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Growth Hormone and Its Receptor Are Expressed in Human Thymic Cells*

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ABSTRACT

GH has been shown to modulate various functions of the thymus. We now demonstrate the production of human GH (hGH) by human thymic cells, and the expression of GH receptors in thymic epithelial cells (TEC) and in thymocytes at different stages of differentiation. The presence of hGH messenger RNA was shown by RT-PCR in both human thymocytes and in primary cultures of TEC. Moreover, immunoreactive hGH material was detected in culture media of thymocytes and TEC with the use of a sensitive immunoradiometric assay. GH receptor gene expression was shown in TEC in primary cultures and in fetal and postnatal TEC lines as well as in thymocytes. By immunocytochemistry, the presence of GH receptors in the various TEC preparations was confirmed. In cytofluorometric studies with the use of a biotinylated anti-GH receptor monoclonal antibody, we could show that GH receptors are predominantly expressed by immature thymocytes: over 90% of $CD3^-$ $CD4^-$ $CD8^-$ $CD19^-$ $CD34^+$ $CD2^+$ cells (a phenotype characterizing the most immature T cell progenitors in the thymus) were GH receptor positive. Our results provide a molecular basis for an autocrine/paracrine mode of action of GH in the human thymus. (*Endocrinology* **139:** 3837–3842, 1998)

HORMONES and neuropeptides can be potent immunomodulators that participate in various functions of the immune system (1, 2). One of the targets for neuroendocrine control is the thymus gland, a primary lymphoid organ in which bone marrow-derived T cell precursors undergo a complex process of maturation, which involves sequential expression of various membrane proteins and rearrangements in T cell receptor genes (3, 4).

Intrathymic T cell differentiation is driven by the thymic microenvironment, a tridimensional network composed of distinct cell types, such as thymic epithelial cells (TEC), macrophages, and dendritic cells, as well as extracellular matrix elements (3, 4). The thymic epithelium, the major component of the thymic microenvironment, influences differentiating thymocytes by the secretion of various polypeptides, including thymic hormones and cytokines and also by cell-cell contacts: interactions involving major histocompatibility complex gene products expressed by thymic epithelial cells with the T cell receptor, and interactions through classical adhesion molecules (3, 4). Lastly, TEC can bind to and interact with maturing thymocytes by means of extracellular matrix ligands and receptors (5).

Numerous recent studies demonstrate that distinct intrathymic cellular interactions are under neuroendocrine control (6); in particular, GH has been shown to modulate thymus physiology. The secretion of thymulin, a chemically defined thymic hormone produced by TEC, is up-regulated by GH in different species, including mouse (7), dog (8), and man (9, 10). GH also increases TEC proliferation *in vitro* as well as the expression of extracellular matrix ligands and receptors with consequent modulation of extracellular matrix-mediated TEC/thymocyte interactions (9, 11). *In vivo* experiments evidenced changes in thymocyte differentiation under GH influence. It was shown that GH injections in aging rats increased total thymocyte number and the percentage of CD3-bearing cells (12, 13). Accordingly, we showed enhanced concanavalin A mitogenic response as well as interleukin-6 production by thymocytes from GH-treated aging animals (7). Moreover, in dwarf mice, long term treatment with GH restored the thymic hypoplasia and decreased number of $CD4^+CD8^+$ thymocytes observed in these animals (14). Lastly, recombinant human GH (hGH) was shown to stimulate human peripheral blood lymphocyte engraftment and migration of T cells into the thymus of SCID (severe combined immunodeficiency) mice (15).

It has been difficult to identify GH receptors (GHRs) in thymic cells, probably because of the low receptor number. Initial studies revealed GH binding sites in murine TEC (16). More recently, the presence of GHR was demonstrated in murine thymocyte subsets by means of cytofluorometry (17). Concerning the human thymus, *in situ* hybridization studies revealed a positive signal for the GHR messenger RNA (mRNA) throughout the cortex as well as the medullary TEC (18).

