235).

Receptors for sex steroids have also been demonstrated in animal and human thymus. Pioneer studies by Grossman and co-workers (236–238) showed, by binding assays, that specific receptors for estrogen, progesterone, and androgen are present particularly in thymic preparations obtained from nonlymphoid cells. Other groups confirmed the presence of androgen and progesterone receptors in TEC-enriched fractions (239–242). In one study, a quantitative RT-PCR approach for detecting androgen receptor mRNA in the thymus showed 6-fold more mRNA in the TEC than in the thymocytes (243). Immunocytochemically based flow cytometry analysis of freshly isolated thymocytes revealed androgen receptor expression in all classes of CD4/CD8-defined thymocyte subsets, with the highest levels observed in the $CD4^-CD8^-$ subset, including the most immature cells (244).

With respect to progesterone receptor, direct binding of peroxidase-coupled ligands onto thymus sections (245), as well as immunocytochemistry, revealed the colocalization of progesterone receptor-positive and estrogen receptor-positive cells with TEC-specific cytokeratin staining (246).

The expression of estrogen receptor in thymocytes was determined though binding assay (247), as well as by immunoblotting and flow cytometry (248). According to previous histochemical analyses obtained with human thymic specimens, the binding is located in the reticulo-epithelial stroma rather than the thymocytes (249).

More recently, a novel form of the human estrogen receptor, the ERβ form, was cloned, and the corresponding mRNA was found in the thymus (250).

Initial observations indicated that thymocytes exhibit specific binding sites for thyroid hormones (251). Nuclear T_3 receptors (NT_3-R) were identified in both TEC and thymocytes by immunochemical approaches using a specific anti-NT_3-R monoclonal antibody, revealing a 57-kDa protein similar to the NT_3-R originally described in the

TABLE 4. Expression of peptidic hormones and neuropeptides as well as their corresponding receptors by thymic cells

Hormone	Site of production		Receptor expression		References
	Thymocytes	TEC	Thymocytes	TEC	
Thyroid hormones					
T_3/T_4	ND	ND	+	+	251, 252
Hypothalamo/pituitary hormones					
PRL	+	ND	+	+	253–255, 256, 287–291
GH	+	+	+	+	137, 257–262, 265, 287, 292, 293, 295, 296
ACTH	ND	+	ND	+	297, 298, 302, 326
LH	+	ND	ND	ND	140, 297, 298
LH-RH	+	ND	ND	ND	140, 323
GH-RH	+	ND	+	ND	265
CRH	+	ND	ND	ND	324–328
TRH	+	ND	ND	ND	266
Somatostatin	−	+	+	+	125, 267, 268, 313, 314, 334, 335
Vasopressin	−	+	+	ND	263, 264, 317–321
Oxytocin	−	+	+	ND	263, 264, 316–322
Other hormones and neuropeptides					
Insulin	ND	+	−	+ or −	311–315
Met/Enkephalin	+	+	ND	ND	269, 270, 329–333
β-Endorphin	+	+	+	+	269, 270, 329–331
VIP	+	+	+	ND	215, 271–274, 335–338
PACAP	ND	ND	+	ND	145, 275, 276
CGRP	ND	ND	+	+	145, 273

ND, Not determined.

liver. Although flow cytometry analysis defining the expression of NT_3-R as a function of the degree of thymocyte differentiation is still lacking, *in situ* immunocytochemical labeling clearly showed that most thymocytes express this receptor (252).

Expression of NT_3-R by the murine thymic epithelium was also determined in various TEC preparations including TNC and TEC lines. Interestingly, NT_3-R expressed by TEC seems to be functional since the epitope recognized by the anti-NT_3-R monoclonal antibody (mAb) was transiently downregulated by treatment of TEC cultures with T_3 (252). More recently, the presence of NT_3-R was also demonstrated in cultured human TEC (our unpublished data).

B. Expression of PRL and GH receptors by thymocytes and TEC

Receptors for various pituitary hormones have also been defined in distinct thymic cell types. Taking the PRL receptors as an example, we first showed that they are expressed on TEC *in situ* and *in vitro*, as defined by immunocytochemistry, immunoblotting, and Northern blotting. Their functional activity was evidenced by modulation of both thymulin production and TEC growth with appropriate agonistic doses of anti-PRL receptor antibodies (253). Additionally, PRL receptors were expressed by most thymocytes, independently of their CD4/CD8-defined maturation stage. The receptor density on cell membranes was enhanced after *in vitro* concanavalin A mitogenic stimulation (254, 255). Interestingly, high levels of circulating PRL, as occur in lactation, enhance the levels of PRL receptors in thymic cells, as revealed by binding assay and semiquantitative RT-PCR (256).

Expression of GH receptor by cultured human TEC was initially shown by means of binding assay (257) and more recently by immunocytochemistry and RT-PCR (258). This is in keeping with *in situ* immunocytochemical data in the avian thymus, showing colocalization of the GH receptor with epithelial cells, defined by cytokeratin labeling (259). Additionally, *in situ* hybridization studies in the rat thymus suggested epithelial labeling for the GH receptor mRNA (260).

GH receptor expression in murine thymocytes was particularly seen in the $CD4^-CD8^-$ immature subset (261). Similar findings were made in human thymocytes in which GH receptor expression was mainly restricted to the very immature differentiation stage defined by the phenotype $CD34^+CD2^+CD3^-CD4^-CD8^-$ (258), suggesting that the direct effects of GH on thymocytes precede events related to the selection of the T cell repertoire. It should be noted, however, that in bovine fetal thymus, GH receptor was found in both $CD4^+$ and $CD8^+$ single positive thymocytes (262). Such differences can be ascribed to species specificity, the fact that the latter study was conducted in early stages of thymus development, and/or sensitivity to the distinct reagents used for detecting the GH receptor by flow cytometry.

Both the receptor and GH binding proteins were found in the thymus of mammalian and avian thymuses (259, 260).

C. Receptors for neuropeptides in thymic cells

Intrathymic expression of receptors for various neuropeptides has been shown in thymocytes, with fewer studies carried out in TEC and other components of the thymic microenvironment. This is the case for oxytocin and vasopressin receptors, determined on thymocyte-derived membrane preparations by means of ligand binding and PCR (263, 264). GH-RH receptors have also been shown in rat thymocytes by means of binding assay and direct coupling of the radioactive ligand onto electrophoresed thymocyte-derived extracts, revealing two protein bands (43 and 27 kDa) similar to those in pituitary gland-derived preparations (265), whereas the TRH receptor has been shown in thymus extracts by RT-PCR (266). The somatostatin receptor family has been identified in mouse thymocytes, as ascertained by gene expression of the receptor subtypes SSTR2A, SSTR2B, and SSTR3 (267). Autoradiographic analysis of radioactive ligands directly bound to human thymus sections indicated that the family is also present in TEC (268). This was confirmed by various methodological strategies including binding assay and RT-PCR (125). It was also demonstrated that somatostatin receptors in human TEC are functional since both somatostatin and its analog octreotide significantly inhibited *in vitro* the proliferation of human TEC (125).

Receptors for endogenous opioids such as β-endorphin and met-enkephalin have been shown by means of binding assays on membrane preparations from total thymus extracts (269). These findings confirmed previous data using the same methodological approach, revealing opioid receptors on thymocyte membranes (270). The presence of such receptors in thymic microenvironmental cells remains to be determined.

The expression of receptors for other neuropeptides has also been demonstrated in the thymus. For example, the mRNA coding for the VIP_1 receptor was detected in thymocytes (271), particularly those bearing the most immature phenotype, the $CD4^-CD8^-$ cells (272). Interestingly, if the expression of VIP_1 receptor is constitutive, thymocytes can express the VIP_2 receptor only after mitogenic stimulation with anti-CD3 antibodies (215). More recently, VIP1 receptor has been identified in human TEC by means of immunocytochemistry, cytofluorometry, RT-PCR, and Southern blotting (273). This receptor is functional since it stimulates cAMP production by cultured TEC, which is keeping with the *in situ* radiolabeling detection of VIP receptor in both cortex and medulla of the human thymus (274).

Receptors for other members of the VIP family, such as the PACAP peptides, have been identified by binding assay (275), although the same research group did not detect PACAP 27 binding sites in thymus autoradiographs (276). Binding sites for CGRP were also detected in thymocyte membranes (145); their presence in the thymic microenvironment has not been studied so far. More recently, the CGRP receptor was demonstrated biochemically and functionally in human TEC (273). Finally, melatonin-binding sites have been detected in thymocyte preparations as well (277, 278).

IX. Intrathymic Expression of Hormones and Neuropeptides

The examples cited above clearly indicate that a classical endocrine pathway is partially implicated in various effects of hormones and neuropeptides on thymic lymphoid and microenvironmental compartments and that target cells are triggered via specific receptors. Nevertheless, another point deserving discussion in respect to neuroendocrine control of the thymus is related to the intrathymic production of several hormones and neuropeptides.

A. Intrathymic production of corticosterone: role in the shaping of the T cell repertoire

Pioneer work by Ashwell and colleagues (279) revealed that not only do TEC bear the enzyme machinery necessary for corticosteroid biosynthesis, but, at least in the murine thymus, corticosterone is actually produced by TEC. More recently, two independent research groups confirmed these results. Additionally, by transfecting a target cell with a plasmid containing two glucocorticoid-responsive elements plus a luciferase reporter gene, the authors showed that secretion of TEC-derived glucocorticoids was enhanced by ACTH and blocked by the glucocorticoid receptor antagonist RU486 as well as two blockers of corticosterone biosynthesis, trilostane and metyrapone (280). Interestingly, in a further recent study, it was shown that, although the whole set of steroidogenic enzymes and cofactors for the synthesis of glucocorticoids could be detected in murine thymic tissue, intact thymic architecture was necessary for glucocorticoid production, since 11β-hydroxylase was not detected in irradiated thymus or in a TEC line (281).

Since low or moderate doses of glucocorticoids were found to prevent anti-CD3-induced apoptosis, it was hypothesized that thymus-derived glucocorticoids might play a physiological role in positive selection of the T cell repertoire. Addition of metyrapone (a selective inhibitor of corticosteroid biosynthesis) to fetal thymus organ cultures enhanced TCR-mediated thymocyte deletion, an effect that could be reversed by exogenous addition of corticosterone to the cultures (279). More recently, using the same model of fetal thymus organ cultures from mice bearing transgenic $\alpha\beta$TCRs, it was shown that thymus-derived glucocorticoid hormones prevent thymocyte apoptosis only when the TCR is capable of recognizing the self-antigen/MHC complex with sufficient avidity to normally undergo positive selection (192). In a second vein, transgenic mice expressing antisense transcripts for the glucocorticoid receptor exhibited thymic atrophy particularly due to $CD4^+CD8^+$ cell depletion and enhanced susceptibility to TCR-mediated apoptosis (282). When this transgene is transferred into the MRL mice that spontaneously develop autoimmunity, there is a decrease in the TCR-Vβ bearing cells that are otherwise positively selected, and such animals then exhibit lower autoantibody production and milder symptoms of the autoimmune disease (283).

Although dealing with transgenic animals, these findings indicate that, under normal conditions, endogenous glucocorticoids might prevent thymocyte apoptosis after TCR/peptide-MHC interaction. In fact, similar data were recently reported with nontransgenic normal syngeneic mice (284).

However, this issue deserves further investigation, since in other studies, it was found that RU 486, an antagonist of the glucocorticoid receptor, blocks anti-CD3 induced apoptosis in newborn-derived thymus organ cultures (285). Additionally, *in vivo* treatment with RU 486 in mice transgenic for one particular TCR able to recognize ovalbumin also prevented thymocyte apoptosis induced by specific antigen stimulation (286).

Whether other steroid hormones, including sex steroids, are produced intrathymically remains to be determined. This is a potentially important issue, given the well established sexual dimorphism of the immune response in normal and autoimmune conditions.

B. Expression of "classic" adenopituitary hormones by thymic cells

The expression of PRL and GH by cells of the immune system, including the thymus, has been extensively documented. Since most of the data were recently reviewed (287), it will be only briefly discussed herein.

PRL gene expression has been detected by distinct research groups in thymocyte-derived preparations (288, 289). The detection of a PRL-immunoreactive molecule (290, 291) indicates that thymocytes constitutively produce PRL. However, the possible roles of thymus-derived PRL and of microenvironmental cells in intrathymic PRL production remain to be investigated.

Intrathymic GH expression was defined by detection of the corresponding mRNA by *in situ* hybridization and the peptide by immunocytochemistry (292); positive signals were revealed in cortical epithelial cells and in septal phenotypically-undefined cells, but not in thymocytes. This contrasted with previous detection of GH mRNA and corresponding protein in rat thymocyte-derived preparations (265, 293), and with the detection of an immunoreactive, 22-kDa, biologically active GH from human thymocytes isolated *ex vivo* (137). More recently, we showed the production and secretion of GH by human thymocytes isolated *ex vivo* as well as in primary TEC cultures, using RT/PCR and immunoradiometric assays. GH gene expression by rat thymocytes is up-regulated by GH-RH (265), in keeping with the demonstration of Pit-1/GHF-1 transcription factor (which controls GH expression in the pituitary gland) in human thymic microenvironmental cells (294). Nevertheless, it should be mentioned that, as yet, no experimental data are available concerning whether GH production and release by distinct thymic cell types are under the same Pit-1/GHF-1-dependent control mechanism in normal conditions. Studies in dwarf mice showed that GH expression by thymocytes and bone marrow microenvironmental cells does not depend on Pit-1 (295, 296). From a functional point of view, it was found that thymocyte-derived GH can enhance thymocyte proliferation through an IGF-I-mediated circuit (137).

