Experimental Physiology – *Symposium Report*

Neuroendocrine control of T cell development in mammals: role of growth hormone in modulating thymocyte migration

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The thymus gland is a primary lymphoid organ, in which bone-marrow-derived T cell precursors undergo differentiation, eventually leading to migration of positively selected cells to the peripheral lymphoid organs. This differentiation occurs along with cell migration in the context of the thymic microenvironment, a three-dimensional network formed by epithelial cells, macrophages, dendritic cells, fibroblasts and extracellular matrix components. A series of data clearly shows that growth hormone (GH) pleiotropically modulates thymic functions. For example, GH upregulates proliferation of thymocytes and thymic epithelial cells. Accordingly, GH-transgenic mice, as well as animals and humans treated with exogenous GH, exhibit an enhanced cellularity in the organ. Growth hormone stimulates the secretion of thymic hormones, cytokines and chemokines by the thymic microenvironment, as well as the production of extracellular matrix proteins, leading to an increase in thymocyte migratory responses and intrathymic traffic of developing T cells. In addition, GH stimulates the *in vivo* export of thymocytes from the organ, as ascertained by studies with intrathymic injection of GH in normal mice and with GH-transgenic mice. Moreover, since GH is produced by thymocytes and thymic epithelial cells, which express GH receptors, we should consider that, in addition to the classic endocrine pathway, the GH control of the thymus may include an autocrine/paracrine pathway. Finally, since GH promotes a replenishment of the thymus and an increase of thymocyte export, it could be envisioned as a potential adjuvant therapeutic agent in the treatment of immunodeficiencies associated with thymic atrophy.

(Received 15 May 2007; accepted after revision 3 July 2007; first published online 15 August 2007)
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It is largely accepted that growth hormone (GH) exerts a regulatory role in the immune system (reviewed by Meazza *et al.* 2004). In particular, this review focuses on the role of GH in the physiology of the thymus, more particularly the migration of developing thymocytes. Before going into details on this issue, a brief background on basic thymus physiology is provided, in terms of T cell development in the context of the thymic microenvironment.

T cell differentiation and the thymic microenvironment

The thymus is a primary lymphoid organ, in which bone-marrow-derived T cell precursors undergo differentiation, ultimately leading to migration of positively selected thymocytes to the T cell-dependent areas of peripheral lymphoid organs. This process involves expression of various proteins and rearrangement of the T cell receptor (TCR) genes. Most immature thymocytes express neither TCR nor CD4 or CD8 molecules and are called double-negative ($CD4^-CD8^-$) cells, representing 5% of total thymic lymphocytes. Maturation progresses with the acquisition of CD4 and CD8 markers, generating the $CD4^+CD8^+$ cells, which comprise 80% of the whole population. At this stage, TCR genes are completely rearranged, and productive rearrangements result in TCR expression on the cell membrane. Thymocytes that do not undergo productive TCR gene rearrangement die by apoptosis, whereas those expressing productive TCR will interact with peptides presented by molecules of the major histocompatibility complex (MHC), expressed

 DOI: 10.1113/expphysiol.2007.038422

by microenvironmental cells. This interaction determines the positive and negative selection events, crucial for normal thymocyte differentiation. Positively selected thymocytes progress to the mature $TCR^+CD4^+CD8^-$ or $TCR^+CD4^-CD8^+$ single positive stage, comprising 15% of thymocytes, which ultimately leave the organ to form the peripheral T cell repertoire (reviewed by Savino & Dardenne, 2000; Anderson *et al.* 2006; Ladi *et al.* 2006).

In addition to the TCR/peptide–MHC interaction, the thymic microenvironment influences thymocyte maturation via adhesion molecules and extracellular matrix (ECM); interactions that are relevant for thymocyte migration (Savino *et al.* 2000). Moreover, the thymic microenvironmental cells secrete soluble polypeptides, including cytokines, such as interleukin 1 (IL-1), IL-3, IL-6 and IL-7; various chemokines, including chemokine CX ligand 12 (CXCL12); and thymic hormones (Boehm & Bleul, 2006). These molecules also participate in the general process of thymocyte differentiation.