In addition to GHR expression, intrathymic expression of GH was suggested with the detection of the specific mRNA

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by *in situ* hybridization and of the protein by immunocytochemistry (19, 20).

In the present work, using RT-PCR and immunoradiometric assay, we show the gene expression and the production of hGH in human TEC and thymocytes in culture. Moreover, GHRs are identified at distinct stages of thymocyte differentiation by cytofluorometry and in cultured TEC by immunohistochemistry.

Materials and Methods

Antibodies

Fluorescein isothiocyanate (FITC)-coupled anti-CD2 (clone 39C1.5, rat IgG2a), anti-CD3 (clone UCHT1, mouse IgG1), anti-CD4 (clone 13B8.2, mouse IgG1), anti-CD8 (clone B9.11, mouse IgG1), anti-CD19 (clone J4.119, mouse IgG1), anti-CD34 (clone QBEND-10, mouse IgG1), and phycoerythrin (PE)-conjugated anti-CD8 (clone B9.11, mouse IgG1) monoclonal antibodies (mAbs) were purchased from Immunotech (Marseille, France). Biotinylated anti-CD3 (clone SA-1) was obtained from Caltag (Tebu, Le Perray-en-Yvelines, France). Fab antirabbit IgG conjugated to FITC was purchased from Biosys (Compiegne, France) and polyclonal antihuman cytokeratin was obtained from Dako Corp. (Trappes, France). Anti-GHR monoclonal antibody (10B8 mAb, mouse IgG1) was generated by immunization of mice with recombinant nonglycosylated human GH-binding protein (21). Anti-GHR mAb 263 (mouse IgG1), which has been well characterized (22), was used in dual staining with 10B8 mAb. These reagents were biotinylated in our laboratory as previously described (17). Streptavidin-Cy-Chrome, purchased from PharMingen (Clinisciences, Paris, France), was used in triple labeling cytofluorometry. Isotype-matched antibodies (PharMingen) were used as negative controls.

Culture of TEC

Surgical discarded thymic tissues were obtained from children undergoing cardiac surgery (n = 12), aged from 5 days to 3 yr. The thymic capsula was removed, and the lobules were transfered to a conical tube containing RPMI 1640, supplemented with 10% heat-inactivated FCS, 10 mM HEPES, 1 mM sodium pyruvate, 1% nonessential amino acids, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 mg/ml streptomycin (all from Life Technologies, Cergy-Pontoise, France). Tissue was minced, and the supernatant containing thymocytes was collected and subjected to centrifugation (1800 rpm for 20 min) on Ficoll-Hypaque density gradient. To obtain fibroblast-free TEC primary culture, small thymic explants were cultured in 75-cm^3 flasks in D-valine-containing Eagle's MEM supplemented with 2 mmol/L L-glutamine, 10 mmol HEPES, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% FCS, as previously described for culture of cortical TEC lines from fetal and postnatal human thymi (23). The purity of epithelial cells in culture was ascertained by anticytokeratin immunostaining and was determined to be over 96%.

The two human TEC lines, obtained by an explant technique and limiting dilution cloning, were provided by Dr. M. L. Toribio (Universidad Autonoma de Madrid, Madrid, Spain). One was obtained from a fetal thymus, and the other was derived from explants of a postnatal organ (23). All cultures were maintained using D-valine containing RPMI 1460 medium at 37 C in 5% CO_2. The viability of the cells was more than 95%, as evaluated by trypan blue staining.

RNA extraction from thymic cell preparations

TEC in primary culture (11–14 days) or TEC lines were treated with trypsin/EDTA (Life Technologies) and centrifuged at 4 C (3000 rpm, 10 min) before washing in PBS. Thymocyte suspensions were centrifuged as described above. The pellets were resuspended in 1 ml RPMI for cell counting. Total RNA was prepared using Trizol reagent (Life Technologies) as an improvement to the single step RNA isolation originally described by Chomczynski and Sacchi (24).

