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Immunohistochemical Localization of Interleukin-6 in Peripheral Human Endocrine Glands

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Interleukin-6 (IL-6) is a pleiotropic cytokine with differentiation and growth-promoting effects. Extensive studies in experimental animals denote that IL-6 is produced in various endocrine organs and participates in the local control of endocrine cell function. The expression of this cytokine in human endocrine glands, however, has only been examined in a limited number of studies. We investigated the immunohistochemical expression and localization of IL-6 in a variety of peripheral human endocrine glands. In the adrenals, IL-6 immunoreactivity was detected in all three zones of the cortex. The reticularis and glomerulosa zones were more heavily stained as compared with the slight immunoreactivity of the fasciculata zone. In the adrenal medulla, chromaffin and sustentacular cells were variably positive. A substantial number of follicular thyroid cells were strongly immunoreactive for IL-6 in all normal and hyperplastic thyroids examined. Parafollicular cells were negative. Parathyroid chief cells were mildly positive; selective and more intense staining was observed in acidophilic cells. Pancreatic islet cells were variably positive. In the testis positive staining was selectively observed in both Leydig and Sertoli cells. In conclusion, IL-6 immunoreactivity is present in almost all the human endocrine glands and it expressed in a cell-specific manner. These observations provide further support for the existence of local immune-endocrine interactions.

Key Words: Adrenal; thyroid; pancreas; parathyroid; testis.

Introduction

Interleukin-6 (IL-6) is a 22- to 30-kDa glycoprotein produced by many cell types and has a wide variety of biologic, differentiation, and growth-promoting effects in a variety of target cell types *(1,2)*. Even though it is mainly produced by activated T-lymphocytes, macrophages, fibroblasts, and endothelial cells, there is now accumulating evidence for its production by several other cell types, including cells of various endocrine organs *(3,4)*. So far, studies in experimental animals have indicated the production of IL-6 by several endocrine glands, and there is good evidence that this locally produced cytokine may participate in the regional control of endocrine cell function *(3,4)*. The expression of this cytokine in human endocrine glands, however, has only been examined in a limited number of studies, and a detailed mapping of its origin in various human endocrine cell types has not been established. In the present study, we investigated the immunohistochemical expression and distribution of IL-6 in a variety of nontumorous human peripheral endocrine tissues.

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Results

Positive IL-6 cytoplasmic staining in the various endocrine glands examined was as follows: In the adrenals, IL-6 immunoreactivity was detected in all three zones of the cortex. The reticularis and glomerulosa zones were more heavily stained as compared with the slight immunoreactivity of the fasciculata zone (Fig. 1A,C). In the adrenal medulla, chromaffin cells and scattered cells with morphologic features compatible to sustentacular cells were variably positive (Fig. 2A,B). In addition, immunohistochemistry revealed very few, scattered macrophages reactive for both IL-6 and CD68.

In all thyroids examined, a substantial number of follicular thyroid cells were strongly immunoreactive for IL-6 in both normal and hyperplastic thyroids (Fig. 3A,B). Statistical analysis of positive cell counts showed no significant differences between normal and hyperplastic thyroid glands ($p = 0.2$). Parafollicular C-cells recognized by calcitonin staining in parallel sections were negative (Fig. 4A,B). Substitution of the primary antiserum for IL-6 yielded a negative results (Fig. 4C).

Parathyroid showed heterogeneous immunoreactivity; chief cells were mildly positive in the substantial majority

