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Growth hormone modulates migration of thymocytes and peripheral T cells

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In the context of immunoneuroendocrine cross talk, growth hormone (GH) exerts pleiotropic effects in the immune system. For example, GH-transgenic mice, as well as animals and humans treated with GH, exhibit enhanced cellularity in the thymus. GH also stimulates the thymic microenvironment, augmenting chemokine and extracellular matrix (ECM) production, with consequent increase in ECM- and chemokine-driven thymocyte migratory responses. Peripheral T cell migration triggered by laminin or fibronectin was enhanced in cells from GH-transgenic versus wild-type control adult mice, as seen for $CD4^+$ and $CD8^+$ T cells from mesenteric lymph nodes. Migration of these T lymphocytes, triggered by the chemokine CXCL12, in conjunction with laminin or fibronectin, was also enhanced compared with control counterparts. Considering that GH can be used as an adjuvant therapy in immunodeficiencies, including AIDS, the concepts defined herein, that GH enhances developing and peripheral T cell migration, provide new clues for future GH-related immune interventions.

Keywords: Growth hormone; thymocytes; cell migration; integrins; chemokines; lymph nodes

Introduction

In the context of crosstalk between the neuroendocrine and immune systems, growth hormone (GH) exerts pleiotropic effects in central as well as peripheral compartments of the immune system.[1,2] For example, in the thymus, a primary lymphoid organ where T cells differentiate, GH upregulates proliferation of distinct cell types, such as 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.[3,4] Conversely, there is a severe thymic atrophy in GH receptor-deficient mice.[5] GH also stimulates the secretion of thymic hormones, cytokines, and chemokines by the thymic microenvironment, as well as the production of extracellular matrix (ECM) proteins, including laminin and fibronectin.[1,3] Importantly, these effects are largely mediated by an IGF-1/IGF-1 receptor interaction.[6] Herein, we will summarize recent data on the role of GH upon the migration of thymic and peripheral T lymphocytes.

Migration of developing T lymphocytes is enhanced by GH

From the entry of bone marrow-derived T cell precursors, to the exportation of mature T cells from the thymus to the periphery of the immune system, migration of developing T cells is a key process for normal thymopoiesis.[7]

T cells develop in the thymus from bone marrow–derived precursors that continuously seed the organ via blood vessels. Interestingly, it has been demonstrated in a xenogeneic model that GH stimulates the chemotaxis and the adhesion of human peripheral T cells into the thymus of severe combined immunodeficiency mice, an event partially mediated by integrin-type cell adhesion receptors.[8]

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Within the thymus, the journey of developing thymocytes is controlled by various cell migration-related ligand/receptor pairs, such as those involving ECM proteins and chemokines.[9,10] Accordingly, we postulated that the oriented cell movement of developing thymocytes is a multivectorial process in which each vector corresponds to a given ligand/receptor pair interaction that contributes to the global biological event of cell migration.[11]

Since intratissue lymphocyte migration depends on sequential events of cell adhesion/de-adhesion, we tested whether GH could modulate adhesion of thymocytes to cultured thymic epithelial cells (TEC), the major component of the thymic microenvironmental tridimensional network, which supports the general process of intrathymic T cell differentiation.[12] Treatment of cultured human TEC with GH resulted in increase of thymocyte/TEC adhesion, and the effect was due to the enhancement of the amounts of fibronectin and laminin, together with the expression of their corresponding receptors, VLA-5 and VLA-6, on TEC membranes.[13] Accordingly, when we treated growing TEC with neutralizing antibodies either to ECM ligands or to the corresponding integrin-type receptors, we were able to abrogate the enhancement of thymocyte adhesion to TEC monolayers. In the same vein, the production of laminin by thymic nurse cells (TNCs, special lymphoepithelial niches in the outer cortex of the thymic lobules) derived from GH-transgenic mice was also increased compared with wild-type controls, and thymocyte release from TNCs was faster in GH-transgenic animals.[3] Moreover, we found that laminin deposition was enhanced in GH-treated normal mice and in GH-transgenic animals, compared with respective controls.[3,14] Nevertheless, it remains to be determined whether the production of various laminin isoforms is upregulated by GH, or if such an effect is restricted to a given isoform. In any case, the responsiveness of thymocytes to laminin-111 is apparent. We showed that in GH-transgenic mice thymocyte adhesion to laminin was higher than in control mice, and an enhancing effect was also observed *ex vivo* when thymocytes were allowed to migrate through laminin-111–coated transwell chambers. The specificity of these effects was demonstrated by showing that they could be blocked with an anti-CD49f monoclonal antibody, which recognizes the alpha-6 chain of the integrin-type laminin receptor VLA-6.[3,14] Interestingly, membrane expression of VLA-6 (which recognizes distinct laminin isoforms) on