RT-PCR

Various quantities of thymocyte- or TEC-derived total RNA were denatured for 10 min at 68 C and cooled at 4 C. RT was performed in solution containing 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM deoxy-NTP, 1 U/ml RNasin, and 10 U/ml Moloney murine leukemia virus reverse transcriptase (Life Technologies) at 37 C for 1 h. The RT reaction was finished by heating specimens at 96 C for 5 min and cooling at 4 C. Oligonucleotide sequences of the different primers used are presented in Table 1. Ten microliters of RT products were used in PCR mixture, with 25 pmol of each primer, sense and antisense, and 1 U *Taq* polymerase (Perkin-Elmer/Cetus, Norwalk, CT), diluted in 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, and 200 mM deoxy-NTP in a final volume of 50 or 30 μl for thymocytes or TEC, respectively. The PCR profile for GH complementary DNA (cDNA) amplification consisted of denaturation at 94 C for 5 min followed by 30 cycles at 94 C for 30 sec, 60 C for 1 min, and 72 C for 1 min. For GHR cDNA amplification, 36 cycles were performed at 94 C for 30 sec, 56 C for 1 min, and 72 C for 1 min and 15 sec. Both cDNA amplifications were completed by heating at 72 C for 10 min. Amplified PCR products were electrophoresed in 1% ethidium bromide-agarose gel and visualized under a UV transluminator. RT-PCR in the absence of RNA served as a negative control, whereas RNA prepared from human hypophysis and liver were used as positive controls for GH and GHR gene expression, respectively.

GH assay

Cell media were collected after 24 h of culture in serum-free conditions.

Supernatants of thymocyte- or TEC-derived cultures were concentrated 20-fold using Centripep-30 Amicon filters (Grace, Epernon, France). The samples were analyzed by a commercial immunoradiometric assay (Kit ^{125}I hGH U Coatria, BioMerieux, Lyon, France) according to instructions of the manufacturer. This technique has been used for the measurement of urinary GH (25), and the detection limit has been evaluated to be 0.5 pg/ml. Results are expressed as picograms per ml concentrated medium.

Immunocytochemistry

Cultures of epithelial cells were submitted to an indirect immunofluorescence technique (26). Briefly, cells were fixed with absolute ethanol for 5 min, incubated with PBS-0.2% BSA for 1 h, and further washed with PBS. Material was then subjected to biotinylated anti-GHR antibody (mAb 10B8, diluted 1:10) for 1 h, washed with PBS, and subjected to streptavidin-FITC (diluted 1:100) for 1 h. After an additional PBS washing, samples were mounted and analyzed under a Leitz Ortoplan fluorescence microscope (Leitz, Rockleigh, NJ). To ascertain the epithelial nature of the primary TEC cultures, anticytokeratin serum (diluted 1:20) was used; it was revealed with the FITC-labeled goat antirabbit IgG serum.

Flow cytometric analysis and cell sorting

Triple fluorescence immunostaining of thymocytes was performed by incubating 1×10^6 cells in PBS supplemented with 2% FCS and 0.1% sodium azide in the presence of biotinylated, PE- or FITC-conjugated specific antibodies or isotype controls at appropriate concentrations. After PBS washing, cells were incubated with streptavidin-Cy-Chrome and postfixed in 1% formaldehyde. Incubations were performed in a final volume of 20 μl in 96-well microtiter plates for 20 min at 4 C. In some experiments, dual immunostaining was carried out using two different anti-GHR monoclonal antibodies; biotinylated 263 mAb was

TABLE 1. Primers used for GH and GH receptor cDNA amplification

Name	Oligonucleotide sequence	Position
hGH-16	5′-GCTCCGCGCCCATCGTCTGC-3′	Exon 2
hGH-146	5′-GTCGAACTTGCTGTAGGTCTGC-3′	Exon 5
GHR-15	5′-CCCTATATTGACAACATCAGTTCC-3′	Exon 7
GHR-23	5′-TTTCCTTCCTTGAGGAGATCTGG-3′	Exon 9/10