If the production of PRL and GH by thymic cells is well documented, evidence for intrathymic expression of other adenopituitary hormones is still scarce. An immunoreactive LH peptide was extracted from human thymocytes (140), and

it was shown that PHA-stimulated mitogenic response of these cells was blocked in the presence of anti-LH antiserum. Additionally, immunocytochemical findings indicate that TSH, FSH, and ACTH may be produced within the thymus, particularly by the epithelial component (297, 298). The POMC gene also appears to be constitutively expressed in the thymus, as ascertained by Northern blotting, RT-PCR, and immunocytochemistry (299–301). The latter study, performed in the chicken thymus, indicated the presence of POMC in TEC and dendritic cells.

Despite the findings discussed above, further studies are needed to define whether these pituitary hormones are actually secreted intrathymically and how they are regulated. It is interesting that the degree of intrathymic hormone production can be disrupted in some pathological conditions, as exemplified by the high quantities of ACTH detected in some carcinoid tumors (302).

C. Is there a functional autocrine/paracrine IGF-I-mediated circuitry in the thymus?

As partially discussed above, converging data now strongly indicate that IGF-I is involved in the effects of GH on the thymus (reviewed in Ref. 287): 1) control of thymulin secretion and TEC/thymocyte adhesion by GH can be prevented *in vitro* by treating TEC cultures with monoclonal antibodies specific for IGF-I or IGF-I receptor (56, 84); and 2) IGF-I alone can replace GH in stimulating thymulin production by cultured TEC and in increasing TEC/thymocyte adhesion (56, 84). Moreover, the enhanced concanavalin-A mitogenic response and IL-6 production by thymocytes observed in GH-treated aging animals (162) can be detected in animals treated with IGF-I (303). In the same vein, it was shown that cyclosporin A-induced thymic atrophy was restored by *in vivo* treatment with recombinant GH or IGF-I (148), and that IGF-I was able to induce repopulation of the atrophic thymus from diabetic rats (304). Taken together, these findings strongly suggest that GH triggers or enhances a functional circuitry in the thymus involving IGF-I and its receptor, thus implying the intrathymic production of these molecules. Recent findings showing that exogenous or thymus-derived GH promotes thymocyte proliferation via IGF-I production by these cells are noteworthy (137, 305). This is in keeping with the demonstration of IGF-I receptors in thymocytes (306, 307).

In a second vein, we recently provided immunochemically based evidence regarding the expression of IGF-I and IGF-I receptor by murine and human TEC, as ascertained by immunocytochemistry, immunoblot, and immunodot analyses. Both molecules are constitutively expressed by TEC, but their densities can be increased after GH treatment (our unpublished data). However, recent findings in experiments using ribonuclease protection assay in human TEC extracts failed to detect mRNA for the IGF-I receptor (308). At present, we cannot explain such contrasting results. They may be due either to the methodology or to the samples used for running the various assays.

In any case, it remains plausible that GH up-regulates a functional IGF-I/IGF-I receptor circuitry in both lymphoid and microenvironmental compartments of the thymus. However, the relative contribution of thymus-derived *vs.* pituitary gland-derived GH to the regulation of IGF-I and its receptor in thymocytes or TEC is unknown at the moment.

IGF-I is not the only member of the insulin family expressed in the thymus. Immunocytochemical evidence suggests that IGF-II may be much more expressed intrathymically than IGF-I (309). Considering that IGF-II receptors have been shown in the rat and the human thymus (306, 308, 310), together with the demonstration of various IGF binding proteins in this tissue (308), the existence of an intrathymic biological circuit mediated by IGF-II and its receptor is also conceivable.

Insulin expression has also been demonstrated in the human thymus (311, 312) and confirmed in the mouse along with other pancreatic hormones (313, 314). Particularly in the mouse, we showed that insulin expression is restricted to thymic dendritic cells (313). This was further confirmed by RT-PCR in studies using FTOC, in which insulin gene expression was not found in MHC class II-positive TEC (315). By contrast, other "classic" pancreatic hormones, such as glucagon and somatostatin, appear to be expressed intrathymically by macrophages (313). It is noteworthy that in the NOD mouse (that spontaneously develops autoimmune insulin-dependent diabetes), there is a decrease in the expression of the proinsulin genes, as compared with normal mouse strains, whereas no changes were observed in glucagon or somatostatin (316). This finding raised the hypothesis that decreased intrathymic expression of insulin might favor the escape of thymocytes potentially able to recognize insulin in the periphery.

D. Neuropeptide expression by the thymic microenvironment

One of the first demonstrations of intrathymic production of neuropeptides was the observation of vasopressin and oxytocin, as well as of their corresponding mRNA transcripts, in the human thymus (317). Further work established the thymic epithelium (including TNCs) as a source of these neurohormones both *in vivo* and *in vitro* (318–320). Moreover, in the thymus, the biosynthesis of oxytocin and arginine-vasopressin also results from cleavage of corresponding neurophysins, similar to what happens in the hypothalamus (321). Whether these hormones are actually secreted has not been determined. Recent biochemical and ultrastructural findings suggest that after processing, oxytocin is directly exported to the surface membrane rather than directed to secretory vesicles (322). This finding indicates that, at least in part, fragments of these peptidic hormones are presented to differentiating thymocytes in the context of the MHC molecules expressed on the TEC surface. From a conceptual viewpoint, in addition to being paracrinally secreted, intrathymic expression of peptidic hormones may also be related to negative selection of those thymocytes that could potentially recognize these molecules in the periphery of the immune system, causing antihormone T cell autoreactivity.

In addition to "classical" neurohypophyseal hormones, neuropeptides typically found and secreted in the hypothalamus have been detected in thymic cells. Using ligand binding as well as ELISA, LH-RH was found in human thymocytes (140). It is noteworthy that the control of thymic LH-RH

may differ from what occurs in the hypothalamus. As studied in rats, an increase in the LH-RH contents in the thymus after castration was found, and such an increase was prevented by testosterone replacement (323). This contrasted with the decrease in the hypothalamic contents of LH-RH in castrated animals.

The intrathymic production of CRH has also been demonstrated by several experimental approaches (324–326) and appears to be phylogenetically conserved, as ascertained by immunocytochemistry (327). In one double labeling immunocytochemical study, macrophages were incriminated as the cell source of intrathymic CRH (328). Together with findings showing the production of ACTH and corticosterone by TEC, this raises the hypothesis that a whole circuit, similar to that seen in the hypothalamus-pituitary-adrenal axis, may occur intrathymically. Nevertheless, in terms of biological responses, the existence of such a cascade and how it might be regulated has not been completely demonstrated in the thymus.

In keeping with the expression of the POMC gene is the detection of β-endorphin in thymus extracts, as ascertained by RIA, as well as in TEC (329–331). Intrathymic opioid production apparently is not restricted to β-endorphin, since met-enkephalin and leu-enkephalin have been detected by immunocytochemistry in both lymphoid and microenvironmental compartments of the rat thymus (329–332), a finding in keeping with evidence for pro-enkephalin A gene expression in the organ (333). Somatostatin gene expression has also been identified in the rat thymus, at the levels of mRNA and translated peptide (334, 335). Recent data suggest that somatostatin is expressed in macrophages and restricted to the medulla (313, 314).

Cloning of a TRH precursor has recently been reported in the rat thymus. Prepro-TRH mRNA was initially defined by RT-PCR using specific nucleotide primers, and the amplified gene product was then sequenced. The TRH peptide itself was further revealed by reverse phase HPLC analysis (266). Unfortunately, this study did not determine which cell type is responsible for intrathymic TRH production. In any case, considering the local production of TRH together with the intrathymic expression of the TRH receptor and the fact that this neuropeptide is able to enhance BrDu uptake by thymocytes (152), it is plausible that an autocrine/paracrine physiological TRH-mediated circuit also exists in the thymus.

Lastly, intrathymic gene expression for VIP should be noted. Various methodological approaches have shown that $CD4^+CD8^+$ double-positive as well as $CD4^+$ and $CD8^+$ single-positive thymocytes express this neuropeptide (276). This agrees with previous findings from the same research group showing *in situ* immunoreactivity for VIP in many thymocytes (335). As discussed earlier, the autocrine/paracrine secretion of VIP by thymocytes, together with its role in counteracting apoptosis induced by glucocorticoid hormones, places this neuropeptide as one further player in the general process of generating the T cell repertoire in the thymus (336). This hypothesis is further supported by data indicating that VIP enhances the antigen-induced differentiation of $CD4^+CD8^+$ immature thymocytes to the $CD4^+$ simple positive mature stage after interacting with specific antigen presenting cells (337). Similarly, enhancement of VIP production upon mitogenic and antigenic stimulation has been recently shown (338).

The intrathymic expression of various hormones and neuropeptides is summarized in Tables 2 and 4.

X. Conclusions and Major Questions to Be Addressed

The findings discussed herein clearly indicate that the thymus is physiologically under neuroendocrine control. It is apparent that circulating levels of distinct hormones and neuropeptides are necessary to maintain various biological functions related to both microenvironmental and lymphoid cells of the organ (summarized in Fig. 8). However, such neuroendocrine control of the thymus appears to be far more complex, with possible intrathymic biological circuitry involving *in situ* production of these mediators, as well as the influence of neurotransmitters, which were not targeted herein for discussion, being extensively reviewed elsewhere (3, 339).

Independent of which pathway(s) is triggered, such neuroendocrine control comprises modulation of the expression of several genes in different cell types. It will be important to amplify this point using fetal thymus organ cultures and *in vivo* experiments designed to evaluate the relative influence of each mediator on each type of heterotypic interaction occurring between thymocytes and microenvironmental cells. It should certainly be useful to define to what extent hormone-mediated paracrine circuits play a role in thymus physiology.

In conjunction with this point, another still unresolved question is how hormone-mediated paracrine circuits are regulated.

Since one major function of the thymus is to generate a T cell repertoire, simultaneously bearing diversity but not autoreactivity, it is crucial to have precise knowledge of the extent to which such a process is under neuroendocrine control. Phenotypic analysis of the Vβ and Vδ gene rearrangements in thymocytes derived from fetal thymus organ cultures subjected to distinct hormones or neuropeptides will be of interest. We anticipate that the use of different models of knockout mice as well as animals in which hormone-specific transgenes are coupled to thymus-specific promoters may be useful in further determining the relative role of each hormone or neuropeptide in the general process of shaping the T cell repertoire. In this context, it is worthwhile recalling that conflicting results are likely to occur when a role for a given hormone is approached in mice in which the corresponding gene (or the gene for the corresponding receptor) has been inactivated. This is apparently the case of PRL or PRL receptor knockout mice, which apparently develop an efficient immune system, with a normal thymocyte differentiation profile (177, 178). This clearly indicates that PRL/PRL receptor-mediated interaction is not crucial for thymus development. Yet, such data should be viewed with caution, as these mice never had functional PRL or PRL receptor. As in several other redundant systems, it is possible that further biological pathway(s) replace(s) the one triggered by PRL-mediated interactions. Perhaps a better model would be a genetically engineered animal in which the

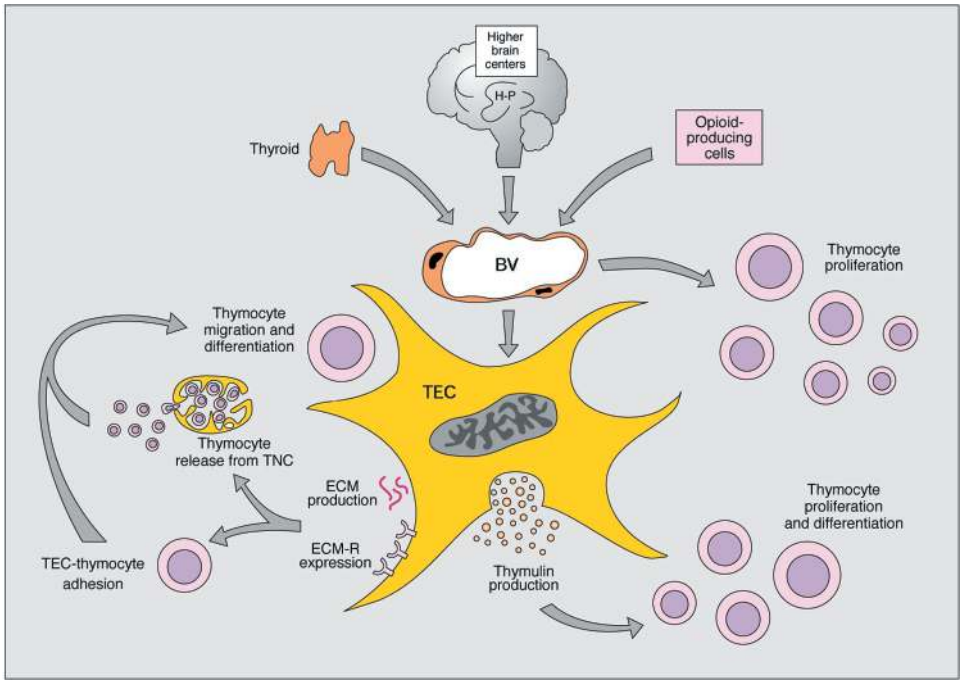

FIG. 8. Pleiotropic nature of the neuroendocrine control of thymus physiology. In this schematic representation, neuroendocrine stimuli are provided by the hypothalamus-pituitary axis, thyroid gland, or opioid-producing cells. Hormones and neuropeptides can act directly on thymocytes, modulating their proliferation rate. Additionally, intrathymic T cell maturation can be indirectly controlled via changes in the behavior of the thymic microenvironment, exemplified herein by TEC. Accordingly, thymic hormone production and expression of ECM ligands and receptors can be hormonally regulated, with consequent effects on proliferation, differentiation, and migration of thymocytes.

receptor can be activated or de-activated by gene manipulation in adult life. In chickens deprived of major neuroendocrine centers by surgical removal of embryonic prosencephalon, thymocyte development ceases with accumulation of immature $CD4^-CD8^-$ thymocytes within the organ (340). This picture is strikingly reversed by embryonic pituitary gland engraftment or by supplying recombinant PRL, thus emphasizing that PRL may be active, particularly in relation to thymocyte differentiation.