The thymic epithelial cell (TEC) network is the major component of the thymic microenvironment, being responsible for positive selection of thymocytes. It also produces cytokines, chemokines and ECM (Savino & Dardenne, 2000). It is a heterogeneous tissue, and cells in different locations within the thymic lobules may be related to specific steps in T cell maturation. One lymphoepithelial complex, the thymic nurse cell (TNC), has been isolated, being formed by one TEC that harbours variable amounts of maturing thymocytes, located in the cortical region of thymic lobules. Once settled in culture, TNCs spontaneously release thymocytes, and TNC-derived epithelial cells can reconstitute lymphoepithelial complexes after being co-cultured with immature thymocytes. These findings place TNCs as an *in vitro* model of thymocyte migration within the TEC context (Savino *et al.* 2002*a*).

Thymocyte differentiation occurs as cells migrate within the thymic lobules. Immature $TCR^-CD4^-CD8^-$ and $TCR^+CD4^+CD8^+$ cells are cortically located, whereas mature $TCR^+CD4^+CD8^-$ and $TCR^+CD4^-CD8^+$ lymphocytes are found in the medulla. Such migration is complex, being controlled by several molecular interactions, including those mediated by ECM, chemokines and semaphorins (Savino *et al.* 2002*a*; 2004; Takahama, 2006; Lepelletier *et al.* 2007).

Interestingly, a number of studies clearly revealed that distinct aspects related to the thymic microenvironment and thymocyte development are modulated by GH.

Growth hormone is a pleiotropic modulator of thymus physiology

Previous studies demonstrated that GH modulates several functions, including thymocyte proliferation and migration, as well as secretion of ECM, chemokines and thymic hormones by TEC. For example, in recent studies we evaluated *in vivo* the effects of high levels of GH in the thymus by using two distinct approaches: intrathymic injection of GH in BALB/c mice and GH-transgenic mice. In both conditions, thymocyte numbers were higher than in the corresponding control animals. This is in keeping with the data obtained with GH-injected mice, which presented 10–15% more cells in the Synthesis + G2 mitosis (S+G2M) phases, compared with denatured GH-injected animals (Smaniotto *et al.* 2004, 2005).

Other functions of GH in the thymus are described in reviews published in the last few years (Welniak *et al.* 2002; Savino *et al.* 2002*b*, 2003). The present review focuses on the role of GH in modulating thymocyte migration.

Growth hormone upregulates thymocyte migration via ECM-mediated interactions

It was demonstrated that treatment of cultured TEC with GH yielded an increase in the amounts of fibronectin and laminin, and in the expression of their corresponding receptors, VLA-5 and VLA-6 (de Mello-Coelho *et al.* 1997).

Since thymocyte/TEC adhesion is partly mediated by ECM (Savino *et al.* 2000), it was tested whether GH was able to modulate this interaction. In fact, thymocyte adhesion to cultured TEC is enhanced by the hormone, an effect that can be abrogated by anti-GH or anti-GH receptor antibodies. This effect could be prevented in TEC cultures pretreated with anti-ECM or anti-ECM receptor antibodies (de Mello-Coelho *et al.* 1997).

Together, these data indicate that GH influences ECM-mediated TEC/thymocyte adhesion, which is crucial for intrathymic T cell traffic.

Growth hormone enhances the entrance of T cell precursors into the thymus

Although few studies addressed the issue of a neuroendocrine control of T cell precursors into the thymus, it was demonstrated that GH increases human T cell engraftment into the thymus of severe combined immunodeficiency syndrome (SCID) mice. This influence is mediated by adhesion molecules, since it can be abrogated with anti-$\beta1$ or anti-$\beta2$ integrin antibodies, and GH-treated cells exhibit an increase in adhesion to vascular cell adhesion molecule (VCAM-1), fibronectin and intracellular adhesion molecule (ICAM-1) (Taub *et al.* 1994). This is in keeping with data showing that the entrance of bone-marrow-derived progenitors into the organ is partly mediated by the VCAM-1/fibronectin receptor, the integrin $\alpha4\beta1$ (Scimone *et al.* 2006).

Modulation of thymocyte traffic in thymic nurse cells

Since thymocyte traffic in the context of thymic nurse cells is mediated by ECM (Villa-Verde *et al.* 1994), and given the fact that GH is able to enhance ECM production by TEC, we investigated whether GH could modulate intra-TNC thymocyte traffic. Compared with control cells, GH significantly accelerates thymocyte release from TNCs and enhances the entrance of thymocytes to reconstitute new lymphoepithelial complexes. Interestingly, insulin-like growth factor 1 (IGF-1) promotes a similar effect (de Mello-Coelho *et al.* 1997).