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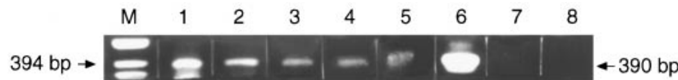

FIG. 1. Expression of GH mRNA in human thymic cells. Thymic tissue was from a 2.5-month-old girl. RNA samples were subjected to RT-PCR, and PCR products were visualized on agarose gel (1%) by ethidium bromide staining. PCR products are from 10 μg (lane 1), 8 μg (lane 2), 4 μg (lane 3), and 2 μg (lane 4) thymocyte RNA; from 10 μg cultured TEC RNA (lane 5); and from 2 μg human pituitary RNA (lane 6). In lanes 7 and 8 are negative controls (in the absence of RNA) of RT and PCR, respectively. M, Mol wt markers.

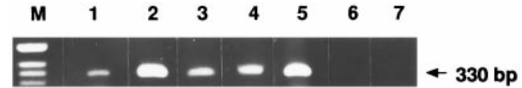

FIG. 2. Expression of GHR mRNA in human thymic cells. RT-PCR was performed using total RNA: 10 μg thymocyte RNA (lane 1), 2 μg TEC RNA (lane 2), and 0.5 μg fetal (lane 3) or postnatal (lane 4) TEC RNA. PCR products were visualized at 330 bp on 1% agarose gel by ethidium bromide staining. Four micrograms of total RNA from human liver were used as a positive control (lane 5), and reaction in the absence of RNA was used as a negative control for RT (lane 6) or PCR (lane 7). M, Mol wt markers.

revealed by streptavidin-PE, whereas 10B8 mAb was directly coupled to FITC. Cell acquisition was performed in a FACScan flow cytometer, and data were analyzed using Lysis II software (Becton Dickinson, Grenoble, France). Forward light scatter gates were set to exclude dead cells and debris.

For analysis of $CD34^+$ $CD2^+$ triple negative (TN) cells, thymocytes were first depleted of CD3-expressing cells by incubation with biotinylated anti-CD3 mAb (Caltag) followed by one cycle of streptavidin microbeads (Miltenyi, Tebu, Le Perray-en-Yvelines, France). The remaining cells were incubated with PE-labeled anti-CD2 mAb and FITC-conjugated anti-CD3, -CD4, -CD8, and -CD19 mAbs (Immunotech). $CD2^+$ $CD3^-$ $CD4^-$ $CD8^-$ $CD19^-$ cells were then sorted with a FACS Vantage cell sorter (Becton Dickinson). Reanalysis of sorted cells indicated a purity of 98% or more.

Results

Production of GH by human thymocytes and TEC

RT-PCR was carried out on total RNA prepared from either freshly isolated or cultured cells, using primers derived from the hGH cDNA sequence. Expression of hGH mRNA (Fig. 1) was detected in freshly isolated thymocytes (lanes 1–4) and in primary culture of TEC (lane 5), where the signal was weaker. A PCR product of similar size (390 bp) was amplified with RNA extracted from human pituitary (lane 6). No signal was detected for controls (RT-PCR in the absence of RNA; lanes 7 and 8).

To evaluate whether thymocytes and TEC secrete hGH, media of 24-h cultures were concentrated and analyzed using a sensitive immunoradiometric assay (Table 2). Immunoreactive hGH was detected in the media of seven different thymocyte cultures with large variations in the hormone concentration. The high value (264 pg/ml) found in one thymocyte culture medium was confirmed by assaying serial dilutions of the medium. In addition, three media of primary TEC cultures contained immunoreactive hGH. However, no GH gene expression was found in fetal and postnatal TEC lines, and no GH-immunoreactive material was detected in the culture media of the cells (not shown).

TABLE 2. hGH assay in culture media of thymic cells

Donor		Thymocytes	TEC
Age	Sex		
5 days	F	2.7	ND
20 days	M	2.5	ND
2.5 months	F	23.6	ND
2.5 months	F	24.5	ND
6.5 months	F	<2.0	24.5
3 yr	F	4.9	8.2
3 yr	M	264	21.3

Cell media were collected after 24 h of culture, in serum-free conditions. Media were concentrated 20-fold. hGH assays were performed in duplicate, for two dilutions of each medium. Data are expressed as picograms per ml. ND, Not done.