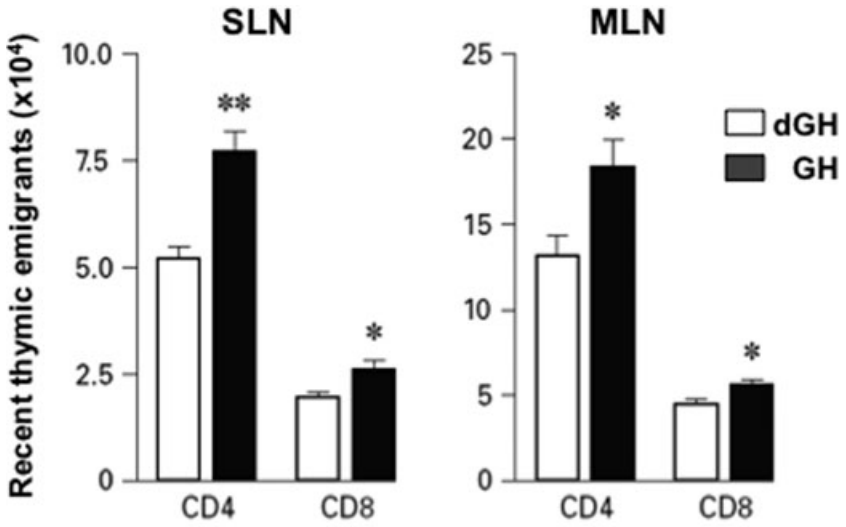

Figure 1. GH treatment enhances *in vivo* homing of $CD4^+$ T cells to lymph nodes. The graphics reveal that normal mice intrathymically treated with GH exhibit more $CD4^+$ and $CD8^+$ recent thymic emigrants in both subcutaneous (SLN) and mesenteric (MLN) lymph nodes, compared with controls injected with heat-induced denaturated growth hormone (dGH). In both cases, recent thymic emigrants were defined by cytofluorometry following intrathymic injection of fluoresceinisothiocyanate, harvest of lymph node-derived cells and labeling with anti-CD4 or anti-CD8 fluorochrome-labeled monoclonal antibodies. Statistical significance: $^*P < 0.05$; $^{**}P < 0.01$. Modified from Ref. 27.