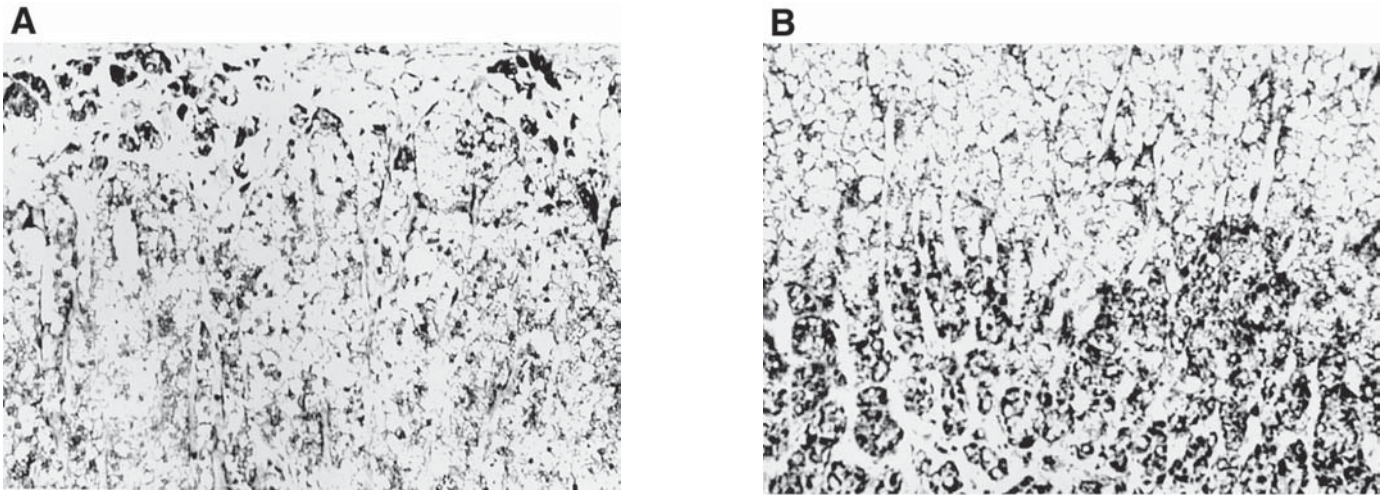

Fig. 1. (**A**) IL-6 immunoreactivity in adrenal cortex. The zona glomerulosa is more heavily stained as compared to the slight immunoreactivity of zona fasciculata (magnification: ×40). (**B**) IL-6 immunoreactivity predominates in the zona reticularis of the adrenal cortex (magnification: ×40).