GHR gene expression in human TEC and thymocytes

Expression of GHR mRNA was analyzed in the different cell populations by PCR after RT of total RNA. Figure 2 shows the PCR products revealed in 1% agarose gel by ethidium bromide staining. A band of the expected size of 330 bp was visualized in thymocytes (lane 1), TEC (lane 2), as well as fetal (lane 3) and postnatal (lane 4) TEC lines. The negative controls represent amplification of the reverse transcribed products lacking RNA, and the positive control represents the amplification of 4 μg total RNA from human liver after reverse transcription (lane 5).

Immunocytochemical detection of GHRs in TEC

The GHR protein was detected in the three TEC preparations by immunofluorescence using the anti-GHR antibody (mAb 10B8; Fig. 3). The signal for GHR was very weak at the cell surface. A strong labeling was visualized in the perinuclear region of the cells (Fig. 3, b–d). As expected, control IgG did not generate significant fluorescent signal (Fig. 3a). Double labeling with anticytokeratin or anti-GHR mAb was also performed by immunocytochemistry on fixed primary TEC or on fetal or postnatal TEC lines. Over 96% of the cells were positive to cytokeratin detection (not shown).

Differential expression of GHRs in thymocyte subsets

The expression of GHRs was low in 10 human thymuses analyzed; 2–10% of positive cells were found in total cell suspensions (Table 3). Comparable results (not shown) were obtained through dual immunostaining using two different anti-GHR monoclonal antibodies, allowing the use of either of the two antibodies to study GHR expression.

We analyzed the expression of GHR in the four thymocyte subsets defined by CD3, CD4, and CD8 expression. In 9 of 10 tissues, the highest percentage of GHR^+ cells was found within the most immature $CD4^-$ $CD8^-$ subset, not expressing the CD3:T cell receptor complex, designated TN cells (Fig. 4 and Table 3). However, although higher frequencies of GHR-positive cells were found within this immature population, GHR density on cell membrane appeared similar in the distinct CD4/CD8-defined subsets.

To better define the identity of GHR-expressing cells among the heterogeneous population of TN immature thymocytes, we further characterized these cells by the expression of the CD2 molecule, an early marker of T cell lineage.

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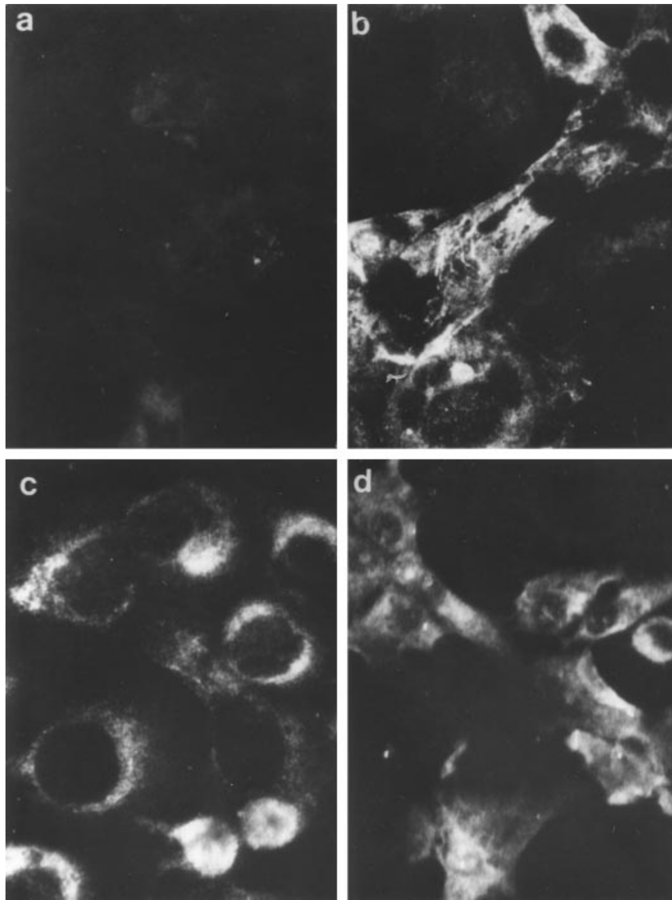