In a second vein, several aspects of thymus physiology have not yet been studied in PRL receptor knockout animals. One example is cell traffic, *e.g.*, thymocyte traffic. We have recently noted that, in spite of a normal CD4/CD8-defined differentiation pattern, the expression of fibronectin receptors by thymocytes from GH receptor null animals is altered compared with the corresponding age-matched wild-type mice, suggesting that T cell migration may be altered in these mice (our unpublished data).

A third aspect to be recalled is the paucity of information regarding the direct effects of hormones upon the thymus in humans. Although thymulin production is definitively upregulated by several hormones including T_4, PRL, and GH (76, 84), the various aspects of intrathymic T cell differentiation in humans have not been directly assessed (reviewed in Ref. 341). Unfortunately, in one study on long-term treatment of monkeys with GH, the thymus was not analyzed (342).

If we consider that abnormal generation and/or expansion of autoreactive T cells from the thymus can be influenced by the neuroendocrine system, it will be important to develop therapeutic strategies based on the neuroendocrine-mediated manipulation of T cells when they are undergoing intrathymic differentiation.

Acknowledgments

The authors thank Dr. F. Homo-Delarche for helpful discussion, Mrs. Martine Netter and Heloisa Nogueira Diniz for computer drawings, Mrs Catherine Slama for typing the manuscript, and Mrs. D. Broneer for English review.

References

1. **Blalock ED** 1994 The syntax of immune-neuroendocrine communication. Immunol Today 15:504–511
2. **Savino W, Dardenne M** 1995 Immunoneuroendocrine interactions. Immunol Today 7:318–322
3. **Madden KS, Felten DL** 1995 Experimental basis for neural-immune interactions. Physiol Rev 75:77–106
4. **Besedovsky HO, Del Rey A** 1996 Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev 17:64–102
5. **Anderson G, Moore NC, Owen JJ, Jenkinson EJ** 1996 Cellular interactions in thymocyte development. Annu Rev Immunol 14: 73–99
6. **Ritter MA, Palmer DB** 1999 The human thymic microenvironment: new approaches to functional analysis. Semin Immunol 11:13–21
7. **Poulin JF, Viswanathan MN, Harris JM, Komanduri KV, Wieder E, Ringuette N, Jenkins M, McCure JM, Sekaly RP** 1999 Direct evidence for thymic function in adult humans. J Exp Med 190: 479–486
8. **Benoit C, Mathis D** 1999 T-lymphocyte differentiation and biology. In: Paul W (ed) Fundamental Immunology, ed 4. Lippincott-Raven, Philadelphia. pp 367–409
9. **Haks MC, Oostervegel MA, Blom B, Spits H, Kruisbeek A** 1999 Cell-fate decisions in early T cell development: regulation by cytokine receptors and the pre-TCR. Semin Immunol 11:23–37
10. **Res P, Spits H** 1999 Developmental stages in the human thymus. Semin Immunol 11:39–46
11. **Haynes BF, Telen MJ, Hale LP, Denning SM** 1989 CD44—a molecule involved in leukocyte adhesion and T-cell activation. Immunol Today 10:423–428
12. **von Boehmer H, Teh HS, Kisielow P** 1989 The thymus selects the useful, neglects the useless and destroys the harmful. Immunol Today 10:57–61
13. **Nossal GJ** 1994 Negative selection of lymphocytes. Cell 76:229–239

14. **Nonoyama S, Nakayama M, Shiohara T, Yata J** 1989 Only dull CD3+ thymocytes bind to thymic epithelial cells. The binding is elicited by both CD2/LFA-3 and LFA-1/ICAM-1 interactions. Eur J Immunol 19:1631–1635
15. **Vollger LW, Tuck DT, Springer TA, Haynes BF, Singer KH** 1987 Thymocyte binding to human thymic epithelial cells is inhibited by monoclonal antibodies to CD2 and LFA-3 antigens. J Immunol 138:358–363
16. **Patel DD, Haynes BF** 1993 Cell adhesion molecules involved in intrathymic T cell development. Semin Immunol 5:282–292
17. **Savino W, Villa-Verde DMS, Lannes-Vieira J** 1993 Extracellular matrix proteins in intrathymic T cell migration and differentiation? Immunol Today 14:158–161
18. **Lannes Vieira J, Chammas R, Villa Verde DMS, Vannier dos Santos MA, de Mello-Coelho V, Souza SJ, Brentani RR, Savino W** 1993 Thymic epithelial cells express laminin receptors that may modulate interactions with thymocytes. Int Immunol 5:1421–1430
19. **Villa Verde DMS, Lagrota Candido JM, Vannier-Santos MA, Chammas R, Brentani RR, Savino W** 1994 Extracellular matrix components of the mouse thymic microenvironment. IV. Thymic nurse cells express extracellular matrix ligands and receptors. Eur J Immunol 24:659–664
20. **Savino W, Dardenne M, Carnaud C** 1996 The conveyor belt hypothesis for intrathymic cell migration. Immunol Today 17:97–98
21. **Alves LA, Campos-de-Carvalho AC, Cirne-Lima EO, Rocha-e-Souza CM, Dardenne M, Spray DC, Savino W** 1995 Functional gap junctions in thymic epithelial cells are formed by connexin 43. Eur J Immunol 25:431–437
22. **Alves LA, Campos de Carvalho AC, Savino W** 1998 Gap junctions: a novel route for cell-cell communication in the immune system? Immunol Today 19:269–275
23. **Le PT, Tuck DT, Dinarello CA, Haynes BF, Singer KH** 1987 Human thymic epithelial cells produce IL-1. J Immunol 138:2520–2526
24. **Le PT, Singer KH** 1993 Human thymic epithelial cells: adhesion molecules and cytokine production. Int J Clin Lab Res 23:56–60
25. **Schluns KS, Grutkoski PS, Cook JE, Engelmann GL, Le PT** 1995 Human thymic epithelial cells produce TGF-β and express TGF-β receptors. Int Immunol 7:1681–1690
26. **Muegge K, Vila MP, Durum SD** 1993 Interleukin-7: a cofactor for V (D) J rearrangement of the T cell receptor β gene. Science 261: 93–95
27. **Rich BE, Leder P** 1995 Transgenic expression of interleukin 7 restores T cell populations in nude mice. J Exp Med 181:1223–1228
28. **Kim CH, Broxmeyer HE** 1999 Chemokines: signal lamps for trafficking of T and B cells for development and effector function. J Leukoc Biol 65:6–15
29. **Suzuki G, Sawa H, Kobayashi Y, Nakata Y, Nakagawa K-I, Uzawa A, Sakiyama H, Kakinuma S, Iwabuchi K, Nagashima M** 1999 Pertussis toxin-sensitive signal controls the trafficking of thymocytes across the corticomedullary junction in the thymus. J Immunol 162:5981–5985
30. **Kim CH, Pelus LM, White JR, Broxmeyer HE** 1998 Differential chemotactic behavior of development T cells in response to thymic chemokines. Blood 91:4434–4443
31. **Savino W, Dardenne M, Papiernik M, Bach JF** 1982 Thymic hormone containing cells. Characterization and localization of serum thymic factor in young mouse thymus studied by monoclonal antibodies. J Exp Med 156:628–633
32. **Savino W, Dardenne M** 1984 Thymic hormone containing cells. VI. Immunohistologic evidence for the simultaneous presence of thymulin, thymopoietin and thymosin-1 in normal and pathological human thymuses. Clin Exp Immunol 14:987–991
33. **Fabien N, Auger C, Monier JC** 1988 Immunolocalization of thymosin α 1, thymopoietin and thymulin in mouse thymic epithelial cells at different stages of culture: a light and electron microscopic study. Immunology 63:721–727
34. **Ben-Efraim S, Keisari Y, Ophir R, Pecht M, Trainin N, Burstein** 1999 Immunopotentiating and immunotherapeutic effects of thymic hormones and factors with special emphasis on thymic humoral factor THF-2. Crit Rev Immunol 19:261–284
35. **Hadden JW** 1998 Thymic endocrinology. Ann NY Acad Sci 840: 352–358
36. **Bodey B, Siegel SE, Kaiser HE** 2000 Review of thymic hormones in cancer diagnosis and treatment. Int J Immunopharmacol 22: 261–273
37. **Dardenne M, Nabarra B, Lefrancier P, Pléau JM, Bach JF** 1982 Contribution of zinc and other metals to the biological activity of serum thymic factor (FTS). Proc Natl Acad Sci USA 79:5370–5375
38. **Dardenne M, Savino W, Berrih S, Bach JF** 1985 A zinc-dependent epitope on the molecule of thymulin, a thymic hormone. Proc Natl Acad Sci USA 82:7035–7038
39. **Safieh-Garabedian B, Kendall MD, Khamashta MA, Hughes GR** 1992 Thymulin and its role in immunomodulation. J Autoimmun 5:547–555
40. **Savino W, Dardenne Bach JF** 1983 Thymic hormone containing cells. II. Evolution of cells containing the serum thymic factor (FTS or thymulin) in normal and autoimmune mice, as revealed by anti-FTS monoclonal antibodies. Relationship with Ia-bearing cells. Clin Exp Immunol 52:1–6
41. **Molinero P, Soutto M, Benot S, Hmadcha A, Guerrero JM** 2000 Melatonin is responsible for the nocturnal increase observed in serum and thymus of thymosin α1 and thymulin concentrations: observations and rat and humans. J Neuroimmunol 103:180–189
42. **Berrih S, Arenzana-Seisdedos F, Cohen S, Devos R, Charron D, Virelizier JL** 1985 Interferon-gamma modulates HLA class II expression on cultured human thymic epithelial cells. J Immunol 135:1165–1172
43. **Farr AG, Hosier S, Braddy SC, Anderson SK, Eisenhardt DJ, Yan ZJ, Robles CP** 1989 Medullary epithelial cell lines from murine thymus constitutively secrete IL-1 and hematopoietic growth factors and express class II antigens in response to recombinant interferon-γ. Cell Immunol 119:427–444
44. **Lannes Vieira J, van der Meide PH, Savino W** 1991 Extracellular matrix components of the mouse thymus microenvironment. II. *In vitro* modulation of basement membrane proteins by interferon-γ: relationship with thymic epithelial cell proliferation. Cell Immunol 137:329–340
45. **Lagrota Candido JM, Villa Verde DMS, Vanderlei Jr FH, Savino W** 1996 Extracellular matrix components of the mouse thymic microenvironment. V. Interferon-modulates thymic epithelial cell/thymocyte interactions via extracellular matrix ligands and receptors. Cell Immunol 170:235–244
46. **Boyd RL, Tucek CL, Godfrey DI, Izon DJ, Wilson TJ, Davidson NJ, Bean AG, Ladyman HM, Ritter MA, Hugo P** 1993 The thymic microenvironment. Immunol Today 14:445–459
47. **Wekerle H, Ketelsen UP, Ernst M** 1980 Thymic nurse cells. Lymphoepithelial cell complexes in murine thymuses: morphological and serological characterization. J Exp Med 151:925–944
48. **van Ewijk W** 1988 Cell surface topography of thymic microenvironments. Lab Invest 59:579–590
49. **Pezzano M, King KD, Philp DD, Adeyemi A, Gardiner B, Yang J, Samms M, Boto W, Guyden JC** 1998 A thymic nurse cell-specific monoclonal antibody. Cell Immunol 185:123–133
50. **Li Y, Pezzano M, Philp D, Reid V, Guyden J** 1992 Thymic nurse cells exclusively bind and internalize CD4+CD8+ thymocytes. Cell Immunol 140:495–506
51. **Villa-Verde DMS, de Mello-Coelho V, Lagrota-Candido JM, Chammas R Savino W** 1995 The thymic nurse cell complex: an *in vitro* model for extracellular matrix-mediated intrathymic T cell migration. Braz J Med Biol Res 28:907–912
52. **Penninger J, Wick G** 1992 Thymic nurse cell lymphocytes react against self major histocompatibility complex. Eur J Immunol 22: 79–83
53. **Aguilar LK, Aguilar-Cordova E, Cartwright Jr J, Belmont JW** 1994 Thymic nurse cells are sites of thymocyte apoptosis. J Immunol 152:2645–2651
54. **Cotta-de-Almeida V, Bertho AL, Villa-Verde DMS, Savino W** 1997 Phenotypic and functional alterations of thymic nurse cells following acute *Trypanosoma cruzi* infection. Clin Immunol Immunopathol 82:125–132
55. **Defresne MP, Humblet C, Rongy AM, Boniver J** 1989 Control of lymphoepithelial interactions within thymic nurse cells by γ-interferon and tumor necrosis factor α. Possible role in the modulation of intrathymic education? Thymus 13:201–204
56. **de Mello Coelho V, Villa Verde DMS, Dardenne M, Savino W**