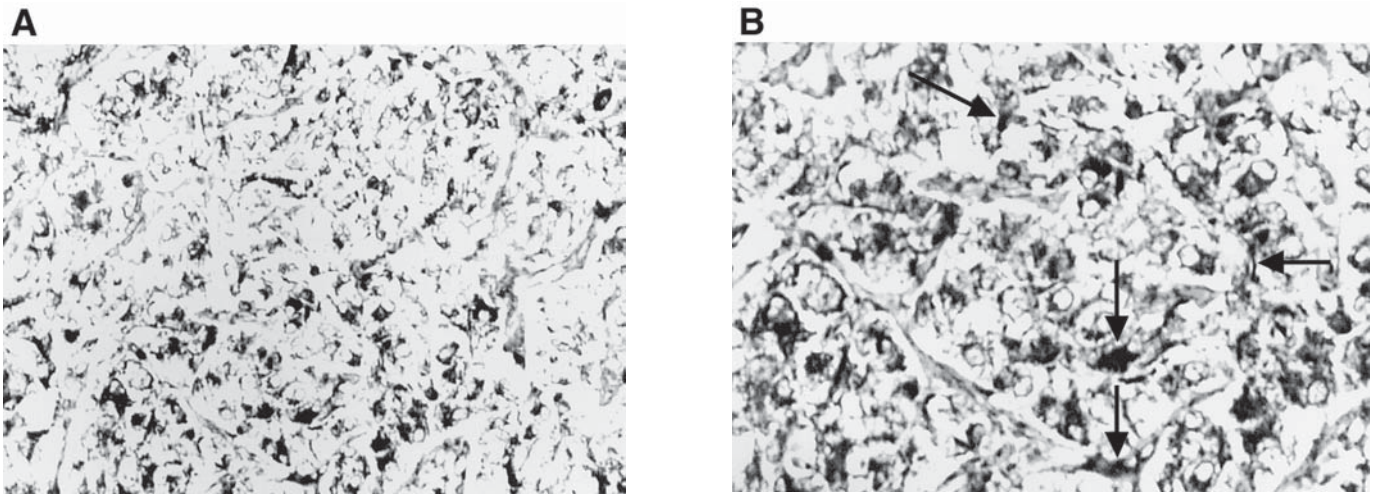

Fig. 2. (A) A few chromaffin cells and scattered stellate cells with morphologic features compatible to sustentacular cells are positive for IL-6 in the adrenal medulla (magnification: ×40). (**B**) This higher magnification of the adrenal medulla shows IL-6 immunoreactivity in sustentacular cells (arrows) mostly located at the periphery of the cell clusters (magnification: ×100).

of parenchyma, mostly with subplasmallemal localization of IL-6. More intense and selective staining with even and diffuse cytoplasmic distribution was observed in acidophilic cells (Fig. 5A,B).

Pancreatic islet cells were variably positive. In some specimens, IL-6-immunopositive cells were mostly localized in the periphery of the islet (Fig. 6A). However, parallel sections stained with pancreatic hormones did not conclusively reveal specific colocalization with any of the pancreatic islet cell types (Fig. 6A). In addition, a very few scattered cells within acini were IL-6 positive.

In the testis a selective positive staining was observed in both Leydig stromal cells and Sertoli cells; the latter were recognized from their triangular shape with their base attached to the wall of seminiferous tubules; their cytoplasm was diffusely immunopositive (Fig. 7A,B). No germinal cells showed conclusive immunoreactivity for IL-6. Seminiferous tubules contained scarce DC-68- and IL-6-positive macrophages, mostly located within the lumen.

Several endothelial cell lymphocytes and plasma cells were immunoreactive for IL-6 and, therefore, served as internal positive controls.

Discussion

In the present study, systemic immunohistochemical investigation of various endocrine tissues by using a highly specific antibody for human IL-6 demonstrated a widespread localization of IL-6 in almost all the major human endocrine glands. Thus, endocrine cells expressing this cytokine were found in the adrenals, the thyroid, the parathyroids,