FIG. 3. Immunocytochemical detection of GHRs in human TEC. Ethanol-fixed cultured TEC were probed by immunocytochemistry with anti-GHR (mAb 10B8). No significant fluorescent labeling was seen when an IgG1-matched unrelated mAb was used (a). A positive signal is present in primary TEC culture (b) as well as in fetal and postnatal cell lines (c and d). Magnification, ×900.

TN $CD2^+$ cells were sorted according to the gating illustrated in Fig. 5. Moreover, as CD2 has been recently shown not to be restricted to T cell lineage, but is also expressed on thymic B cells bearing the $CD19^+$ marker (27), $CD19^+$ cells were also separated by sorting. Sorted thymocytes expressing the $CD2^+$ $CD3^-$ $CD4^-$ $CD8^-$ $CD19^-$ phenotype were then examined for their expression of CD34, a marker for intrathymic T cell progenitors (28) and GHR. Over 90% of cells with the $CD34^+$ $CD2^+$ phenotype expressed GHR at the cell membrane, as shown in Fig. 5.

Discussion

In the present work, we demonstrate that the production of GH as well as the expression of GHRs in human thymus occur at the level of both lymphoid and microenvironmental compartments of the organ.

Intrathymic production of GH has originally been reported on the basis of immunocytochemical and *in situ* hybridization data (19, 29), which revealed positive signals in cortical epithelial cells and in septal, phenotypically undefined cells, but not in thymocytes. However, immunoreactive and biologically active GH was shown to be produced by isolated human and rat thymocytes (30, 31). In another study, a GH-specific RT-PCR product was revealed in total thymus extracts (but not in distinct cell types), and *in situ* hybridization signal was found in epithelial cells and in some thymocytes (20). Our data represent the first demonstration of the presence of GH mRNA in human thymocytes and TEC, with the secretion of a GH-like peptide by both cell types. However, the subpopulation of GH-producing cells in human thymus remains to be identified.

The fact that the two TEC lines presented neither detectable hormone production nor GH gene expression remains intriguing. It is unlikely that nonepithelial contaminants in TEC primary cultures are responsible for GH expression because 1) very few contaminant cells are present in the cultures; and 2) previous findings, using immunocytochemistry, have demonstrated subseptal TEC positive for anti-GH labeling (19). A more likely explanation is loss of the ability of immortalized TEC to constitutively produce GH.

The physiological relevance of the locally produced GH needs to be further studied. In addition to actions of pituitary-derived GH, aspects of thymus physiology, including thymulin production (6), and some thymocyte/microenvironmental interactions (11) as well as progress of thymocyte differentiation may be under the control of thymus-derived GH. In a recent work, Sabharwal and Varma showed that GH is produced by human thymic cells and is able to stimulate the proliferation of cultured thymocytes. The authors also provided evidence favoring an indirect GH effect, mediated by locally produced insulin-like growth factor I (IGF-I) (31). It has also been reported that exogenous GH is able to stimulate the production of thymulin and IGF-I as well as the proliferation of TEC (6). All of these results suggest that locally synthesized GH and IGF-I could play important roles as growth factors for thymocytes and TEC.