1997 Pituitary hormones modulate cell-cell interactions between thymocyte and thymic epithelial cells. J Neuroimmunol 76:39–49
57. **von Boehmer H** 1990 Developmental biology of T cells in T cell-receptor transgenic mice. Annu Rev Immunol 8:531–556
58. **Ceredig R** 1988 Differentiation potential of 14-day fetal mouse thymocytes in organ culture. Analysis of CD4/CD8-defined single-positive and double-positive cells. J Immunol 141:355–362
59. **Benfield MR, Vail A, Bucy RP, Weigent DA** 1997 Growth hormone induces interferon γ production and may play a role in the presentation of alloantigens *in vitro*. Neuroimmunomodulation 4:19–27
60. **Benfield MR, Parker KL, Waldo FB, Overstreet SL, Kohaut EC** 1993 Growth hormone in the treatment of growth failure in children after renal transplantation. Kidney Int Suppl 44:S62–S64
61. **Sacedon R, Vicente A, Varas A, Jimenez V, Muños JJ, Zapata AG** 1999 Glucocorticoid-mediated regulation of thymic dendritic function. Int Immunol 11:1217–1224
62. **Sacedon R, Vicente A, Jiménez E, Munoz JJ, Vicente A, Zapata AG** 1999 Accelerated maturation of the thymic stroma in the progeny of adrenalectomized pregnant rats. Neuroimmunomodulation 6:23–30
63. **Sacedon R, Vicente A, Varas A, Jimenez E, Munoz JJ, Zapata AG** 1999 Early maturation of T-cell progenitors in the absence of glucocorticoids. Blood 94:2819–2826
64. **Lannes-Vieira J, Dardenne M, Savino W** 1991 Extracellular matrix components of the mouse thymus microenvironment. I. Ontogenetic studies and modulation by glucocorticoid hormones. J Histochem Cytochem 39:1539–1546
65. **Shu S, Naylor P, Touraine JL, Hadden JW** 1996 IL-1, ICAM-1, LFA-3 and hydrocortisone differentially regulate cytokine secretion by human fetal thymic epithelial cells. Thymus 24:89–99
66. **Villa-Verde DMS, de Mello-Coelho V, Farias-de-Oliveira DA, Dardenne M, Savino W** 1993 Pleiotropic influence of triiodothyronine on thymus physiology. Endocrinology 133:867–875
67. **Head GM, Mentlein R, Kranz A, Downing JE, Kendall MD** 1997 Modulation of dye-coupling and proliferation in cultured rat thymic epithelium by factors involved in thymulin secretion. J Anat 191:355–365
68. **Head GM, Mentlein R, Von Patay B, Downing JE, Kendall MD** 1998 Neuropeptides exert direct effects on rat thymic epithelial cells in culture. Dev Immunol 6:95–104
69. **Savino W, Villa-Verde DMS, Alves LA, Dardenne M** 1998 Neuroendocrine control of the thymus. Ann NY Acad Sci 840:470–479
70. **Alves LA, Nihei OK, Fonseca PC, Campos-de-Carvalho AC, Savino W** 2000 Gap junction modulation by extracellular signaling molecules: the thymus model. Braz J Med Biol Res 33:457–465
71. **Napolitano M, Bellavia C, Maroder M, Farina M, Vacca A, Frat L, Gulino A, Screpanti I** 1997 Modulation of cytokine gene expression by thymic lympho-stromal cell to cell interactions: effect of retinoic acid. Thymus 24:247–258
72. **Tseng YH, Kessler MA, Schuler LA** 1997 Regulation of interleukin (IL)-1a, IL-1β and IL-6 expression by growth hormone and prolactin in bovine thymic stromal cells. Mol Cell Endocrinol 128: 117–127
73. **Martens H, Malgrange B, Robert F, Charlet C, De Groote D, Heymann D, Godard A, Soulillou JP, Moonen G, Gennen V** 1996 Cytokine production by human thymic epithelial cells: control by the immune recognition of the neurohypophysial self-antigen. Regul Pept 67:39–45
74. **Savino W, Wolf B, Aratan-Spire S, Dardenne M** 1984 Thymic hormone containing cells. IV. Fluctuations in the thyroid hormone levels *in vivo* can modulate the secretion of thymulin by the epithelial cells of young mouse thymus. Clin Exp Immunol 55:629–635
75. **Fabris N, Mocchegiani E** 1985 Endocrine control of thymic serum factor production in young adult and old mice. Cell Immunol 91:325–335
76. **Fabris N, Mocchegiani E, Mariotti S, Pacini F, Pinchera A** 1986 Thyroid function modulates thymic endocrine activity. J Clin Endocrinol Metab 62:474–478
77. **Mocchegiani E, Amadio L, Fabris N** 1990 Neuroendocrine-thymus interactions. I. *In vitro* modulation of thymic factor secretion by thyroid hormones. J Endocrinol Invest 13:139–147
78. **Dardenne M, Savino W, Gagnerault MC, Itoh T, Bach JF** 1989 Neuroendocrine control of thymic hormonal production. I. Prolactin stimulates *in vivo* and *in vitro* the production of thymulin by human and murine thymic epithelial cells. Endocrinology 125:3–12
79. **Timsit J, Safieh B, Gagnerault MC, Savino W, Lubetzki J, Bach JF, Dardenne M** 1990 Increase of circulating levels of thymulin in hyperprolactinemia and acromegaly. C R Acad Sci III 310:7–13
80. **Pelletier M, Montplaisir S, Dardenne M, Bach JF** 1976 Thymic hormone activity and spontaneous autoimmunity in dwarf mice and their littermates. Immunology 30:783–788
81. **Dardenne M, Savino W** 1990 Neuroendocrine control of the thymic epithelium: modulation of thymic endocrine function, cytokeratin expression and cell proliferation by hormones and neuropeptides. Prog Neuroendocrinimmunol 3:18–25
82. **Mocchegiani E, Sartorio A, Santarelli L, Ferrero S, Fabris N** 1996 Thymulin, zinc and insulin-like growth factor-I (IGF-I) activity before and during recombinant growth hormone (rec-GH) therapy in children and adults with GH deficiency. J Endocrinol Invest 19:630–637
83. **Mocchegiani E, Paolucci P, Balsamo A, Cacciari E, Fabris N** 1990 Influence of growth hormone on thymic endocrine activity in humans. Horm Res 33:248–255
84. **Timsit J, Savino W, Safieh B, Chanson P, Gagnerault MC, Bach JF, Dardenne M** 1992 Growth hormone and insulin-like growth factor-I stimulate hormonal function and proliferation of thymic epithelial cells. J Clin Endocrinol Metabol 75:183–188
85. **Savino W, Gagnerault MC, Bach JF, Dardenne M** 1990 Neuroendocrine control of thymic hormonal production. II. Stimulatory effects of endogenous opioids on thymulin production by cultured human and murine thymic epithelial cells. Life Sci 46:1687–1697
86. **Dardenne M, Savino W, Duval D, Kaiserlian D, Hassid J, Bach JF** 1986 Thymic hormone containing cells. VII. Adrenals and gonads control the *in vivo* secretion of thymulin and its plasmatic inhibitor. J Immunol 136:1303–1308
87. **Savino W, Bartoccioni E, Homo-Delarche F, Gagnerault MC, Itoh T, Dardenne M** 1988 Thymic hormone containing cells. IX. Steroids *in vitro* modulate thymulin secretion by human and murine thymic epithelial cells. J Steroid Biochem 30:479–484
88. **Savino W, Dardenne M, Bach JF** 1983 Thymic hormone containing cells. III. Evidence for a feedback regulation of the secretion of the serum thymic factor (FTS) by thymic epithelial cells. Clin Exp Immunol 52:7–12
89. **Cohen S, Berrih S, Dardenne M, Bach JF** 1986 Feedback regulation of the secretion of a thymic hormone (thymulin) by human thymic epithelial cells in culture. Thymus 8:109–119
90. **Cousins RJ, Leinart AS** 1988 Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J 2:2884–2890
91. **Coto JA, Hadden EM, Sauro M, Zorn N, Hadden JW** 1992 Interleukin 1 regulates secretion of zinc-thymulin by human thymic epithelial cells and its action on T-lymphocyte proliferation and nuclear protein kinase C. Proc Natl Acad Sci USA 89:7752–7756
92. **Besedovsky HO, Sorkin E** 1974 Thymus involvement in female sexual maturation. Nature 249:356–358
93. **Bonomo A, Kehn PI, Shevach EM** 1995 Post-thymectomy autoimmunity: abnormal T-cell homeostasis. Immunol Today 16:61–67
94. **Ruitenberg EJ, Berkvens JM** 1977 The morphology of the endocrine system in congenitally athymic (nude) mice. J Pathol 121: 225–231
95. **Daneva T, Spinedi E, Hadid R, Gaillard RC** 1995 Impaired hypothalamo-pituitary-adrenal axis function in Swiss nude athymic mice. Neuroendocrinology 62:79–86
96. **Rebar RW, Miyake A, Low TL, Goldstein AL** 1981 Thymosin stimulates secretion of luteinizing hormone-releasing factor. Science 214:669–671
97. **Zaidi SA, Kendall MD, Gillham B, Jones MT** 1988 The release of luteinizing hormone from pituitaries perifused with thymic extracts. Thymus 12:253–264
98. **Hadley AJ, Rantle CM, Buckinham JC** 1997 Thymulin stimulates corticotropin release and cyclic nucleotide formation in the rat anterior pituitary gland. Neuroimmunomodulation 4:62–69
99. **Badamchian M, Spangelo BL, Damavandy T, MacLeod RM, Goldstein AL** 1991 Complete amino acid sequence analysis of a

peptide isolated from the thymus that enhances release of growth hormone and prolactin. Endocrinology 128:1580–1588
100. **Angioni S, Iori G, Cellini M, Sardelli S, Massolo F, Petraglia F, Genazzani AR** 1992 Acute β-interferon or thymopentin administration increases plasma growth hormone and cortisol levels in children. Acta Endocrinol (Copenh) 127:237–241
101. **Malaise MG, Hazee-Hagelstein MT, Reuter AM, Vrinds-Gevaert Y, Goldstein G, Franchimont P** 1987 Thymopoietin and thymopentin enhance the levels of ACTH, β-endorphin and β-lipotropin from rat pituitary cells *in vitro*. Acta Endocrinol (Copenh) 115: 455–460
102. **Goya RG, Sosa YE, Brown OA, Dardenne M** 1994 *In vitro* studies on the thymus-pituitary axis in young and old rats. Ann NY Acad Sci 741:108–114
103. **Brown OA, Sosa YE, Dardenne M, Pléau JM, Goya RG** 1999 Growth hormone-releasing activity of thymulin on pituitary somatotropes is age dependent. Neuroendocrinology 69:20–27
104. **Milenkovic L, McCann SM** 1992 Effects of thymosin α-1 on pituitary hormone release. Neuroendocrinology 55:14–19
105. **Milenkovic L, Lyson K, Aguila MC, McCann SM** 1992 Effect of thymosin α 1 on hypothalamic hormone release. Neuroendocrinology 56:674–679
106. **Prepin J** 1991 Fetal thymus and thymulin stimulate the *in vitro* proliferation of oogonia in the fetal rat ovary. C R Acad Sci III 313:407–411
107. **Prepin J** 1993 Fetal thymus and thymulin stimulate *in vitro* proliferation of gonocytes in the fetal testis in rats. C R Acad Sci III 316:451–454
108. **Prepin J, Le Vigouroux P, Dadoune JP** 1994 Effects of thymulin on *in vitro* incorporation of ^{3}H-thymidine into gonocytes of newborn rat testes. Reprod Nutr Dev 34:289–294
109. **Prepin J, Le Vigouroux P** 1997 Inhibition by TGF-β 1 of the *in vitro* thymulin-stimulated proliferation of gonocytes from fetal rat testes. Reprod Nutr Dev 37:203–206
110. **Uzumcu M, Akira S, Lin YC** 1992 Stimulatory effect of thymic factor(s) on steroidogenesis in cultured rat granulosa cells. Life Sci 51:1217–1228
111. **Hinojosa L, Chavira R, Dominguez R, Rosas P** 1999 Effects of thymulin on spontaneous puberty and gonadotrophin-induced ovulation in prepubertal normal and hypothymic mice. J. Endocrinol 163:255–260
112. **Wise T, Ford JJ** 1999 Effects of the thymic peptide thymulin on *in vitro* and *in vivo* testicular steroid concentrations in white composite and Meishan boars. J Anim Sci 77:2240–2251
113. **Safieh-Garabedian B, Jalakhian RH, Saade NE, Haddad JJ, Jabbur SJ, Kanaan AS** 1996 Thymulin reduces hyperalgesia induced by peripheral endotoxin injection in rats and mice. Brain Res 717: 179–183
114. **Safieh-Garabedian B, Kanaan SA, Jalakhian RH, Poole S, Jabbur SJ, Saade NE** 1997 Hyperalgesia induced by low doses of thymulin injections: possible involvement of prostaglandin E2. J Neuroimmunol 73:162–168
115. **Safieh-Garabedian B, Ahmed K, Khamashta MA, Taub NA, Hughes GR** 1993 Thymulin modulates cytokine release by peripheral blood mononuclear cells: a comparison between healthy volunteers and patients with systemic lupus erythematosus. Int Arch Allergy Immunol 101:126–131
116. **Saadé NE, Major SC, Jabbur SJ, Atweh SF, Kanaan SA, Safieh-Garabedian B** 1998 Involvement of capsaicin sensitive primary afferents in thymulin-induced hyperalgesia. J Neuroimmunol 91: 171–179
117. **Saadé NE, Lawand HF, Safieh-Garabedian B, Kanaan SA, Atweh SF, Jabbur SJ** 1999 Thymulin induces c-fos expression in the spinal cord of rats which is reversed by meloxicam and morphine. J Neuroimmunol 97:16–24
118. **Menzaghi F, Heinrichs SC, Vargas-Cortes M, Goldstein G, Koob GF** 1996 IRI-514, a synthetic peptide analogue of thymopentin, reduces the behavioral response to social stress in rats. Physiol Behav 60:397–401
119. **Pellow S, Chopin P, File SE, Briley M** 1985 Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167
120. **Klusa V, Kiivet RA, Muceniece R, Liepa I, Harro J, Svirskis S, Andermanis A, Rago L** 1990 Thymopentin antagonizes stress-induced changes of GABA/benzodiazepine receptor complex. Regul Pept 27:355–365
121. **Morley BJ, Murphy R** 1993 Effects of neonatal thymectomy on the densities of nicotinic cholinergic receptors in skeletal muscle and brain. Exp Neurol 120:277–282
122. **Dardenne M** 1999 Role of thymic peptides as transmitters between the neuroendocrine and immune systems. Ann Med 31[Suppl 2]:34–39
123. **Lempereur L, Cantarella G, Murabito P, Chiarenza A, Fiore L, Zappala G, Bernardini R** 1999 Thymic hormones, cancer and behavioural adaptive responses. Ann Med 31[Suppl 2]:45–45
124. **Tsuji Y, Kinoshita Y, Hato F, Lin B** 1997 Marked differences in proliferative response to stimulation of growth hormone and insulin-like growth factor-I between thymoma- and normal thymus-derived epithelial cell lines. Cell Mol Biol (Noisy-le-grand) 43:149–156
125. **Ferone D, van Hagen PM, van Koetsveld PM, Zuijderwijk J, Mooy DM, Lichtenauer-Kaligis EGR, Colao A, Bogers AJ, Lombardi G, Lamberts SW, Hofland LJ** 1999 *In vitro* characterization of somatostatin receptors in human thymus and effects of somatostatin and octreotide on cultured human thymic epithelial cells. Endocrinology 140:373–380
126. **Guerin S, Mari B, Fernandez E, Belhacene N, Toribio ML, Auberger P** 1997 CD10 is expressed on human thymic epithelial cells lines and modulates thymopentin induced cell proliferation. FASEB J 11:1003–1011
127. **Sakabe K, Kawashima I, Urano R, Seiki K, Itoh T** 1994 Effects of sex steroids on the proliferation of thymic epithelial cells in a culture model: a role of protein kinase C. Immunol Cell Biol 72: 193–199
128. **Kurz B, von Gaudecker B, Krisch B, Mentlein R** 1996 Rat thymic epithelial cells *in vitro* and *in situ*: characterization by immunocytochemistry and morphology. Cell Tissue Res 283:221–229
129. **Winczyk K, Pawlikowski M** 1992 Metoclopramide increases the number of solid epithelial islands in the adult rat thymuses. Thymus 20:123–126
130. **Scheiff JM, Cordier AC, Haumont S** 1997 Epithelial cell proliferation in thymic hyperplasia induced by triiodothyronine. Clin Exp Immunol 27:516–521
131. **Kurz B, Feindt J, von Gaudecker B, Kranz A, Loppnow H, Mentlein R** 1997 β-Adrenoceptor-mediated effects in rat cultured thymic epithelial cells. Br J Pharmacol 120:1401–1408
132. **Villa-Verde DMS, Defresne MP, Greimers R, Dardenne M, Savino W, Boniver J** 1991 Induction of thymocyte proliferation by supernatants from a mouse thymic epithelial cell line. Cell Immunol 136:113–121
133. **Lin B, Kinoshita Y, Hato F, Tsuji Y** 1997 Enhancement of thymic lymphocyte proliferation by the culture supernatant of thymus epithelial cells stimulated by prolactin. Cell Mol Biol (Noisy-le-grand) 43:361–367
134. **Lin B, Kinoshita Y, Hato F, Tsuji Y** 1997 Enhancement of DNA synthetic activity of thymic lymphocytes by the culture supernatant of thymus epithelial cells stimulated by growth hormone. Cell Mol Biol (Noisy-le-grand) 43:351–359
135. **Postel-Vinay MC, de Mello-Coelho V, Gagnerault MC, Dardenne M** 1997 Growth hormone stimulates the proliferation of activated mouse T lymphocytes. Endocrinology 138:1816–1820
136. **Dialynas E, Brown-Borg H, Bartke A** 1999 Immune function in transgenic mice overexpressing growth hormone (GH) releasing hormone, GH or GH antagonist. Proc Soc Exp Biol Med 221: 178–183
137. **Sabharwal P, Varma S** 1996 Growth hormone synthesized and secreted by human thymocytes acts via insulin-like growth factor I as an autocrine and paracrine growth factor. J Clin Endocrinol Metab 81:2663–2669
138. **Hinton PS, Peterson CA, Dahly EM, Ney DM** 1998 IGF-1 alters lymphocyte survival and regeneration in thymus and spleen after dexamethasone treatment. Am Physiol 274:R912–R920
139. **Bazzoni N, Ambrosi B, Arosio JM, Barbareschi M, Losa M, Faglia G** 1990 Acromegaly and thymic hyperplasia: a case report. J Endocrinol Invest 11:931–935
140. **Sabharwal P, Varma S, Malarkey WB** 1992 Human thymocytes