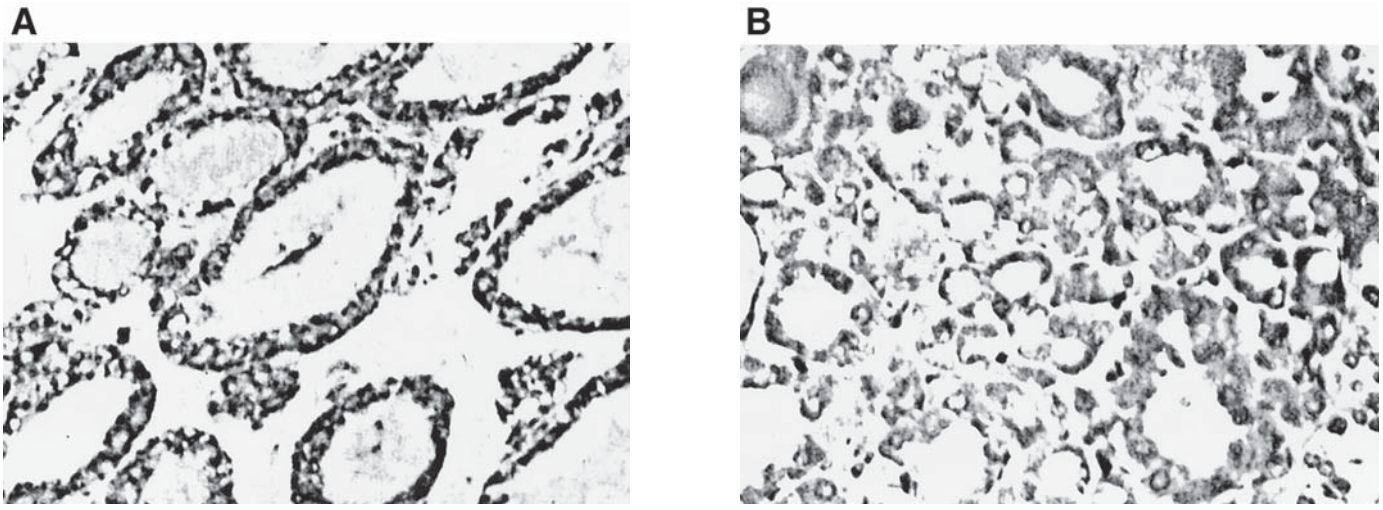

Fig. 3. (**A**) IL-6 immunoreactivity in apparently normal thyroid parenchyma (magnification: ×100). (**B**) follicular cells of hyperplastic thyroid gland strongly immunoreactive for IL-6 (magnification: ×100).

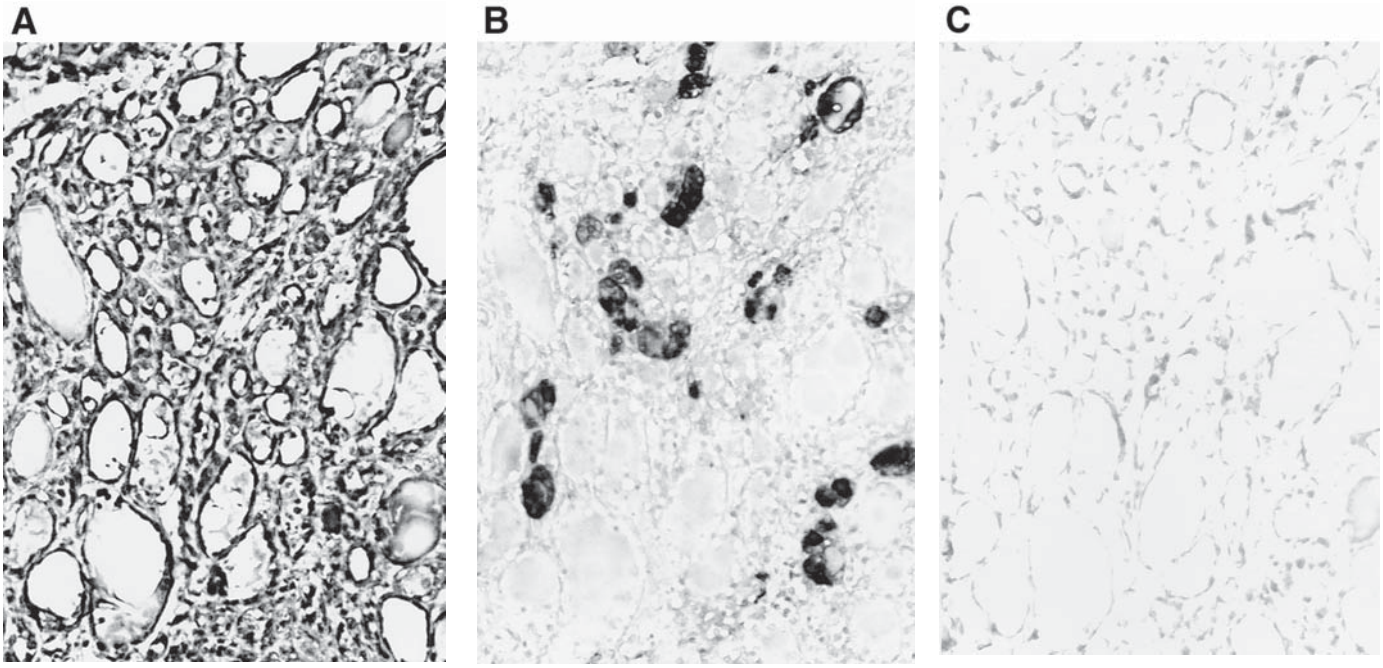

Fig. 4. Parallel sections stained for IL-6 (**A**) and calcitonin (**B**). Several C-cells are positive for calcitonin (B). No colocalization of calcitonin with IL-6 is noted. The negative control (**C**), where the primary antiserum for IL-6 was substituted with phosphate-buffered saline (PBS), is immunonegative (magnification: ×80).

the endocrine pancreas, and the testis. Furthermore, this study provided evidence for the specific cell types, which are responsible for its local production.