The biological effects of GH are mediated by receptors located in target cell membranes. The presence of GH-binding sites on cultured murine TEC was shown initially (16). Using RT-PCR followed by Southern analysis, GHR mRNA was identified in rat thymus, and *in situ* hybridization experiments revealed the presence of GHR mRNA on human medullary microenvironmental cells and thymocytes (18). More recently, using cytofluorometry, the expression of GHRs was demonstrated on murine thymocytes (17). Nonetheless, the expression of GHRs in human thymocytes had not been reported. We demonstrate that GHRs are expressed in thymocytes, and that the expression is restricted to a small percentage of cells, which is in contrast with the large expression of PRL receptors in most thymocytes (32). Despite comparable effects of PRL and GH on the thymic microenvironment (6, 11), direct targeting of these two hormones on the lymphoid compartment of the organ might be differential.

Human thymocytes have been shown to differentiate from immature TN $CD3^-$ $CD4^-$ $CD8^-$ precursors to mature single positive $CD4^+$ or $CD8^+$ populations expressing high levels of CD3. Our data demonstrate that GHR are mainly present in these immature $CD3^-$ $CD4^-$ $CD8^-$ TN precursors. However, TN thymocytes that lack the markers CD3, CD4, and CD8 constitute a heterogeneous population of precursors with various capacities for differentiation (33). Convincing evidence has now been obtained that the most immature

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TABLE 3. Proportion of GHR-positive cells in CD4/CD8-defined human thymocyte subpopulations, using flow cytofluorometry

Donor		% of positive thymocytes				
Age	Sex	Total	$CD4^+CD8^-$	$CD4^-CD8^+$	$CD4^+CD8^+$	$CD4^-CD8^-$
20 days	M	2.4	0.9	0.6	2.4	4.8
2.5 months	F	8.8	11.0	5.1	7.2	59.0
2.5 months	F	10.0	14.1	5.3	8.0	69.0
6 months	F	3.5	5.0	3.1	2.3	25.9
6 months	F	6.3	0.6	13.5	6.1	4.4
6.5 months	F	6.9	4.9	3.5	6.9	13.3
9 months	M	4.3	2.7	1.9	4.2	20.4
10 months	M	9.3	14.7	6.0	1.7	26.1
10 months	F	3.2	7.0	1.1	2.2	19.3
3 yr	F	2.9	6.8	2.6	1.6	38.7

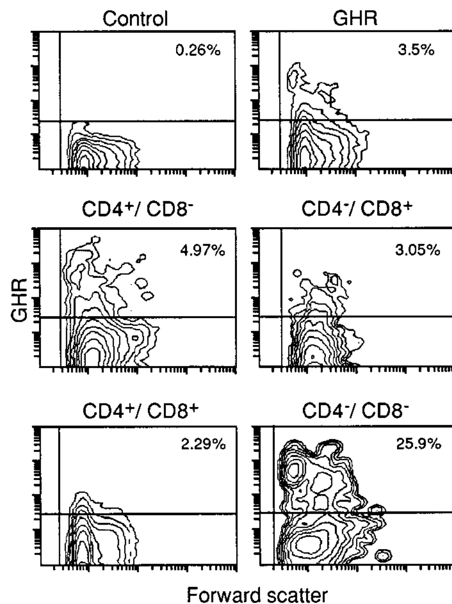

FIG. 4. Distribution of GHRs in thymocyte subpopulations. Thymocytes were stained with biotinylated anti-GHR (10B8 mAb) followed by SAV-Cy-Chrome and FITC- and PE-conjugated mAbs (tricolor immunofluorescence staining) after elimination of dead cells. In the *upper panels*, GHR expression is shown in total viable thymocytes (*right*), and the IgG-negative control is on the *left*. The *lower panels* show the GHR^+ cells within each CD4/CD8-defined thymocyte subset. The percentage of GHR-bearing cells is indicated in each panel. One representative profile of 10 human thymuses studied is presented.