More recently, we noticed that the production of laminin by TNCs derived from GH-transgenic mice is increased compared wild-type control mice. Accordingly, thymocyte release from TNCs was also faster in GH-transgenic animals (Smaniotto *et al.* 2005).

Growth hormone stimulates thymocyte migration by a combined effect of laminin and CXCL12

The effects of GH upon thymocyte migration were also approached *ex vivo* by using transwell migration chambers (reviewed by Savino *et al.* 2002*b*). We evaluated the effects of intrathymic high levels of GH in two distinct models: intrathymic injection of GH in BALB/c mice; and GH-transgenic mice. We showed that thymocyte adhesion to laminin is higher in GH-treated and in GH-transgenic animals compared with control mice. An enhancing effect was also observed in relation to the numbers of migrating cells in laminin-coated transwells (Smaniotto *et al.* 2005). Interestingly, the membrane expression of the laminin receptor VLA-6 on thymocytes from GH-injected or GH-transgenic mice does not change significantly (Smaniotto *et al.* 2005), indicating that the activation levels of this integrin are higher when GH contents are high. Interestingly, fibronectin-driven migration remained unchanged in the presence of high GH levels.

Considering that intrathymic laminin deposition is also enhanced *in vivo* upon GH stimulation, it is likely that hyper-responsive thymocytes, encountering higher amounts of laminin, accelerate their traffic within the organ.

Growth hormone also modulates other interactions related to thymocyte migration. In fact, thymocyte

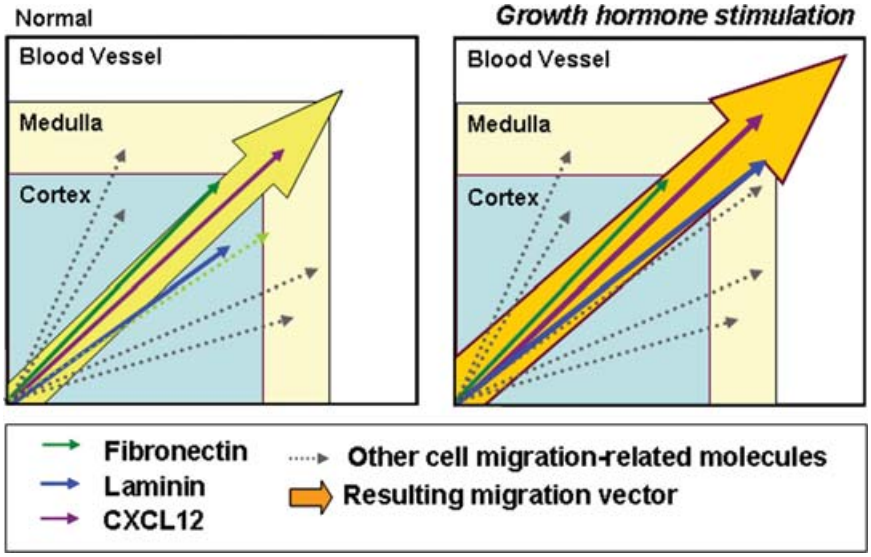

Figure 1. Thymocyte migration is upregulated by growth hormone
This figure illustrates how GH can modulate intrathymic T cell migration, and it is based on a multivectorial model to describe thymocyte migration. Accordingly, intrathymic T cell migration results from a balance of several interactions involving cell migration modulators and respective receptors. Once assembled, these individual vectors form a resulting vector that leads thymocytes throughout the thymic lobules, from the cortex towards the medulla, with mature subsets ultimately being exported from the organ through the blood vessel walls. This pattern can be altered by acute and chronic high levels of growth hormone, exemplified *in vivo* by intrathymic injection of GH into normal mice and by GH-transgenic mice, respectively. Narrow arrows represent individual migration vectors induced by specific stimuli, such as fibronectin (green), laminin (blue), CXCL12 (violet) and other molecules (grey). Large arrows represent the resulting migration vector in control *versus* GH-transgenic mice (yellow and orange, respectively). Compared with control animals, thymocyte migration in GH-transgenic (or GH-treated) mice is altered concerning the interactions of at least two of the molecules analysed, namely laminin and CXCL12. Such changes are related to an increased thymocyte migration, with larger numbers of cells being exported from the organ to lymph nodes.

migratory response triggered by the chemokine CXCL12 is higher in GH-transgenic and GH-treated mice compared with control animals, although the membrane levels of CXCR4 remained unchanged in both transgenic and wild-type animals (Smaniotto *et al.* 2005). These data suggest that CXCR4 is spontaneously activated in GH-transgenic thymocytes.