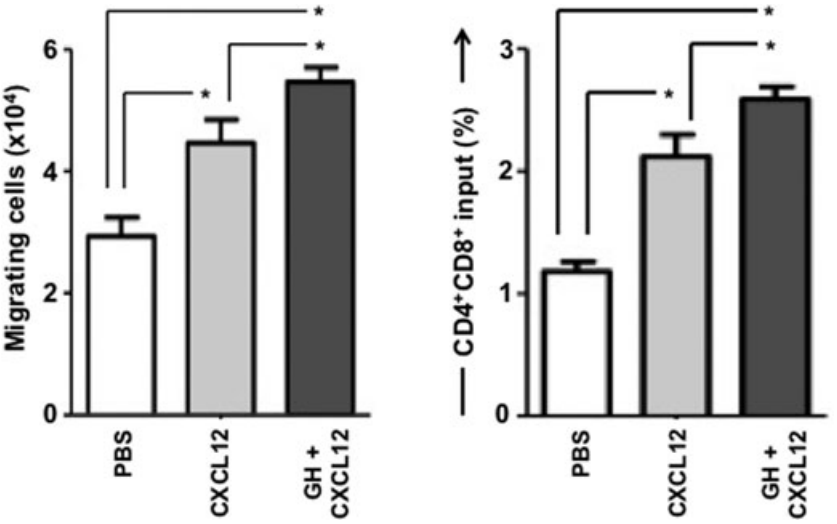

Figure 2. **GH enhances CXCL12-induced transendothelial migration of thymocytes. The left panel depicts the absolute numbers of thymocytes that migrated in each experimental condition. The right panel shows the percentage of input for each CD4/CD8-defined thymocyte subset. Transendothelial thymocyte migration was performed in transwell culture inserts. For that, a murine thymic endothelial cell line was cultured onto the inserts of the transwell chambers for 48 hours. Freshly isolated thymocytes (pretreated or not with GH 10^{-8} M for 1 h were then added to thymic endothelial cell monolayer. CXCL12 (100 ng/mL) was applied in the bottom of the transwell chambers. Two million thymocytes were allowed to migrate for 18 h, and migrating cells were harvested in the bottom of the transwell chambers, counted, and phenotyped by flow cytometry. Each bar represents the mean ± standard error of one of three independent experiments. $^{*}P < 0.05$.**

thymocytes from GH-transgenic mice did not change significantly, indicating that GH enhances the activation levels of this integrin rather than increases corresponding gene expression.

Since thymocyte migration is also influenced by chemokines—CXCL12 being one major component—[7,9] we sought to determine whether GH could modulate intrathymic CXCL12-driven migration. In fact, when CXCL12 was applied, thymocyte migration was consistently higher in GH-transgenic mice, compared with age-matched wild-type control animals. This response was blocked by *B. pertussis* toxin, which is known to inhibit various G-protein coupled chemokine receptors. Moreover, we found that when CXCL12 was applied in conjunction with laminin-111, there was a synergic enhancing effect upon migration of GH-transgenic mouse-derived thymocytes. Of note, an increase in CXCL12 production was seen in TEC from GH-transgenic animals, compared with wild-type counterparts, both *in situ* and *in vitro*, as defined at the mRNA and protein levels.[3]

Thymocyte export also seems to be upregulated by GH. We recently evaluated transendothelial migration of GH-treated thymocytes freshly isolated from normal C57BL/6 mice, in the presence of the chemokine CXCL12 (Fig. 1). When we pretreated thymocytes with GH and led them to migrate toward CXCL12, we observed an increase in the numbers of migrating thymocytes—in particular, those bearing the $CD4^+CD8^+$ phenotype—when compared with untreated thymocytes.[4] In addition, *in vivo* experiments showed that GH favors the trafficking of naive $CD4^+CD8^-$ recent thymic emigrants to the peripheral lymph nodes, as could be demonstrated in both normal mice treated with GH intrathymically and in GH-transgenic mice,[3,14] as illustrated in Figure 2. Moreover, in acromegalic patients there is an increase in the relative numbers of circulating $CD4^+$ T lymphocytes, compared with age-matched healthy individuals.[15] Of note, an increase in the export of $CD4^+$ T lymphocytes from the thymus was seen in a cohort of HIV-infected patients that received GH as an adjuvant treatment to highly active antiretroviral therapy.[16,17] Accordingly, in these subjects the thymus significantly increased in size, as ascertained *in vivo*.[16]

It has been shown that HIV-positive children with GH-deficiency exhibit diminished thymus volume and reduced circulating $CD4^+$ T cells,

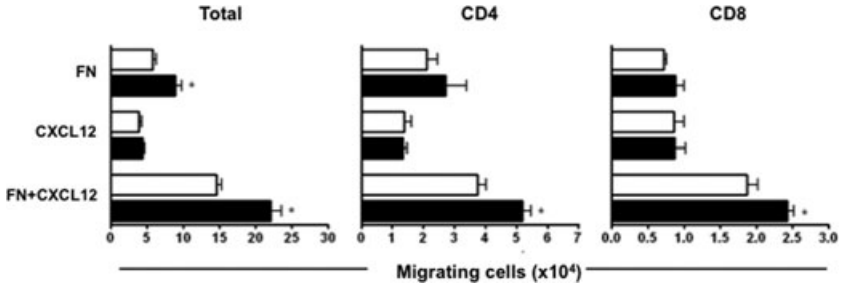

Figure 3. Enhanced migration of mesenteric lymph node derived T cells from GH-transgenic mice. Migration values correspond to the numbers of cells that migrated toward each specific stimulus (fibronectin, CXCL12, or fibronectin combined with CXCL12) subtracted from the values obtained when cells were led to migrate through BSA, applied as an unrelated protein. Experiments were done using two- to three-month-old mice, with at least five animals evaluated per group. The unpaired Student's *t*-test was applied for statistical analyses. Results are expressed as mean ± SEM, and differences between wild-type and transgenic groups were considered statistically significant when $^{*}P < 0.05$.

compared with HIV-positive children without GH deficiency.[18] Taken together, these data show that replenishment of the peripheral pool of $CD4^{+}$ T lymphocytes can be enhanced by GH-based therapy.