secrete luteinizing hormone: an autocrine regulator of T cell proliferation. Biochem Biophys Res Commun 187:1187–1192
141. **Mukherjee P, Mastro AM, Hymer WC** 1990 Prolactin induction of interleukin-2 receptors on rat splenic lymphocytes. Endocrinology 126:88–94
142. **Hosokawa Y, Onga T, Nakashima K** 1994 Induction of D2 and D3 cyclin-encoding genes during promotion of G1/S transition by prolactin dependent Nb2 cells. Gene 147:249–252
143. **Soder O, Hellstrom PM** 1987 Neuropeptide regulation of human thymocyte, guinea pig T lymphocyte and rat B lymphocyte mitogenesis. Int Arch Allergy Appl Immunol 84:205–211
144. **Mascardo RN, Barton RW, Sherline P** 1984 Somatostatin has an antiproliferative effect on concanavalin-A activated rat thymocytes. Clin Immunol Immunopathol 33:131–139
145. **Bulloch K, McEwens BS, Diwa A, Radojcic T, Hausman J, Baird S** 1994 The role of calcitonin gene-related peptide in the mouse thymus revisited. Ann NY Acad Sci 741:129–136
146. **Freitas CS, Dalmau SR, Savino W** 1998 Epidermal growth factor (EGF) modulates fetal thymocyte growth and differentiation: partial reversal by insulin, mimicking by specific inhibitors of EGF receptor tyrosine kinase activity, and differential expression of CD45 phosphatase isotypes. J Immunol 161:3384–3392
147. **Kelley KW, Brief S, Westly HJ, Novakofski T, Bechtel PJ, Simon J, Walker EB** 1986 GH3 pituitary adema cells can reverse thymic aging in rats. Proc Natl Acad Sci USA 83:5663–5667
148. **Beschorner WE, Divic J, Pulido H, Yao X, Kenworthy P, Bruce G** 1991 Enhancement of thymic recovery after cyclosporine by recombinant human growth hormone and insulin-like growth factor I. Transplantation 52:879–884
149. **Van Buul-Offers SC, De Haan K, Reijnen-Gresnigt MG, Meinsma D, Jansen M, Oei SL, Bonte EJ, Sussenbach JS, Van den Brande JL** 1995 Overexpression of human insulin-like growth factor-II in transgenic mice causes increased growth of the thymus. J Endocrinol 144:491–502
150. **Van der Ven LTM, Roholl PJM, Reijnen-Gresnigt MG, Bloemen RJ, Van Buul-Offers SC** 1997 Expression of insulin-like growth factor II (IGF-II) and histological changes in the thymus and spleen of transgenic mice overexpressing IGF-II. Histochem Cell Biol 107: 193–203
151. **Kooijman R, van Buul-Offers SC, Scholtens LE, Schuurman HJ, Van den Brande LJ, Zegers BJ** 1995 T cell development in insulin-like growth factor-II transgenic mice. J Immunol 154:5736–5745
152. **Pawlikowski M, Zerek-Melen G, Winczyk K** 1992 Thyroliberin (TRH) increases thymus cell proliferation in rats. Neuropeptides 23:199–202
153. **Wynczyk K, Pawlikowski M** 2000 Time of day-dependent effects of thyroliberin and thyrotropin on thymocyte proliferation in rats. Neuroimmunomodulation 7:89–91
154. **Blacker CM, Ataya KM, Savoy-Moore RT, Subramanian MG, Mutchnick MG, Dunbar JC** 1991 The gonadotropin-releasing hormone agonist leuprolide affects the thymus and other non-reproductive systems of female rats. Acta Endocrinol (Copenh) 125: 581–589
155. **Plotnikoff NP, Murgo AJ, Faith RE** 1984 Neuroimmunomodulation with enkephalins: effects on thymus and spleen weights in mice. Clin Immunol Immunopathol 32:52–56
156. **Pierpaoli W, Regelson W** 1994 Pineal control of aging: effect of melatonin and pineal grafting on aging mice. Proc Natl Acad Sci USA 91:787–791
157. **Maestroni G, Conti A** 1990 The pineal neurohormone melatonin stimulates activated $CD4^+$ Thy1+ cells to release opioids agonist(s) with immunoenhancing and anti-stress properties. J Neuroimmunol 28:167–176
158. **Sainz RM, Mayo JC, Reiter RJ, Antolin I, Esteban MM, Rodriguez C** 1999 Melatonin regulates glucocorticoid receptor: an answer to its antiapoptotic action in thymus. FASEB J 13:1547–1556
159. **Olsen NJ, Kovacs WJ** 1996 Gonadal steroids and immunity. Endocr Rev 17:369–384
160. **Olsen NJ, Viselli SM, Shults K, Stelzer G, Kovacs WJ** 1994 Induction of immature thymocyte proliferation after castration of normal male mice. Endocrinology 134:107–113
161. **Greenstein BD, Fitzpatrick FT, Adcock IM, Kendall MD, Wheeler MJ** 1986 Reappearance of the thymus in old rats after orchidectomy: inhibition of regeneration by testosterone. J Endocrinol 110: 417–422
162. **Goya RG, Gagnerault MC, De Moraes MC, Savino W, Dardenne M** 1992 *In vivo* effects of growth hormone on thymus function in aging mice. Brain Behav Immun 6:341–354
163. **Torres BA, Johnson HM** 1988 Arginine vasopressin (AVP) replacement of helper cell requirement in IFN-γ production. Evidence for a novel AVP receptor on mouse lymphocytes. J Immunol 140:2179–2183
164. **Rameshwar P, Gascon P, Ganea D** 1992 Immunoregulatory effects of neuropeptides: stimulation of interleukin-2 production by substance P. J Neuroimmunol 37:65–74
165. **Ganea D** 1996 Regulatory effects of vasoactive intestinal peptide on cytokine production in central and peripheral lymphoid organs. Adv Neuroimmunol 6:61–74
166. **Tang H, Sun L, Xin Z, Ganea D** 1996 Down-regulation of cytokine expression in murine lymphocytes by PACAP and VIP. Ann NY Acad Sci 805:768–778
167. **Li YM, Brunke DL, Dantzer R, Kelley KW** 1992 Pituitary epithelial cell implants reverse the accumulation of $CD4^-CD8^-$ lymphocytes in thymus glands of aged rats. Endocrinology 130:2703–2709
168. **Murphy WJ, Durum SK, Longo DL** 1992 Role of neuroendocrine hormones in murine T cell development. Growth hormone exerts thymopoietic effects *in vivo*. J Immunol 149:3851–3857
169. **Okuyama R, Abo T, Seki S, Ohteki T, Sugiura K, Kusumi A, Kumagai K** 1992 Estrogen administration activates extrathymic T cell differentiation in the liver. J Exp Med 175:661–669
170. **Hirahara H, Ogawa M, Kimura M, Ital T, Tsuchida M, Hanawa H, Watanabe Abo T** 1994 Glucocorticoid independence of acute thymic involution induced by lymphotoxin and estrogen. Cell Immunol 153:401–411
171. **Screpanti I, Morrone S, Meco D, Santoni A, Gulino A, Paolini R, Crisanti A, Mathieson BJ, Frati L** 1989 Steroid sensitivity of thymocyte subpopulations during intrathymic differentiation. Effects of 17β-estradiol and dexamethasone on subsets expressing T cell antigen receptor or IL-2 receptor. J Immunol 142:3378–3383
172. **Rijhsinghani AG, Thompson K, Bhatia SK, Waldschmidt TJ** 1996 Estrogen blocks early T cell development in the thymus. Am J Reprod Immunol 36:269–277
173. **Screpanti I, Meco D, Morrone S, Gulino A, Mathieson BJ, Frati L** 1991 *In vivo* modulation of the distribution of thymocyte subsets: effects of estrogen on the expression of different T cell receptor V β gene families in CD4-, CD8- thymocytes. Cell Immunol 134: 414–426
174. **Nakayama M, Otsuka K, Sato K, Hasegawa K, Osman Y, Kawamura T, Abo T** 1996 Activation by estrogen of the number and function of forbidden T-cell clones in intermediate T-cell receptor cells. Cell Immunol 172:163–171
175. **Hosokawa Y, Yang M, Kaneko S, Tanaka M, Nakashima K** 1996 Prolactin induces switching of T cell receptor gene expression from α to γ in rat NB2 pre-T lymphoma cells. Biochem Biophys Res Commun 220:958–962
176. **Montgomery DW, Krumenacker JS, Buckley AR** 1998 Prolactin stimulates phosphorylation of the human T cell antigen receptor complex and ZAP-70 tyrosine kinase: a potential mechanism for its immunomodulation. Endocrinology 139:811–814
177. **Horseman ND, Zhao W, Montecino-Rodriguez E, Tanaka M, Nakashima K, Engle SJ, Smith F, Markoff E, Dorshkind K** 1997 Defective mammopoiesis, but normal hematopoiesis in mice with a targeted disruption of the prolactin gene. EMBO J 16:6926–6935
178. **Bouchard B, Ormandy CJ, Di Santo JP, Kelly PA** 1999 Immune system development and function in prolactin receptor-deficient mice. J Immunol 163:576–582
179. **Cohen JJ, Duke RC, Fadok VA, Sellins KS** 1992 Apoptosis and programmed cell death in immunity. Annu Rev Immunol 10: 267–293
180. **McConkey DJ, Orrenius S, Okret S, Jondal M** 1993 Cyclic AMP potentiates glucocorticoid-induced endogenous endonuclease activation in thymocytes. FASEB J 7:580–585
181. **Jeon SH, Kang MG, Kim YH, Jin YH, Lee C, Chung HY, Kwon H, Park SD, Seong RH** 1997 A new mouse gene, SRG3, related to the SWI3 of *Saccharomyces cerevisiae*, is required for apoptosis in-