In previous studies in human tissues, only the pituitary and the adrenals were thoroughly examined for the immunohistochemical presence of IL-6. In the pituitary, previous studies showed the presence of IL-6 protein, IL-6 mRNA, and IL-6 receptor in hormone-secreting cells of the normal and tumorous anterior pituitary gland *(5–10)*.

Previous studies in the human adrenals have shown the presence of IL-6 mRNA by *in situ* hybridization and of IL-6 receptor by immunohistochemistry in steroid-secreting cells from all cortical zones and in particular the zona reticularis *(11,12)*. The immunohistochemical presence of IL-6 was also shown in cultures of adrenocortical cells *(12)*. In good agreement with these studies, we also observed the immunohistochemical presence of IL-6 in steroid-producing cells from all cortical zones. We also noticed that the

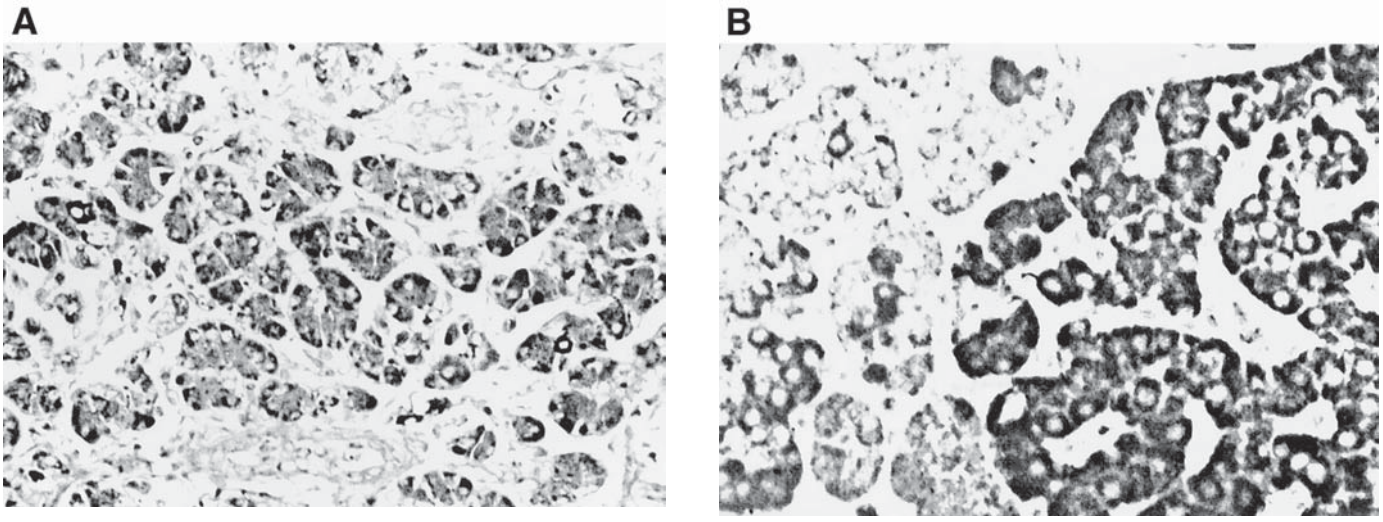

Fig. 5. (**A**) Acidophilic cells of parathyroid parenchyma selectively stained for IL-6 (magnification: ×80). (**B**) Chief cells (**left**), mildly positive for IL-6, mostly with subplasmallemal localization in the cytoplasm. In this higher magnification, acidophilic cells (**right**) are more heavily stained with diffuse cytoplasmic distribution of immunoreactivity. The unstained nuclei are shown as round empty spaces in the cell center (magnification: ×160).