We then evaluated whether GH could enhance the combined effects of laminin plus CXCL12. When both molecules were applied, the numbers of migrating cells were higher than the summation of each stimulus alone, an effect that essentially targets $CD4^+CD8^+$ cells.

Conjointly, these data indicate that the enhanced thymocyte migration seen in GH-transgenic and in GH-injected mice results at least partly from a combined action of laminin and CXCL12. Taking into account that thymocyte migration appears to result from various molecular interactions, it is possible that GH modulates the expression of other cell migration-related molecules that are involved in the generation of a resulting migration vector. Such a concept is described schematically in Fig. 1.

Growth hormone modulates thymocyte export

One direct strategy to evaluate thymocyte export is the evaluation of recent thymic emigrants (RTEs). It is well established that intrathymic injection of fluorescein isothiocyanate (FITC) randomly labels thymocyte cell membranes. This allows the recovery of the $FITC^+$ cells that have recently been exported from the thymus (Savino *et al.* 2002*a*, 2004). Following intrathymic injection of normal mice with GH in combination with FITC, we found a significant increase in the percentages of $CD4^+CD8^-FITC^+$ cells in the lymph nodes. Rather similar data were obtained when FITC was injected within the thymus of GH-transgenic animals (Smaniotto *et al.* 2004, 2005).

These data are in keeping with previous reports showing an increase in human RTEs in the blood of Acquired immunodeficiency syndrome (AIDS) patients who received GH in conjunction with specific antiretrovirus therapy (Napolitano *et al.* 2002). With respect to AIDS patients, we reported a severe thymic atrophy (Savino *et al.* 1986), whereas other researchers showed a decrease in GH production (Barbey-Morel *et al.* 2002). Accordingly, long-term GH treatment augmented thymus volume concomitantly with an enhanced export of $CD4^+$ T cells (Napolitano *et al.* 2002).

Is there an autocrine/paracrine circuitry in the thymus involving local GH and IGF-1 production?

In addition to the endocrine effects of GH upon the thymus, we should consider the possibility of an autocrine/paracrine GH-dependent pathway. As expected, the expression of GH receptors by thymocytes and microenvironmental cells has been demonstrated by various methods. Moreover, we and others showed that GH itself is produced by thymocytes and thymic epithelial cells (reviewed by Savino & Dardenne, 2000).

The effects of GH within the thymus are mediated by an IGF-1–IGF-1 receptor circuitry. Not only can the effects of GH be seen by the use of IGF-1, but they can be abrogated by anti-IGF-1 or anti-IGF-1 receptor antibodies (de Mello-Coelho *et al.* 1997). Moreover, GH enhances the expression of IGF-1 and IGF-1 receptor by cultured thymic epithelium (de Mello-Coelho *et al.* 2002), which is in keeping with the fact that GH-transgenic mice have high levels of circulating IGF-1 (Sotelo *et al.* 1998). Also, it was demonstrated that administration of IGF-1 together with bone marrow cells resulted in an increase in thymus cellularity, compared with transfer of bone marrow cells alone, and that IGF-1 potentiates the colonization of fetal thymus organ cultures with T cell precursors (Montecino-Rodriguez *et al.* 1998), indicating that IGF-1 also enhances the entrance of cell precursors into the thymus.

Taken together, these suggest an autocrine/paracrine circuitry, involving the intrathymic production of GH and IGF-1.

Conclusions

It has been demonstrated that GH exerts a pleiotropic role upon the thymus. In addition to enhancing proliferation of thymic cells, this hormone upregulates cytokine production by the thymic microenvironment and increases the ECM/chemokine-driven intrathymic T cell traffic, as well as thymocyte export. In this respect, the effects of GH appear to occur via distinct molecular interactions, involving at least laminin and CXCL12, with their corresponding receptors. Since GH promotes a replenishment of the thymus and increases intrathymic T cell traffic, ultimately modulating thymocyte export, it could be envisioned as a potential adjuvant therapeutic agent in the treatment of immunodeficiencies that are associated with thymic atrophy.

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Acknowledgements

This work was funded by Fiocruz, CNPq and Faperj (Brazil), being developed in the context of the Franco-Brazilian International Laboratory of Immunology-Immunopathology, by Fiocruz (Brazil) and CNRS (France).