duced by glucocorticoids in a thymoma cell line. J Exp Med 185: 1827–1836
182. **Schreiber L, Eshel I, Meilin A, Sharabi Y, Shoham J** 1991 Analysis of thymic stromal cell subpopulations grown *in vitro* on extracellular matrix in defined medium. III. Growth conditions of human thymic epithelial cells and immunomodulatory activities in their culture supernatant. Immunology 74:621–629
183. **Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T** 1993 p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362:847–849
184. **Neamati N, Fernandez A, Wright S, Kiefer J, McConkey DJ** 1995 Degradation of lamin B1 precedes oligonucleosomal DNA fragmentation in apoptotic thymocytes and isolated thymocyte nuclei. J Immunol 154:3788–3795
185. **Marchetti P, Decaudin D, Macho A, Zamzami N, Hirsch T, Susin SA, Kroemer G** 1997 Redox regulation of apoptosis: impact of thiol oxidation status on mitochondrial function. Eur J Immunol 27: 289–296
186. **Squier MK, Cohen JJ** 1997 Calpain, an upstream regulator of thymocyte apoptosis. J Immunol 158:3690–3697
187. **Hughes Jr FM, Bortner CD, Purdy GD, Cidlowski JA** 1997 Intracellular K^+ suppresses the activation of apoptosis in lymphocytes. J Biol Chem 272:30567–30576
188. **Cole TJ, Blendy JA, Monagham AP, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K, Schutz G** 1995 Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes Dev 9:1608–1621
189. **Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schütz G** 1998 DNA binding of the glucocorticoid receptor is not essential for survival. Cell 93:531–541
190. **Zacharchuk CM, Mercep M, Chakraborti P, Simons SS, Ashwell JD** 1990 Programmed T cell death: cell activation- and steroid-induced pathways are mutually antagonistic. J Immunol 145:4037–4045
191. **Iwata M, Hanaoka S, Sato K** 1991 Rescue of thymocytes and T cell hybridomas from glucocorticoid-induced apoptosis by stimulation via the T cell receptor/CD3 complex: a possible *in vitro* model for positive selection of the T cell repertoire. Eur J Immunol 21:643–648
192. **Vacchio MS, Ashwell JD** 1997 Thymus-derived glucocorticoids regulate antigen-specific positive selection. J Exp Med 185:2033–2038
193. **Leceta J, Fernandez-Galaz C, Sanchez C, Navarro R, Gomariz RP, Zapata A, Tejero A** 1988 Effects of neonatal treatment with estrogens on the development of the thymus in rats. Dev Comp Immunol 12:375–383
194. **Martin A, Alonso L, Gomez del Moral M, Zapata AG** 1994 Morphometrical changes in the rat thymic lymphoid cells after treatment with two different doses of estradiol benzoate. Histol Histopathol 9:281–286
195. **Martin A, Alonso LM, Gomez del Moral M, Zapata AG** 1994 Ultrastructural changes in the adult rat thymus after estradiol benzoate treatment. Tissue Cell 26:169–179
196. **Aboussaouira T, Marie C, Brugal G, Idelman S** 1991 Inhibitory effect of 17β-estradiol on thymocyte proliferation and metabolic activity in young rats. Thymus 17:167–180
197. **Gilbody JS, Wheeler MJ, Wolstencroft R, Greenstein BD** 1992 Dose-related effects of oestradiol on rat thymic and splenic T-lymphocyte responsiveness to mitogens. Int J Immunopharmacol 14:167–172
198. **Shinomiya N, Tsuru S, Tsugita M, Katsura Y, Takemura T, Rokutanda M, Nomoto K** 1991 Thymic depletion in pregnancy: kinetics of thymocytes and immunologic capacities of the hosts. J Clin Lab Immunol 34:11–22
199. **Clarke AG, Kendall M** 1994 The thymus in pregnancy: the interplay of neural, endocrine and immune influences. Immunol Today 15:545–551
200. **Kumar N, Shan LX, Hardy MP, Bardin CW, Sundaram K** 1995 Mechanism of androgen-induced thymolysis in rats. Endocrinology 136:4887–4893
201. **Olsen NJ, Viselli SM, Fan J, Kovacs WJ** 1998 Androgens accelerate thymocyte apoptosis. Endocrinology 139:748–752
202. **Rijhsinghani AG, Thompson K, Bhatia SK, Waldschmidt TJ** 1996 Estrogen blocks early T cell development in the thymus. Am J Reprod Immunol 36:269–277
203. **Staples JE, Gasiewicz TA, Fiore NC, Lubahn DB, Korach KS, Silverstone AE** 1999 Estrogen receptor a is necessary in thymic development and estradiol-induced thymic alteractions. J Immunol 163:4168–4174
204. **May M, Holmes E, Rogers W, Poth M** 1990 Protection from glucocorticoid induced thymic involution by dehydroepiandrosterone. Life Sci 46:1627–1631
205. **Blauer KL, Poth M, Rogers WM, Bernton EW** 1991 Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. Endocrinology 129:3174–3179
206. **Fletcher-Chiappini SE, Compton MM, La Voie HA, Day EB, Witorsch RJ** 1993 Glucocorticoid-prolactin interactions in Nb2 lymphoma cells: antiproliferative *vs.* anticytolytic effects. Proc Soc Exp Biol Med 202:345–352
207. **Montgomery DW, Krumenacker JS, Buckley AR** 1998 Prolactin stimulates phosphorylation of the human T-cell antigen receptor complex and ZAP-70 tyrosine kinase: a potential mechanism for its immunomodulation. Endocrinology 139:811–814
208. **Delgado M, Garrido E, Martinez C, Leceta J, Gomariz RP** 1996 Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptides (PACAP 27 and PACAP 38) protect $CD4^+CD8+$ thymocytes from glucocorticoid-induced apoptosis. Blood 12:5152–5161
209. **Sainz RM, Mayo JC, Uria H, Kotler M, Antolin I, Rodriguez C, Menendez-Pelaez A** 1995 The pineal neurohormone melatonin prevents *in vivo* and *in vitro* apoptosis in thymocytes. J Pineal Res 19:178–188
210. **Provinciali M, Di Stefano G, Bulian D, Tibaldi A, Fabris N** 1996 Effect of melatonin and pineal grafting on thymocyte apoptosis in aging mice. Mech Ageing Dev 90:1–19
211. **Taub DD, Tsarfaty G, Lloyd AR, Durum SK, Longo DL, Murphy WJ** 1994 Growth hormone promotes human T cell adhesion and migration to both human and murine matrix proteins *in vitro* and directly promotes xenogeneic engraftment. J Clin Invest 94:293–300
212. **Montecino-Rodriguez E, Clark R, Dorshkind K** 1998 Effects of insulin-like growth factor administration and bone marrow transplantation on thymopoiesis in aged mice. Endocrinology 139:4120–4126
213. **Bomberger CE, Haar JL** 1992 Dexamethasone and hydrocortisone enhance the *in vitro* migration of prethymic stem cells to thymus supernatant. Thymus 20:89–99
214. **Martin A, Vicente A, Torroba M, Moreno C, Jimenez E, Zapata AG** 1996 Increased numbers of CD5+ B cells in the thymus of estradiol benzoate-treated rats. Thymus 24:111–127
215. **Delgado M, De la Fuente M, Martinez C, Gomariz RP** 1995 Pituitary adenylate cyclase-activating polypeptides (PACAP27 and PACAP38) inhibit the mobility of murine thymocytes and splenic lymphocytes: comparison with VIP and implication of cAMP. J Neuroimmunol 62:137–146
216. **Savino W, de Mello-Coelho V, Dardenne M** 1995 Control of the thymic microenvironment by growth hormone/insulin-like growth factor-I-mediated circuits. Neuroimmunomodulation 2:313–318
217. **Scollay R, Butcher EC, Weissmann L** 1980 Thymus cell migration. Quantitative aspects of cellular traffic from the thymus to the periphery to mice. Eur J Immunol 10:210–218
218. **Gabor MJ, Godfrey DI, Scollay R** 1997 Recent thymic emigrants are distinct from most medullary thymocytes. Eur J Immunol 27: 2010–2015
219. **Ribeiro-Carvalho MM, Smaniotto S, Savino W, de Mello-Coelho V** 1999 Hormonal influence on T cell migration. Neuroimmunomodulation 6:465–466
220. **Savino W, Smaniotto S, de Mello-Coelho V, Dardenne M,** Is there a role for growth hormone upon intrathymic T cell migration? Ann NY Acad Sci, in press
221. **Morale MC, Batticane N, Gallo F, Barden N, Marchetti B** 1995 Disruption of hypothalamic-pituitary-adrenocortical system in transgenic mice expressing type II glucocorticoid receptor antisense ribonucleic acid permanently impairs T cell function: effets

on T cell trafficking and T cell responsiveness during posnatal development. Endocrinology 136:3949–3960
222. **Pepin MC, Pothier F, Barden N** 1992 Impaired type II glucocorticoid-receptor function in mice bearing antisense RNA transgene. Nature 355:725–728
223. **Martin A, Casares F, Alonso L, Nieuwenhuis P, Vicente A, Zapata AG** 1995 Changes in the blood-thymus barrier of adult rats after estradiol-treatment. Immunobiology 192:231–248
224. **Bonomo A, Kehn PI, Shevach EM** 1994 Premature escape of double-positive thymocytes to the periphery of young mice. Possible role in autoimmunity. J Immunol 152:1509–1518
225. **Tsawdaroglou NG, Govindan MV, Schmid W, Sekeris CE** 1981 Dexamethasone-binding proteins in cytosol and nucleus of rat thymocytes. Purification of three receptor proteins. Eur J Biochem 114:305–313
226. **Lowy MT** 1989 Quantification of type I and II adrenal steroid receptors in neuronal, lymphoid and pituitary tissues. Brain Res 503:191–197
227. **Okret S, Carlstedt-Duke J, Wrange O, Carlstrom K, Gustafsson JA** 1981 Characterization of an antiserum against the glucocorticoid receptor. Biochim Biophys Acta 677:205–219
228. **Bernard PA, Joh TH** 1984 Characterization and immunocytochemical demonstration of glucocorticoid receptor using antisera specific to transformed receptor. Arch Biochem Biophys 229:466–476
229. **McGimsey WC, Cidlowski JA, Stumpf WE, Sar M** 1991 Immunocytochemical localization of the glucocorticoid receptor in rat brain, pituitary, liver, and thymus with two new polyclonal antipeptide antibodies. Endocrinology 129:3064–3072
230. **Ranelletti FO, Maggiano M, Aiello FB, Carbone A, Larocca LM, Musiani P, Piantelli M** 1987 Glucocorticoid receptors and corticosensitivity of human thymocytes at discrete stages of intrathymic differentiation. J Immunol 138:440–445
231. **Kitraki E, Kittas C, Stylianopoulou F** 1997 Glucocorticoid receptor gene expression during rat embryogenesis. An *in situ* hybridization study. Differentiation 62:21–31
232. **Ben Rhouma K, Sakly M** 1994 Involution of rat thymus: characterization of cytoplasmic glucocorticoid receptors, evidence of glucocorticoid resistant dexamethasone receptor-positive cells. Arch Int Physiol Biochim Biophys 102:97–102
233. **Dardenne M, Itoh T, Homo-Delarche F** 1986 Presence of glucocorticoid receptors in cultured thymic epithelial cells. Cell Immunol 100:112–118
234. **Oakley RH, Webster JC, Sar M, Parker Jr CR, Cidlowski JA** 1997 Expression and subcellular distribution of the beta-isoform of the human glucocorticoid receptor. Endocrinology 138:5028–5038
235. **Sacedon R, Vicente A, Varas A, Morale MC, Barden M, Marchetti B, Zapata G** 1999 Partial blockade of T-cell differentiation during ontogeny and marked alterations on the thymic microenvironments in transgenic mice with impaired glucocorticoid receptor function. J Neuroimmunol 98:157–167
236. **Grossman CJ, Sholiton LJ, Nathan P** 1979 Rat thymic estrogen receptor. I. Preparation, location and physiochemical properties. J Steroid Biochem 11:1233–1240
237. **Grossman CJ, Nathan P, Taylor BB, Sholiton LJ** 1979 Rat thymic dihydrotestosterone receptor: preparation, location and physicochemical properties. Steroids 34:539–553
238. **Grossman CJ, Sholiton LJ, Helmsworth JA** 1983 Characteristics of the cytoplasmic and nuclear dihydrotestosterone receptors of human thymic tissue. Steroids 42:11–22
239. **Pearce P, Khalid BA, Funder JW** 1981 Androgens and the thymus. Endocrinology 109:1073–1077
240. **Pearce PT, Khalid BA, Funder JW** 1983 Progesterone receptors in rat thymus. Endocrinology 113:1287–1291
241. **Fujii-Hanamoto H, Seiki K, Sakabe K, Ogawa H** 1985 Progestin receptor in the thymus of ovariectomized immature rats. J Endocrinol 107:223–229
242. **Fujii-Hanamoto H, Grossman CJ, Roselle GA, Mendenhall CL, Seiki K** 1990 Nuclear progestin receptors in rat thymic tissue. Thymus 15:31–45
243. **Kumar N, Shan LX, Hardy MP, Bardin CW, Sundaram K** 1995 Mechanism of androgen-induced thymolysis in rats. Endocrinology 136:4887–4893
244. **Viselli SM, Olsen NJ, Shults K, Steizer G, Kovacs WJ** 1995 Immunochemical and flow cytometric analysis of androgen receptor expression in thymocytes. Mol Cell Endocrinol 109:19–26
245. **Sakabe K, Seiki K, Fujii-Hanamoto H** 1986 Histochemical localization of progestin receptor cells in the rat thymus. Thymus 8: 97–107
246. **Kawashima I, Sakabe K, Seiki K, Fujii-Hanamoto H, Akatsuka A, Tsukamoto H** 1991 Localization of sex steroid receptor cells, with special reference to thymulin (FTS)-producing cells in female rat thymus. Thymus 18:79–93
247. **Gulino A, Screpanti I, Torrisi MR, Frati L** 1985 Estrogen receptors and estrogen sensitivity of fetal thymocytes are restricted to blast lymphoid cells. Endocrinology 117:47–54
248. **Kohen F, Abel L, Sharp A, Amir-Zaltsman Y, Somjen D, Luria S, Mor G, Knyszynski A, Thole H, Globerson A** 1998 Estrogen-receptor expression and function in thymocytes in relation to gender and age. Dev Immunol 5:277–285
249. **Nilsson B, Bergqvist A, Lindblom D, Ljungberg O, Sodergard R, von Schoultz B** 1986 Characterization and localization of specific oestrogen binding in the human thymus. Gynecol Obstet Invest 21:150–157
250. **Mosselman S, Polman J, Dijkema R** 1996 ERβ: identification and characterization of a novel human estrogen receptor. FEBS Lett 392:49–53
251. **Brtko J, Knopp J** 1983 Rat thymus: demonstration of specific thyroxine receptors in nuclear extract. Endocrinol Exp 17:3–9
252. **Villa-Verde DMS, Defresne MP, Vannier-dos-Santos MA, Dussault JH, Boniver J, Savino W** 1992 Identification of nuclear triiodothyronine receptors in the thymic epithelium. Endocrinology 131:1313–1320
253. **Dardenne M, Kelly PA, Bach JF, Savino W** 1991 Identification and functional activity of prolactin receptors in thymic epithelial cells. Proc Natl Acad Sci USA 88:9700–9704
254. **Gagnerault MC, Touraine P, Savino W, Kelly PA, Dardenne M** 1993 Expression of prolactin receptors in murine lymphoid cells in normal and autoimmune situations. J Immunol 150:5673–5681
255. **Dardenne M, Leite de Moraes MC, Kelly PA, Gagnerault MC** 1994 Prolactin receptor expression in human hematopoietic tissues analysed by flow cytofluorometry. Endocrinology 134:2108–2114
256. **Feng JC, Loh TT, Sheng HP** 1998 Lactation increases prolactin receptor expression in spleen and thymus of rats. Life Sci 63:111–119
257. **Ban E, Gagnerault MC, Jammes H, Postel-Vinay MC, Haour F, Dardenne M** 1991 Specific binding sites for growth hormone in cultured mouse thymic epithelial cells. Life Sci 48:2141–2148
258. **de Mello-Coelho V, Gagnerault MC, Souberbielle JC, Strasburger CJ, Savino W, Dardenne M, Postel-Vinay MC** 1998 Growth hormone and its receptor are expressed in human thymic cells. Endocrinology 139:3837–3842
259. **Hull KL, Thiagarajah A, Harvey S** 1996 Cellular localization of growth hormone receptors/binding proteins in immune tissues. Cell Tissue Res 286:69–80
260. **Mertani HC, Morel G** 1995 *In situ* gene expression of growth hormone (GH) receptor and GH binding protein in adult male rat tissues. Mol Cell Endocrinol 109:47–61
261. **Gagnerault MC, Postel-Vinay MC, Dardenne M** 1996 Expression of growth hormone receptors in murine lymphoid cells analyzed by flow cytofluorometry. Endocrinology 137:1719–1726
262. **Chen HT, Schuler LA, Schultz RD** 1998 Growth hormone receptor and regulation of gene expression in fetal lymphoid organs. Mol Cell Endocrinol 137:21–29
263. **Elands J, Resink A, De Kloet ER** 1990 Neurohypophyseal hormone receptors in the rat thymus, spleen and lymphocytes. Endocrinology 126:2703–2710
264. **Lolait SJ, O'Carroll AM, Mahan LC, Felder CC, Button DC, Young III WS, Mezey E, Brownstein MJ** 1995 Extrapituitary expression of the rat V1b vasopressin receptor gene. Proc Natl Acad Sci USA 92:6783–6787
265. **Guarcello V, Weigent DA, Blalock JE** 1991 Growth hormone releasing hormone receptors on thymocytes and splenocytes from rats. Cell Immunol 136:291–302
266. **Montagne JJ, Ladram A, Nicolas P, Bulant M** 1999 Cloning of thyrotropin-releasing hormone precursor and receptor in rat thymus, adrenal gland and testis. Endocrinology 140:1054–1059