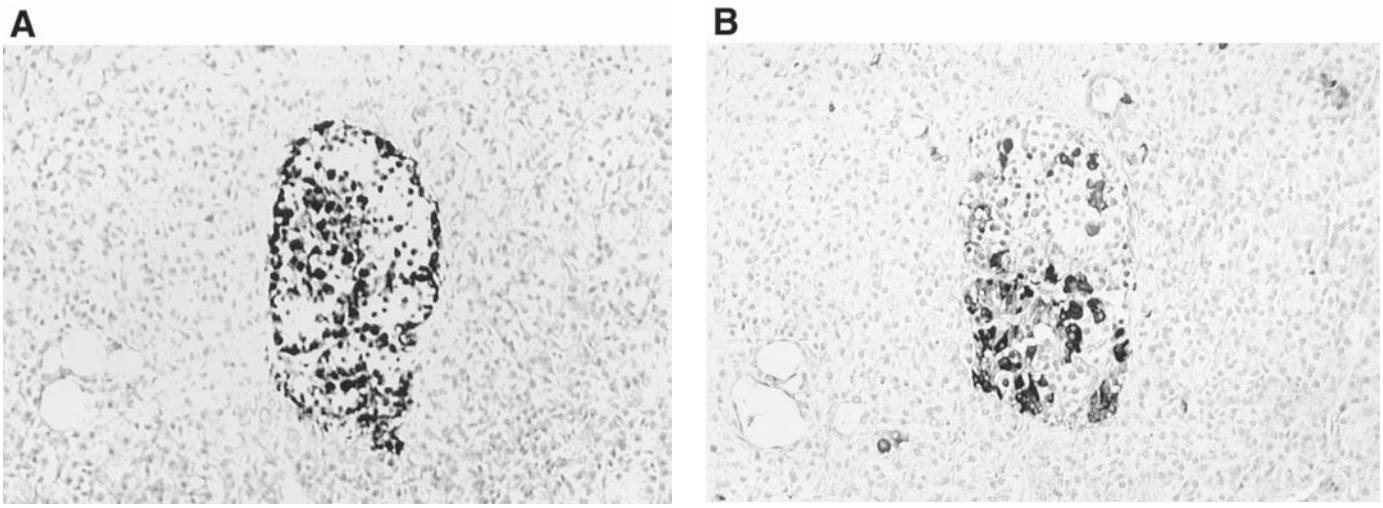

Fig. 6. Parallel sections of pancreatic islet cells immunopositive for IL-6 (**A**) with localization in periphery of islet as compared to immunostain for glucagon (**B**) in same islet (magnification: ×40).

reticularis and the glomerulosa zone were more heavily stained compared to the slight immunoreactivity observed in the zona fasciculata. Note that in contrast to a previous report *(12)*, IL-6 was also detected in scattered cells of the adrenal medulla. Based on morphologic features we recognized IL-6 immunopositivity in both chromaffin and stellate sustentacular cells with the latter cells bearing the highest amount of medullary IL-6 immunoreactivity. Similar findings were observed in the rat adrenal medulla *(13)*. Elevated circulating levels of IL-6 have been observed in several patients with pheochromocytoma that derives from adrenal medulla *(14)*.

In the thyroid, IL-6 mRNA expression and IL-6 production have been demonstrated in primary human follicular cell cultures and thyroid cell lines *(15–17)*. The presence of IL-6 was also shown in thyroid specimens obtained from patients with autoimmune thyroid diseases, nontoxic multinodular goiters, and papillary but not follicular thyroid car-cinomas *(18,19)*. Taken together, these data are compatible with a constitutional production of IL-6 in thyroid epithelial cells. Consistently, in the present study follicular epithelial cells from normal thyroids were IL-6 positive; parafollicular cells did not show IL-6 immunoreactivity. This finding demonstrates that normal follicular thyroid cells selectively express IL-6 and its presence is independent of any autoimmune-related process. In fact, thyroid follicle destruction is associated with elevated IL-6 levels in the circulation of affected patients *(20)*. Elevations of IL-6 levels of a lesser degree