Also, recent data derived from a randomized, placebo-controlled, double-blind study revealed that low-dose GH therapy in highly active antiretroviral therapy (HAART)-treated HIV patients (daily injections of 0.7 mg GH) promoted an increase in thymic emigrants, compared with the placebo group.[19] Moreover, the frequency of recent thymic emigrants, labeled by the detection of T cell receptor excision circles (TREC), and total TREC content significantly increased in the GH group, compared with the placebo group.[19]

GH enhances ECM- and chemokine-driven migratory responses of peripheral T lymphocytes

We recently showed that young and middle-aged GH-Tg mice exhibit higher numbers of B and T lymphocytes in lymph nodes and spleens, compared with wild-type age-matched controls.[20] Similarly, it has been demonstrated that ghrelin, a neuropeptide having a strong GH-releasing activity, induces proliferation of peripheral T lymphocytes.[21] Functionally, we found that migration of T lymphocytes from mesenteric lymph nodes of GH-Tg mice, triggered by the chemokine CXCL12 in conjunction with laminin or fibronectin, was enhanced compared with lymphocytes from wild-type age-matched counterparts[20] (Fig. 3). Since such effects were not correlated with the membrane densities of the corresponding receptors, these findings indicate that GH enhances the activation state of cell migration–related receptors. It should be noted that the contents of CXCL12, fibronectin, and laminin in peripheral lymphoid organs from GH-transgenic animals were higher than what was found in corresponding control animals, as illustrated in Figure 4. Considering that chemokines attached to the extracellular matrix appear to be better presented to lymphocytes, we can conceive that the efficiency of ligand/receptor pair ligation is higher in a given microenvironment simultaneously enriched in chemokines attached to an enhanced ECM-containing network. These findings indicate that within the lymphoid organs of GH-transgenic mice, T cells should migrate more rapidly, leading to a more rapid recirculation of these lymphocytes.

We also evaluated chemotactic migratory responses driven by the chemokine CCL21, largely known to stimulate migration of peripheral T lymphocytes. We found that CCL21-driven migration of spleen-derived T lymphocytes from GH-transgenic mice, triggered by the chemokine CCL21, was enhanced compared with lymphocytes from control mice. Importantly, such an enhancing effect was even higher when the chemokine was applied in conjunction with the ECM proteins fibronectin or laminin.[20]

Conceptually, these data tell us that the GH-induced increase of migratory responses of lymphocytes in peripheral lymphoid organs is at least partially due to a combined action of selected ECM components and chemokines, thus similar to what we had previously demonstrated for thymocytes.[8,9]