267. **Elliot DE, Metwali A, Blum AM, Sandor M, Lynch R, Weinstock JV** 1994 T lymphocytes isolated from the hepatic granulomas of Schistosome-infected mice express somatostatin receptor subtype II (SSTR2) messenger RNA. J Immunol 153:1180–1186
268. **Reubi JC, Waser B, Horisberger U, Krenning E, Lamberts SW, Gebbers JO, Gersbach P, Laissue JA** 1993 *In vitro* autoradiographic and *in vivo* scintigraphic localization of somatostatin receptors in human lymphatic tissue. Blood 82:2143–2151
269. **Maestroni GJ, Conti A** 1991 Anti-stress role of the melatonin-immuno-opioid network: evidence for a physiological mechanism involving T cell-derived, immunoreactive β-endorphin and MET-enkephalin binding to thymic opioid receptors. Int J Neurosci 61: 289–298
270. **Levay G, Gaspar E, Nyakas C, Endroczi E** 1989 Age-related changes in adrenocorticoid and opioid receptor capacity of thymus-derived lymphocytes in rats. Exp Clin Endocrinol 94:262–268
271. **Gomariz RP, Delgado M, Naranjo JR, Mellstrom B, Tormo A, Mata F, Leceta J** 1993 VIP gene expression in rat thymus and spleen. Brian Behav Immun 7:271–278
272. **Delgado M, Martinez C, Leceta J, Garrido E, Gomariz RP** 1996 Differential VIP and VIP_1 receptor gene expression in rat thymocyte subsets. Peptides 17:803–807
273. **Marie JC, Wakkach A, Coudray A, Chastre E, Berrih-Aknin S, Gespach C** 1999 Functional expression of receptors for calcitonin gene-related peptide, calcitonin, and vasoactive intestinal peptide in the human thymus and thymomas from myasthenia gravis patients. J Immunol 162:2103–2112
274. **Reubi JC, Horisberger U, Kappeler A, Laissue JA** 1998 Localization of receptors for vasoactive intestinal peptide, somatostatin, and substance P in distinct compartments of human lymphoid organs. Blood 92:191–197
275. **Gottschall PE, Tatsuno I, Miyata A, Arimura A** 1990 Characterization and distribution of binding sites for the hypothalamic peptide, pituitary adenylate cyclase activating polypeptide. Endocrinology 127:272–277
276. **Shivers BD, Gorcs TJ, Gottschall PE, Arimura A** 1991 Two high affinity binding sites for pituitary adenylate cyclase-activating polypeptide have different tissue distributions. Endocrinology 128: 3055–3065
277. **Poon AM, Liu ZM, Tang F, Pang SF** 1994 Cortisol decreases 2[^{125}I]iodomelatonin binding sites in the duck thymus. Eur J Endocrinol 130:320–324
278. **Rafii-el-Idrissi M, Calvo JR, Pozo D, Harmouch A, Guerrero JM** 1995 Specific binding of 2-[^{125}I]iodomelatonin by rat splenocytes: characterization and its role on regulation of cyclic AMP production. J Neuroimmunol 57:171–178
279. **Vacchio MS, Papadopoulos V, Ashwell JD** 1994 Steroid production in the thymus: implications for thymocyte selection. J Exp Med 179:1835–1846
280. **Pazirandeh A, Xue Y, Rafter I, Sjövall J, Jondal M, Okret S** 1999 Paracrine glucocorticoid activity produced by mouse thymic epithelial cells. FASEB J 13:893–901
281. **Lechner O, Wiegers GJ, Oliveira-Dos-Santos AJ, Dietrich H, Recheis H, Waterman M, Boyd R, Wick G** 2000 Glucocorticoid production in the murine thymus. Eur J Immunol 30:337–346
282. **King LB, Vacchio MS, Dixon K, Hunziker R, Margulies DH, Ashwell JD** 1995 A targeted glucocorticoid receptor antisense transgene increases thymocyte apoptosis and alters thymocyte development. Immunity 3:647–656
283. **Tolosa E, King LB, Ashwell JD** 1998 Thymocyte glucocorticoid resistance alters positive selection and inhibits autoimmunity and lympholiferative disease in MRL-lpr/lpr mice. Immunity 8:67–76
284. **Vacchio MS, Lee JY, Ashwell JD** 1999 Thymus-derived glucocorticoids set the thresholds for thymocyte selection by inhibiting TCR-mediated thymocyte activation. J Immunol 163:1327–1333
285. **Jondal M, Okret S, McConkey D** 1993 Killing of immature $CD4^+CD8+$ thymocytes by anti-CD3 or 5′-(N-ethyl)-carboxamide adenosine is blocked by glucocorticoid receptor antagonist RU 486. Eur J Immunol 23:1246–1250
286. **Xue Y, Murdjeva M, Okret S, McConkey D, Kiuossis D, Jondal M** 1996 Inhibition of I-Ad-, but not Db-restricted peptide-induced thymic apoptosis by glucocorticoid receptor antagonist RU 486 in T cell receptor transgenic mice. Eur J Immunol 26:428–434
287. **Clark R** 1997 The somatogenic hormones and insulin-like growth factor-1: stimulators of lymphopoiesis and immune function. Endocr Rev 18:157–179
288. **O'Neal KD, Montgomery DW, Truong TM, Yu-Lee LY** 1992 Prolactin gene expression in human lymphoid cells. Mol Cell Endocrinol [Suppl] 87: R19–R23
289. **Wu H, Devi R, Malarkey WB** 1996 Expression and localization of prolactin messenger ribonucleic acid in the human immune system. Endocrinology 137:349–353
290. **Montgomery DW, LeFevre JA, Ulrich ED, Adamson CR, Zukoski CF** 1990 Identification of prolactin-like proteins synthesized by normal murine lymphocytes. Endocrinology 127:2601–2603
291. **Montgomery DW, Shen GK, Ulrich ED, Steiner LL, Parrish PR, Zukoski CF** 1992 Human thymocytes express a prolactin-like messenger ribonucleic acid and synthesize bioactive prolactin-like proteins. Endocrinology 131:3019–3026
292. **Maggiano N, Piantelli M, Ricci R, Larocca LM, Capelli A, Ranelletti FO** 1994 Detection of growth hormone-producing cells in human thymus by immunohistochemistry and non-radioactive *in situ* hybridization. J Histochem Cytochem 42:1349–1354
293. **Weigent DA, Blalock JE** 1991 The production of growth hormone by subpopulations of rat mononuclear leukocytes. Cell Immunol 135:55–65
294. **Delhase M, Vergani P, Malur A, Hooghe-Peters EL, Hooghe RJ** 1993 The transcription factor Pit-1/GHF-1 is expressed in hemopoietic and lymphoid tissues. Eur J Immunol 23:951–955
295. **Weigent DA, Blalock JE** 1994 Effect of the administration of growth-hormone-producing lymphocytes on weight gain and immune function in dwarf mice. Neuroimmunomodulation 1:50–58
296. **Kooijman R, Malur A, Van Buul-Offers SC, Hooghe-Peters EL** 1997 Growth hormone expression in murine bone marrow cells is independent of the pituitary transcription factor PIT-1. Endocrinology 138:3949–3955
297. **Batanero E, de Leeuw FE, Jansen GH, van Wichen DF, Huber J, Schuurman HJ** 1992 The neural and neuro-endocrine component of the human thymus. II. Hormone immunoreactivity. Brain Behav Immun 6:249–264
298. **Martin-Fontecha A, Broekhuizen R, de Heer C, Zapata A, Schuurman HJ** 1993 The neuro-endocrine component of the rat thymus: studies on cultured thymic fragments before and after transplantation in congenitally athymic and euthymic rats. Brain Behav Immun 7:1–15
299. **Wajs E, Kutoh E, Gupta D** 1995 Melatonin affects proopiomelanocortin gene expression in the immune organs of the rat. Eur J Endocrinol 133:754–760
300. **Franchini A, Ottaviani E** 1999 Immunoreactive POMC-derived peptides and cytokines in the chicken thymus and bursa of Fabricius microenvironments: age-related changes. J Neuroendocrinol 11:685–692
301. **Lacaze-Masmonteil T, de Keyzer Y, Luton JP, Kahn A, Bertagna X** 1987 Characterization of proopiomelanocortin transcripts in human nonpituitary tissues. Proc Natl Acad Sci USA 84:7261–726
302. **Herbst WM, Kummer W, Hofmann W, Otto H, Heym C** 1987 Carcinoid tumors of the thymus: an immunohistochemical study. Cancer 60:2465–2470
303. **Clarck R, Strasser J, McCabe S, Robbins K, Jardieu P** 1993 Insulin-like growth factor-I stimulation of lymphopoiesis. J Clin Invest 92:540–548
304. **Binz K, Joller P, Froesch P, Binz H, Zapf J, Froesch ER** 1990 Repopulation of the atrophied thymus in diabetic rats by insulin-like growth factor I. Proc Natl Acad Sci USA 87:3690–3694
305. **Yamada M, Hato F, Kinoshita Y, Tominaga K, Tsuji Y** 1994 The indirect participation of growth hormone in the thymocyte proliferation system. Cell Mol Biol (Noisy-le-grand) 40:111–121
306. **Verland S, Gammeltoft S** 1989 Functional receptors for insulin-like growth factors I and II in rat thymocytes and mouse thymoma cells. Mol Cell Endocrinol 67:207–216
307. **Kooijman R, Scholtens LE, Rijkers GT, Zegers BJ** 1995 Type I insulin-like growth factor receptor expression in different developmental stages of human thymocytes. J Endocrinol 147:203–209
308. **Kecha O, Martens H, Franchimont N, Achour I, Hazée-Hagelstein MT, Charlet-Renard C, Geenen V, Winkler R** 1999 Characteriza-