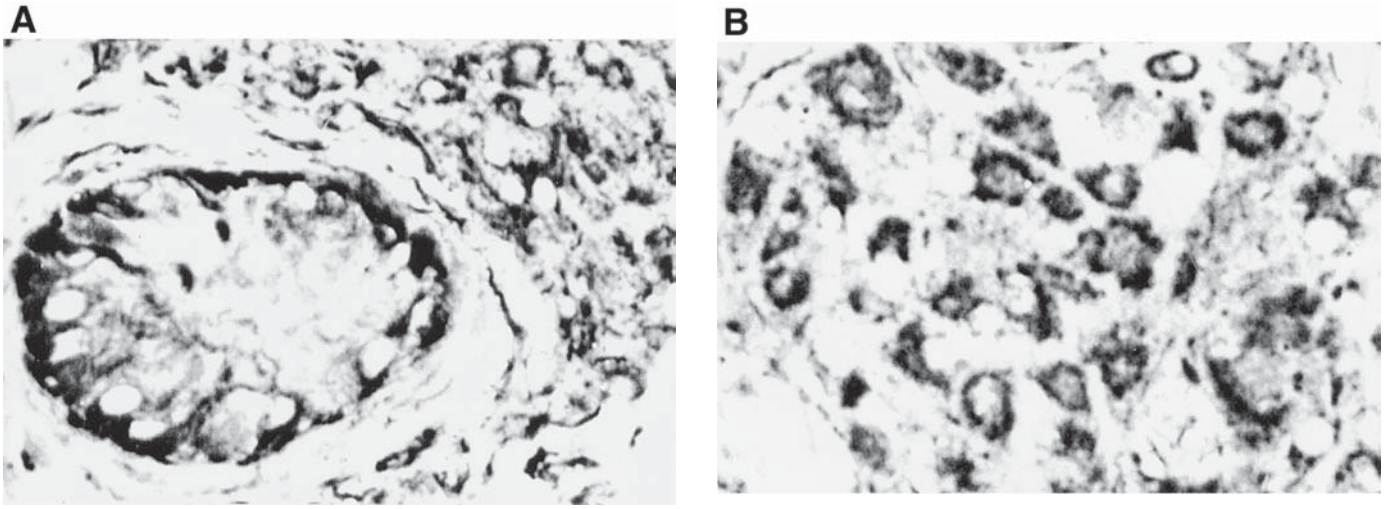

Fig. 7. Both Sertoli cells of the seminiferous tubules (**left**) and Leydig stromal cells (**right**) of testicular parenchyma are immunopositive for IL-6. The empty round spaces correspond to the unstained nuclei (magnification: ×160). (**B**) This higher magnification of the testicular parenchyma shows strong and diffuse cytoplasmic IL-6 immunopositivity (magnification: ×160).

are also found in all forms of hyperthyroidism including Graves disease and toxic multinodular goiter, but such elevations in IL-6 are probably of nonthyroid origin *(21)*.