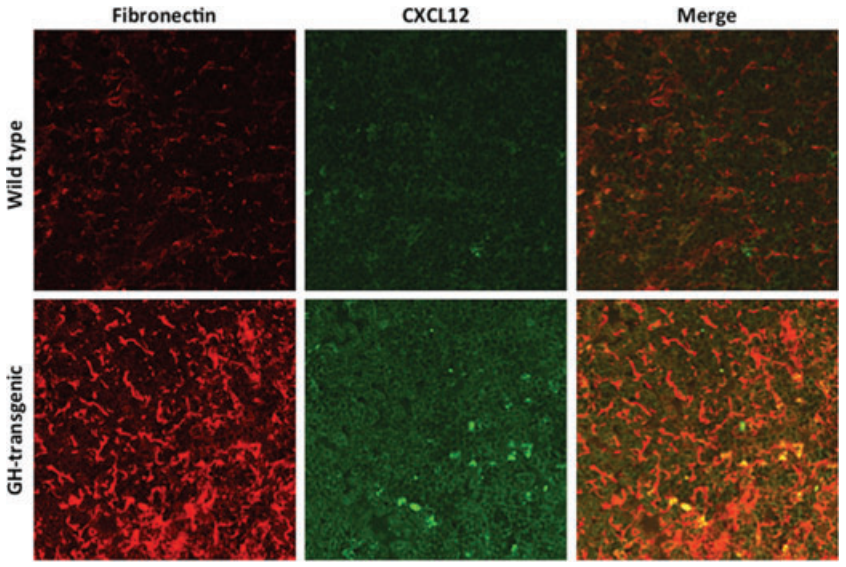

Figure 4. Enhanced deposition of fibronectin and CXCL12 in mesenteric lymph nodes from GH-transgenic mice. The deposition of fibronectin (in red) and CXCL12 (in green) in mesenteric lymph nodes from wild-type and GH-transgenic mice were analyzed by confocal microscopy. Magnification: 400×. Experiments were done using 2- to 3-month-old mice, with three animals being evaluated per group.

In this respect, such data highlight the possibility that the multivectorial concept of cell migration, initially postulated for thymocytes, can be applied for migration of peripheral T lymphocytes within lymphoid tissues, as well as sites of immunological activity. Moreover, the *ex vivo* cell migration data, using fixed concentrations of CXCL12, together with the fact that the presence of such chemokines is actually enhanced within the lymphoid organs suggest that the real *in vivo* effect is likely more important than the one measured *ex vivo*. *In vivo* tracking of these cells will hopefully shed more light on this issue.

Concluding remarks and perspectives

In addition to the well-known role of GH and its secretagogue ghrelin on thymopoiesis in both young and aging individuals,[22] the data summarized here clearly illustrate the role of GH in positively modulating T cell migration, both in the thymus and peripheral lymphoid organs. Yet, from a physiological point of view, there are several issues that deserve to be analyzed. For example, thus far only chemokines and ECM components have been examined as being targets for GH. There are several other interactions involved in the intrathymic trafficking of lymphocytes, including those mediated by semaphorins/neuropilins[23–25] and Ephs/Ephrins,[26] both having chemorepulsive roles upon thymocyte migration. In addition, nothing is known about whether GH plays a role in a key interaction for thymocyte export, namely, the interaction mediated by sphingosine-1-phosphate and the type 1 sphingosine-1-phosphate receptor.[27]

In any case, and considering that GH can be used as an adjuvant therapy in treating immunodeficiencies, including AIDS,[16,17] the concepts defined herein, that GH enhances migration of both developing and mature peripheral T cells, provide new clues for future GH-related immune interventions.

Acknowledgments

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Conflicts of interest

The authors declare no conflicts of interest.

References

1. Savino, W. & M. Dardenne. 2000. Neuroendocrine control of thymus physiology. *Endocr. Rev*. **21:** 412–443.
2. Hattori, N. 2009. Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system. *Growth Hormone IGF Res*. **19:** 187–197.