tion of the insulin-like growth factor axis in the human thymus. J Neuroendocrinol 11:435–440
309. **Geenen V, Achour I, Robert F, Vandersmissen E, Sodoyez JC, Defresne MP, Boniver J, Lefebvre PJ, Franchimont P** 1993 Evidence that insulin-like growth factor 2 (IGF2) is the dominant thymic peptide of the insulin superfamily. Thymus 21:115–127
310. **Funk B, Kessler U, Eisenmenger W, Hansmann A, Kolb HJ, Kiess W** 1992 Expression of the insulin-like growth factor-II/mannose-6-phosphate receptor in multiple human tissues during fetal life and early infancy. J Clin Endocrinol Metab 75:424–431
311. **Pugliese A, Zeller M, Fernandez Jr A, Zalcberg LJ, Bartlett RJ, Ricordi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD** 1997 The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. Nat Genet 15:293–297
312. **Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C** 1997 Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet 15:289–292
313. **Throsby M, Homo-Delarche F, Chevenne D, Goya R, Dardenne M, Pleau JM** 1998 Pancreatic hormone expression in the murine thymus: localization in dendritic cells and macrophages. Endocrinology 139:2399–2406
314. **Smith KM, Olson DC, Hirose R, Hanahan D** 1997 Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance. Int Immunol 9:1355–1365
315. **Heath VL, Moore NC, Parnell SM, Mason DW** 1999 Intrathymic expression of genes involved in organ specific autoimmune disease. J Autoimmun 11:309–318
316. **Throsby M, Pleau JM, Dardenne M, Homo-Delarche F** 1999 Thymic expression of the pancreatic endocrine hormones. Neuroimmunomodulation 6:108–114
317. **Geenen V, Legros JJ, Franchimont P, Baudrihaye M, Defresne MP, Boniver J** 1986 The neuroendocrine thymus: coexistence of oxytocin and neurophysin in the human thymus. Science 232: 508–511
318. **Geenen V, Legros JJ, Franchimont P, Defresne MP, Boniver J, Ivell R, Richter D** 1987 The thymus as a neuroendocrine organ: synthesis of vasopressin and oxytocin in human thymic epithelium. Ann NY Acad Sci 496:56–66
319. **Geenen V, Defresne MP, Robert F, Legros JJ, Franchimont P, Boniver J** 1988 The neurohormonal thymic microenvironment: immunocytochemical evidence that thymic nurse cells are neuroendocrine cells. Neuroendocrinology 47:365–368
320. **Robert F, Geenen V, Schoenen J, Burgeon E, De Groote D, Defresne MP, Legros JJ, Franchimont P** 1991 Colocalization of immunoreactive oxytocin, vasopressin and interleukin-1 in human thymic epithelial neuroendocrine cells. Brain Behav Immun 5: 102–115
321. **Moll UM, Lane BL, Robert F, Geenen V, Legros JJ** 1988 The neuroendocrine thymus: abundant occurence of oxytocin, vasopressin and neurophysin-like peptides in epithelial cells. Histochemistry 89:385–398
322. **Wiemann M, Ehret G** 1993 Subcellular localization of immunoreactive oxytocin within thymic epithelial cells of the male mouse. Cell Tissue Res 273:79–87
323. **Azad N, LaPaglia N, Agrawal L, Steiner J, Uddin S, Williams DW, Lawrence AM, Emanuele NV** 1998 The role of gonadectomy and testosterone replacement on thymic luteinizing hormone-releasing hormone production. J Endocrinol 158:229–235
324. **Redei E** 1992 Immunoreactive and bioactive corticotropin-releasing factor in rat thymus. Neuroendocrinology 55:115–118
325. **Aird F, Clevenger CV, Prystowsky MH, Redei E** 1993 Corticotrophin-releasing factor mRNA in rat thymus and spleen. Proc Natl Acad Sci USA 90:7104–7108
326. **Jessop DS, Renshaw D, Lightman SL, Harbuz MS** 1995 Changes in ACTH and β–endorphin immunoreactivity in immune tissues during a chronic inflammatory stress are not correlated with changes in corticotropin-releasing hormone and arginine vasopressin. J Neuroimmunol 60:29–35
327. **Ottaviani E, Franchini A, Franceschi C** 1998 Presence of immunoreactive corticotropin-releasing hormone and cortisol molecules in invertebrate haemocytes and lower and higher vertebrate thymus. Histochem J 30:61–67
328. **Brouxhon SM, Prasad AV, Joseph SA, Felten DL, Bellinger DL** 1998 Localization of corticotropin releasing factor in primary and secondary lymphoid organs of the rat. Brain Behav Immun 12: 107–122
329. **Piantelli M, Maggiano LM, Larocca R, Ricci R, Ranelletti FO, Lauriola L, Capelli A** 1990 Neuropeptide immunoreactive cells in human thymus. Brain Behav Immun 4:189–197
330. **Jessop DS, Lightman SL, Chowdrey HS** 1994 Effects of a chronic inflammatory stress on levels of pro-opiomelanocortin-derived peptides in the rat spleen and thymus. J Neuroimmunol 49:197–203
331. **von Gaudecker B, Steinmann GG, Hansmann ML, Harpprecht J, Milicevic NM, Muller-Hermelink HK** 1986 Immunohistochemical characterization of the thymic microenvironment. A light and ultrastructural immunocytochemical study. Cell Tissue Res 244: 403–412
332. **Zagon IS, Rhodes RE, McLaughlin PJ** 1986 Localization of enkephalin immunoreactivity in diverse tissues and cells of the developing and adult rat. Cell Tissue Res 246:561–565
333. **Rosen H, Bebar O, Abramsky O, Ovadia O** 1989 Regulated expression of pro-enkephalin A in normal lymphocytes. J Immunol 143:3703–3707
334. **Fuller PJ, Verity K** 1989 Somatostatin gene expression in the thymus gland. J Immunol 143:1015–1017
335. **Gomariz RP, Lorenzo MJ, Cacicedo L, Vicente A, Zapata AG** 1990 Demonstration of immunoreactive vasoactive intestinal peptide (IR-VIP) and somatostatin (IR-SOM) in rat thymus. Brain Behav Immun 4:151–161
336. **Delgado M, Martinez C, Leceta J, Gomariz RP** 1999 Vasoactive intestinal peptide in the thymus: synthesis, receptors and biological actions. Neuroimmunomodulation 6:97–107
337. **Pankhaniya R, Jabrane-Ferrat N, Gaufo GO, Sreedharan SP, Dazin P, Kaye J, Goetzl EJ** 1998 Vasoactive intestinal peptide enhancement of antigen-induced differentiation of a cultured line of mouse thymocytes. FASEB J 12:119–127
338. **Martinez C, Delgado M, Abad C, Gomariz RP, Ganea D, Leceta J** 1999 Regulation of VIP production and secretion by murine lymphocytes. J Neuroimmunol 93:126–138
339. **Felten SY, Madden KS, Bellinger DL, Kruszewska B, Moynihan JA, Felten DL** 1998 The role of the sympathetic nervous system in the modulation of immune responses. Adv Pharmacol 42:583–587
340. **Moreno J, Varas A, Vicente A, Zapata AG** 1998 Role of prolactin in the recovered T-cell development of early partially decapitated chicken embryo. Dev Immunol 5:183–195
341. **Velkeniers B, Dogusan Z, Naessens F, Hooghe R, Hooghe-Peters EL** 1998 Prolactin, growth hormone and the immune system in humans. Cell Mol Life Sci 54:1102–1108
342. **LeRoith D, Yanowski J, Kaldjian EP, Jaffe ES, LeRoith T, Purdue K, Cooper BD, Pyle R, Adler W** 1996 The effects of growth hormone and insulin-like growth factor I on the immune system of aged female monkeys. Endocrinology 137:1071–1079
343. **Bach JF, Dardenne M, Pléau JM, Rosa J** 1977 Biochemical characterization of a serum thymic factor. Nature 266:55–56
344. **Cung MT, Marraud M, Lefrancier P, Dardenne M, Bach JF, Lausac JP** 1988 NMR study of a lymphocyte differentiating thymic factor. An investigation of the Zn(II)-nonapeptide complexes (thymulin). J Biol Chem 263:5574–5580
345. **Savino W, Huang PC, Corrigan A, Berrih S, Dardenne M** 1984 Thymic hormone-containing cells. V. Immunohistological detection of metallothionein within the cells bearing thymulin (a zinc-containing hormone) in human and mouse thymuses. J Histochem Cytochem 32:942–946
346. **Savino W, Dardenne M** 1986 Thymic hormone-containing cells. VIII. Effects of colchicine, cytochalasin B and monensin on secretion of thymulin by cultured human thymic epithelial cells. J Histochem Cytochem 34:1719–1723
347. **Buckingham JC, Safieh B, Singh S, Arduino LA, Cover PO, Kendall MD** 1992 Interactions between the hypothalamo-pituitary adrenal axis and the thymus in the rat: a role for corticotrophin in the control of thymulin release. J Neuroendocrinol 4:295–301
348. **Saha AR, Hadden EM, Hadden JW** 1995 Zinc induces thymulin secretion from human thymic epithelial cells *in vitro* and augments

splenocyte and thymocyte responses *in vivo*. Int J Immunopharmacol 17:729–733
349. **Pléau JM, Fuentes V, Morgat JM, Bach JF** 1980 Specific receptors for the serum thymic factor (FTS) in lymphoblastoid cultured cell lines. Proc Natl Acad Sci USA 77:2861–2865
350. **Gastinel LN, Pléau JM, Dardenne M, Sasaki A, Bricas E, Morgat JL, Bach JF** 1982 High affinity binding sites on plasma membrane obtained from the lymphoblastoid cultured 1301 cell line for highly radioactive serum thymic factor. Biochim Biophys Acta 684: 117–126
351. **Bach JF, Dardenne M, Pleau JM, Bach MA** 1975 Isolation biochemical characteristics and biological activity of a circulating thymic hormone in the mouse and in the human. Ann NY Acad Sci 249:186–210
352. **Bene MC, Faure G, Bordigoni P, Olive D, Duheille J** 1982 *In vitro* induction of monoclonal antibody-defined T cell markers in lymphocytes from immunodeficient children by synthetic serum thymic factor (FTS). Clin Exp Immunol 48:423–428
353. **Chang WP, Marsh JA** 1993 The effect of synthetic thymulin on cell surface marker expression by avian T –cell precursors. Dev Comp Immunol 17:85–96
354. **Palacios R, Fernandez C, Sideras P** 1982 Development and continuous growth in culture of interleukin-2 producer lymphocytes from athymic nu/nu mice. Eur J Immunol 12:777–782
355. **Bordigoni P, Faure G, Bene MC, Dardenne M, Bach JF, Duheille J, Olive D** 1982 Improvement of cellular immunity and IgA production in immunodeficient children after treatment with synthetic serum thymic factor (FTS). Lancet 2:293–297
356. **Inagaki-Ohara K, Kobayashi N, Nishimura H, Sakai T, Matsumoto Y, Hiromatsu K, Awaya A, Yoshikai Y** 1996 Effects of a nonapeptide thymic hormone on intestinal intraepithelial lymphocytes in mice following administration of 5-fluorouracil. Cell Immunol 171:30–40
357. **Matsumoto T, Nakano M** 1980 Non-specific stimulation of anti bacterial resistance by a synthetic thymic factor (FTS) in mice. Microbiol Immunol 24:1185–1197
358. **Matsumoto T, Shibata M** 1982 Activation of polyclonal antibody responses by a synthetic serum thymic factor (FTS) in CBA/N mice. Immunology 45:293–301
359. **Bardos P, Bach JF** 1982 Modulation of mouse natural killer cell activity by the serum thymic factor (FTS). Scand J Immunol 16: 321–325
360. **Kaiserlian D, Bardos P, Bach JF** 1983 *In vitro* modulation of mouse natural killer (NK) cell activity by the serum thymic factor (FTS). Cell Immunol 76:232–242
361. **Muzzioli M, Mocchegiani E, Bressani N, Bevilacqua P, Fabris N** 1992 *In vitro* restoration by thymulin of NK activity of cells from old mice. Int J Immunopharmacol 14:57–61
362. **Kaiserlian D, Dujic A, Dardenne M, Bach JF, Blanot D, Bricas E** 1981 Prolongation of murine skin grafts by FTS and its synthetic analogues. Clin Exp Immunol 45:338–343
363. **Israel-Biet D, Noel LH, Bach MA, Dardenne M, Bach JF** 1983 Marked reduction of DNA antibody production and glomerulopathy in thymulin (FTS-Zn) or cyclosporin A treated (NZB × NZW) F1 mice. Clin Exp Immunol 54:359–365
364. **Faure G, Bene MC, Tamisier JN, Gaucher A, Duheille J** 1984 Thymulin (FTS-Zn) induced *in vitro* modulation of T cell subsets markers on lymphocytes from rheumatoid arthritis and systemic lupus erythematosus patients. Int J Immunopharmacol 6:381–388
365. **Mizutani M, El-Fotoh M, Mori M, Ono K, Doi K, Awaya A, Matsumoto Y, Matsumoto Y, Onodera T** 1996 *In vivo* administration of serum thymic factor (FTS) prevents EMC-D virus induced diabetes and myocarditis in BALB/cAJcl mice. Arch Virol 141: 73–83
366. **Bordigoni P, Bene MC, Faure G, Donner M, Janot C, Dardenne M, Duheille J, Olive D** 1984 Allogeneic bone marrow transplantation in man: *in vitro* activity of FTS-Zn on T-cell markers and functions. Br J Haematol 58:661–669
367. **Parent G, Chevalier P, Zalles L, Sevilla R, Bustos M, Dhenin JM, Jambon B** 1994 *In vitro* lymphocyte-differentiating effects of thymulin (Zn-FTS) on lymphocyte subpopulations of severely malnourished children. Am J Clin Nutr 60:274–278
368. **Okamoto M, Morishita M, Setoguchi C, Nakata K** 1993 Restorative effect of short term administration of thymulin on thymus-dependent antibody production in restraint-stressed mice. Int J Immunopharmacol 15:757–762
369. **Barbour EK, Hamadeh SK, Ghanem DA, Haddad JJ, Safieh-Garabedian B** 1998 Humoral and cell-mediated immunopotentiation in vaccinated chicken layers by thymic hormones and zinc. Vaccine 16:1650–1655
370. **Aono H, Morishita M, Sasano M, Okamoto M, Okahara A, Nakata K, Mita S** 1997 Amelioration of type II collagen induced arthritis in rats by treatment with thymulin. J Rheum 24:1564–1569