The immunohistochemical detection of IL-6 in normal human parathyroids, pancreas and testis, to our knowledge, has not been previously described. In the present study, parathyroid cells were invariably positive for IL-6 immunoreactivity. Acidophilic cells were more intensively stained compared with chief cells. The mechanism underlying such a predominant production of IL-6 by the acidophilic cells in the parathyroids is currently unclear. In previous studies, the presence of IL-6 in human pancreatic islet cells had only been shown on biopsy material from diabetic patients *(22)*. In the present study, by using normal pancreatic tissue, we were able to clearly demonstrate that islet cells represent the major source of IL-6 production in the human pancreas. Despite the previously reported high degree of IL-6 expression in acinar tumorous cell lines *(23)*, we found only scarce acinar cells staining positively for IL-6 in the normal human pancreas. A predilection of IL-6-positive cells in the periphery of the islet was noticed, which could imply a predominant IL-6 production by glucagons-producing cells. In fact, the systemic administration of IL-6 results in a predominant stimulation of glucagons secretion in human subjects *(24)*. However, our parallel staining studies failed to demonstrate a selective expression of IL-6 in glucagons-producing cells of the pancreatic islets. On the basis of these findings, it is suggested that the elevated IL-6 levels of patients with pancreatitis *(25)* are probably owing to leakage of this cytokine in the circulation following massive islet-cell destruction. Regarding the human testis, only indirect evidence had been available for the cells of origin of IL-6. Thus, isolated Leydig or Sertoli cells in culture were previously shown to release IL-6, providing evidence that both cell types are responsible for the presence of this cytokine in the human testis *(26)*. However, because incomplete cell separation or contamination by macrophages could be a source of conflict regarding these findings, our morphologic observations confirmed beyond a doubt the presence of IL-6 in both Leydig and Sertoli cells of the human testis.

In conclusion, the results presented herein clearly demonstrated a selective distribution of IL-6 in several types of human endocrine cells, thus strongly suggesting that these cells have the capacity to produce IL-6. Thus, our data provide further support for the existence in human endocrine glands of local immune-endocrine networks similar to those described in endocrine tissues originating from experimental animals in which IL-6 affects directly their secretory and possibly proliferative status and participates in the local control of their function. However, despite the progress made over recent years in clarifying many of the actions of IL-6 in the endocrine system, the physiologic role served by this and other cytokines, acting in concert with IL-6, remains to be further elucidated.

Materials and Methods

Tissues

The following nontumorous human endocrine glands obtained at surgery or autopsy were examined: 10 adrenals removed during nephrectomy for renal carcinoma; 12 thyroids obtained after thyroidectomy for a cold nodule or nontoxic multinodular goiter—in all cases there was no laboratory or morphologic evidence of autoimmune thyroid disease; 8 parathyroids removed incidentally during total thyroidectomy; 6 pancreases obtained after surgery for suspected insulinoma or duct carcinoma; and 4 testicles removed from

patients with prostatic carcinoma and 4 biopsy specimens from patients with sterility.

Immunohistochemistry

For immunohistochemical detection of IL-6, the standard avidin-biotin-peroxidase complex (ABC) technique was applied on formalin-fixed, paraffin-embedded tissues. Formalin-fixed paraffin-embedded sections (4 μm thick) were incubated overnight at 4ºC with a sheep anti-IL-6 antibody (1:1000, kindly donated by Prof. J. Landon, St. Barth-olomew's Hospital, London). The specificity of the immunostaining was verified by replacing primary antiserum with PBS and normal sheep serum, and by adsorption of antiserum with recombinant human IL-6 *(27)*. The IL-6 antibody showed no crossreactivity with other cytokines including IL-1. Parallel sections were immunostained for calcitonin (1:60, polyclonal) (Dako, A/S, Copenhagen, Denmark) in thyroid specimens, for insulin (1:60, monoclonal) (clone E2E3; Signet, Dedham, MA), glucagon (1:800, polyclonal) (Dako), and somatostatin (1:1500, polyclonal) (Dako) in pancreatic sections. To identify macrophages, immunostaining with a monoclonal anti-CD68 antibody (1:600) (clone KP1; Dako) was performed in parallel sections. Before application of the primary antibodies for IL-6, calcitonin and insulin, sections were pretreated for 10 min with 5 mg/mL of pronase (Sigma, St. Louis, MO). Sections forwarded for glucagon and DC-68 immunohistochemistry were immersed in sodium citrate buffer, pH 6.0, and treated with a high-pressure cooking method for 1 h *(5)*. The antigen-antibody-binding sites were visualized by the elite Vectastain kit (Vector Laboratories, Inc., Burlingame, CA) using as chromogen the diaminobenzidine supplemented with 1 mg/100 mL of nickel chloride (Sigma). No nuclear counter-staining was used.

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