3. Smaniotto, S., V. de Mello-Coelho, D.M. Villa-Verde, *et al*. 2005. Growth hormone modulates thymocyte development *in vivo* through a combined action of laminin and CXCL12. *Endocrinology* **146:** 3005–3017.
4. Smaniotto, S., A.A. Martins-Neto, M. Dardenne & W. Savino. 2011. Growth hormone is a modulator of lymphocyte migration. *Neuroimmunomodulation* **18:** 309–313.
5. Savino, W., S. Smaniotto, N. Binart, *et al*. 2003. In vivo effects of growth hormone on thymic cells. *Ann. N.Y. Acad. Sci*. **992**:179–185.
6. Mello-Coelho, V., D.M.S. Villa-Verde, D.A. Farias-de-Oliveira, *et al*. 2002. Functional IGF-1-IGF-1 receptor-mediated circuit in human and murine thymic epithelial cells. *Neuroendocrinology* **75:** 139–150.
7. Ciofani, M. & J.C. Zúñiga-Pflücker. 2007. The thymus as an inductive site for T lymphopoiesis. *Annu. Rev. Cell Dev. Biol.* **23:** 463–493.
8. Taub, D.D., G. Tsarfaty, A.R. Lloyd, *et al*. 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.
9. Savino W., D.A. Mendes-da-Cruz, J.S. Silva, M. Dardenne, *et al*. 2002. Intrathymic T-cell migration: a combinatorial interplay of extracellular matrix and chemokines? *Trends Immunol*. **23:** 305–313.
10. Savino, W., D.A. Mendes-Da-Cruz, S. Smaniotto, *et al*. 2004. Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix. *J. Leuk. Biol.* **75:** 1–11.
11. Mendes-da-Cruz, D.A., S. Smaniotto, A.C. Keller, *et al*. 2008. Multivectorial abnormal cell migration in the NOD mouse thymus. *J. Immunol.* **180:** 4639–4647.
12. Petrie, H.T. & J.C. Zúñiga-Pflücker 2007. Zoned out: functional mapping of stromal signaling microenvironments in the thymus. *Annu. Rev. Immunol.* **25:** 649–679.
13. de Mello-Coelho, V., D.M.S. Villa-Verde, M. Dardenne & W. Savino. 1997. Pituitary hormones modulate cell-cell interactions between thymocytes and thymic epithelial cells. *J. Neuroimmunol*. **76:** 39–49.
14. Smaniotto, S., M.M. Ribeiro-Carvalho, M. Dardenne, *et al*. 2004. Growth hormone stimulates the selective trafficking of thymic CD4+CD8- emigrants to peripheral lymphoid organs. *Neuroimmunomodulation* **11:** 299–306.
15. Colao, A., D. Ferone, P. Marzullo, *et al*. 2002. Lymphocyte subset pattern in acromegaly. *J. Endocrinol. Invest.* **25:** 125–128.
16. Napolitano, L.A., J.C. Lo, M.B. Gotway, *et al*. 2002. Increased thymic mass and circulating naive CD4 T cells in HIV-1-infected adults treated with growth hormone. *AIDS* **16:** 1103–1111.
17. Napolitano, L.A., D. Schmidt, M.B. Gotway, *et al*. 2008. Growth hormone enhances thymic function in HIV-1-infected adults. *J. Clin. Invest*. **118:** 1085–1098.
18. Vigano, A., M. Saresella, D. Trabattoni, *et al*. 2004. Growth hormone in lymphocyte thymic and postthymic development: a study in HIV-infected children. *J. Pediatrics* **145:** 542–548.
19. Hansen, B.R., L. Kolte, S.B. Haugaard, *et al*. 2009. Improved thymic index, density and output in HIV-infected patients following low-dose growth hormone therapy: a placebo controlled study. *AIDS* **23:** 2123–2131.
20. Smaniotto, S., D.A. Mendes-da-Cruz, C.E. Carvalho-Pinto, *et al*. 2010. Combined role of extracellular matrix and chemokines on peripheral lymphocyte migration in growth hormone transgenic mice. *Brain Behav. Immun.* **24:** 451–461.
21. Xia, Q., W. Pang, H. Pan, *et al*. 2004. Effects of ghrelin on the proliferation and secretion of splenic T lymphocytes in mice. *Regul. Pept.* **122:** 173–178.
22. Taub, D.D., W.J. Murphy & D.L. Longo. 2010. Rejuvenation of the aging thymus: growth hormone-mediated and ghrelin-mediated signaling pathways. *Curr. Opin. Pharmacol*. **10:** 408–424.
23. Lepelletier, Y., S. Smaniotto, R. Hadj-Slimane, *et al*. 2007. Control of human thymocyte migration by Neuropilin-1/Semaphorin-3A mediated interactions. *Proc. Natl. Acad. Sci. U.S.A.* **104:** 5545–5550.
24. Mendes-da-Cruz, D.A., Y. Lepelletier, A.C. Brignier, *et al*. 2009. Neuropilins, semaphorins, and their role in thymocyte development. *Ann. N.Y. Acad. Sci.* **1153:** 20–28.
25. Garcia, F., Y. Lepelletier, S. Smaniotto, *et al*. 2012. Inhibitory effect of semaphorin-3A, a known axon guidance molecule, in the human thymocyte migration induced by CXCL12. *J. Leukoc. Biol*. **91:** 7–13.
26. Stimamiglio, M.A., E. Jiménez, S.D. Silva-Barbosa, *et al*. 2010. EphB2-mediated interactions are essential for proper migration of T cell progenitors during fetal thymus colonization. *J. Leukoc. Biol.* **88:** 483–494
27. Rosen, H. & E.J. Goetzl. 2005. Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network. *Nat. Rev. Immunol.* **5:** 560–70.
28. Savino, W. & M. Dardenne. 2010. Pleiotropic modulation of thymic functions by growth hormone: from physiology to therapy. *Curr. Opinion Pharmacol.* **10:** 434–442.