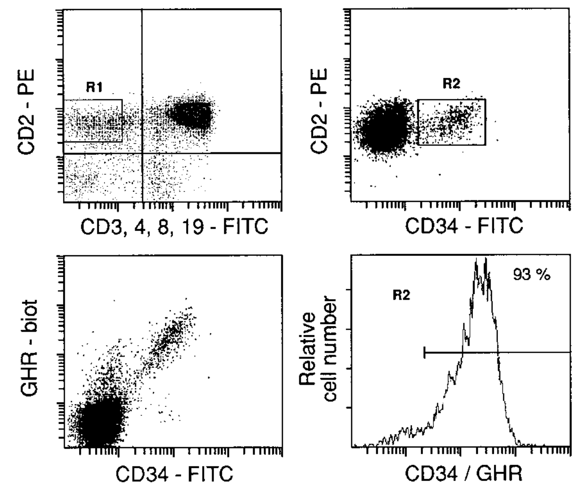

FIG. 5. Cytofluorometric detection of GHRs on $CD34^+CD2^+$ thymocytes. Thymocytes were first $CD3^+$ depleted using the immunomagnetic bead technique. Cells were then stained with anti-CD2-PE and anti-CD3, -CD4, -CD8, and -CD19-FITC antibodies. $CD2^+CD3^-$ $CD4^-$ $CD8^-$ $CD19^-$ cells were sorted using the gate R1 indicated in the figure (*upper panel, left*). $CD2^+$-sorted thymocytes were labeled with biotinylated anti-GHR antibody followed by streptavidin-Cy-Chrome in conjunction with anti-CD34 PE. In the *upper panel* (*right*), $CD2^+$-sorted cells were analyzed for the expression of CD34 (gate R2). In the *lower panels*, the distribution of GHR is shown in the $CD34^+$ $CD2^+$-gated thymocyte subpopulation.

thymocytes able to differentiate in $CD3^+$ cells express the pluripotent marker stem cell CD34 (28, 34). $CD34^+$ TN cells can be further subdivided according to phenotype. The finding that $CD34^+$ coexpress CD2, an early T cell marker, suggests further that these cells are T cell precursors, although the expression of CD2 has been recently shown on some thymic mature B cells (defined by the CD19 marker). Thus, using these three markers and cell sorting, we defined the phenotype of immature thymic precursors expressing GHR and showed, on CD3-depleted cells, that GHR^+ cells were mainly restricted to a subpopulation of TN cells expressing CD34 and CD2, but not CD19. These findings are in agreement with the expression of GHR in immature murine thymic cell subsets (17) and also with *in vivo* data observed in dwarf mice; the animals presented a deficiency in T cell progenitors in the thymus, and GH treatment resulted in the expansion of $CD4^+/CD8^+$ double positive cells (14). Taking into account the comitogenic activity of GH on concanavalin A-treated mouse thymocytes (35), it is conceivable that in man, GH may play a role in the continuous expansion of cells that have not yet been subjected to the process of positive and negative selection of their repertoire. It is of interest to recall the dual effector theory for GH, which was proposed by Green for preadipocytes but can be applied to other cell types (36): GH directly affects the differentiation of cell precursors and indirectly, through IGF-I, has a mitogenic effect on young differentiated cells.

By immunocytochemistry, using biotinylated monoclonal antireceptor antibody, the presence of GHRs is shown in TEC in primary culture and also in two human cell lines. The subcellular distribution of the GHRs is in accordance with the findings of previous studies in other cells; the receptors are more numerous in intracellular compartments than at the cell surface (37).

Constant interactions exist between TEC and differentiating thymocytes, with well known effects of TEC on intra-

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thymic T cell migration/differentiation. Considering that GH can be produced by TEC and thymocytes, and that both cell types express GHRs, a paracrine GH-mediated lymphoepithelial cross-talk is conceivable (38). Indeed, thymocyte-derived cytokines, such as interferon-γ, can modulate TEC proliferation and extracellular matrix secretion with consequent changes in the thymocyte adhesion pattern to epithelial cells (39, 40). Likewise, GH affects various functions of thymocytes and TEC, supporting the hypothesis of a bidirectional GH-mediated cross-talk between the two cell types. It was recently shown that GH is able to regulate the expression of several cytokines in bovine thymic stromal cells (41).

In conclusion, our results support a paracrine/autocrine mode of action for GH in the human thymus.

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