# Pathomorphological and Immunohistochemical Analysis of Autoimmune Processes in the Thyroid Gland

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The morphology and function of TG were studied in residents of an East Kazakhstan region with unfavorable ecology. One-third of the population developed autoimmune processes in this organ. The morphogenesis and regeneratory processes in the thyroid gland against the background cellular infiltration were studied. Irrespective of the morphogenesis stage, the autoimmune processes in the thyroid gland were paralleled by dysregulatory disorders in the follicular epithelium which in many cases acquired immunohistochemical characteristics of the main cells of the parathyroid glands. Structural reorganization was paralleled by changes in the cell composition of the stroma, focal hyperplasia of oncocytes and C-cells, and enhanced angiogenesis.

**Key Words:** *thyroid gland; autoimmune processes; angiogenesis; immunohistochemistry*

Autoimmune diseases of the thyroid gland (TG) are a very prevalent pathology. Numerous studies have shown that none of endocrine diseases depends so much on the environmental status as TG diseases, which can be regarded as markers of ecological problems [3,6]. Autoimmune thyroiditis is diagnosed in 3-4% of population of Russia [2] and the incidence of autoimmune TG diseases in the local population increases [7]. Relationships were detected between deterioration of ecology and increased incidence of autoimmune processes (AIP) in TG observed in recent years in various regions [1,6,7]. This necessitates more profound study of morphological changes in the TG under these circumstances.

The presence of lymphoid elements in TG always indicates the presence of AIP. That is why it is so important to study the incidence and type of cellular infiltration, particularly at the early stages of AIP development. Some data indicate a relationship between autoimmune TG diseases and tumor processes in this organ [9,10]. Therefore, the study of changes in TG parenchyma and stroma in AIP by immunohistochemical methods [5] is an important problem.

We studied some morphofunctional characteristics of TG at different stages of AIP development.

## MATERIALS AND METHODS

The study was carried out on autopsy material from 572 patients dead from diseases not associated with TG pathologies. Of these, 192 cases with AIP in TG were detected, 42 of these were TG with Hashimoto's thyroiditis and 150 with cellular infiltration. The reference groups comprised 40 autopsy specimens from subjects dead from causes other than TG diseases and 32 specimens collected during interventions for TG diseases. The interventions were carried out mainly for nodular formations in TG.

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Stepped sections were prepared by the method of Christeller in Houston (USA) with the assistance of Prof. T. M. Viler and stained with hematoxylin and eosin; the step between two successive sections was 5 mm. The lobes of TG were fixed in 10% formalin. A total of 10,868 sections stained with hematoxylin and eosin were examined. Immunohistochemical analysis was carried out in 72 cases using serial sections of TG stained with various antisera.

Immunomorphological studies were carried out with assistance from Prof. I. Sekine (Japan). Thyroglobulin, calcitonin, parathyroid hormone, T and B lymphocyte markers, mast cells, chromogranin A, somatostatin, S-100 protein, CD34, vimentin, keratin, cyclin D, platelet endothelial growth factor (PEGF; marker of neoangiogenesis and endothelial cell proliferation) were detected by indirect immunoperoxidase method (antibodies from Novocastra Laboratories Ltd.). Apoptosis was detected by immunohistochemical method using an ApoTaq kit (Oncor, Gaithersburg). Immunohistochemical studies were carried out under identical conditions according to a stereotypical protocol, in which only the antiserum dilutions were taken into consideration.

The histological status of TG was evaluated using the protocol for evaluating organ's structure [4] distinguishing 3 structural patterns: 1) round or oval follicles, slightly eosinophilic colloid, and cubical or slightly flattened follicular epithelium; 2) disorganization of irregularly shaped follicles with somewhat enlarged epithelium; and 3) combination of these two variants.

Histological and immunomorphological studies were carried out using a semiquantitative method for evaluating the intensity of reactions and number of structures and cell elements [8]. The data were processed statistically using Data Analysis package from Microsoft Office Excel 2003 software.

## RESULTS

Autoimmune processes in TG were characterized by small foci of lymphoid infiltration or solitary cells in the majority of cases (52.6±3.6%). The majority of these infiltrates were located under TG capsule.

Morphological changes in TG during AIP did not involve the parenchyma or stoma alone; combined involvement of these compartments was characteristic (Fig. 1, *a*). The relationship between cellular infiltration of TG and restructuring of its parenchyma and stroma was confirmed by a significant ($p<0.05$) reduction in the number of observations with normal TG structure (type 1), detected in just 4.2±1.9% cases with cellular infiltration (type 1 structure was detected in 27.0±4.4% TG without cellular infiltration). No cases with normal TG structure were detected in Hashimoto's thyroiditis.

Foci of C-cell hyperplasia positively reacting to antiserum to calcitonin and chromogranin A (Fig. 1, *b*) were detected in TG with cellular infiltration. The count of C-cells in normal adult TG was negligible; they were found as solitary cells in 1 of 10-12 cases.

Small cellular infiltrations consisted mainly of T lymphocytes (Fig. 1, *c*), which diffusely infiltrated the organ tissue. B cells in these cases were just solitary (Fig. 1, *d*). Numerous B cells appeared with progress of cellular infiltration and formation of lymphoid follicles, which was paralleled by significant changes in TG parenchyma.

A relationship between the type of TG structure and oncocytic transformation of follicular epithelium was observed. In samples with oncocytes, type 1 TG structure was extremely rare (in just 1.8±1.2% cases); the number of oncocytes in these cases was low.

A relationship between restructuring of TG parenchyma and oncocytic transformation and cellular infiltration of the stroma was revealed. Disorders in differentiation of follicular epithelium were detected, which manifested in the presence of parathyroid hormone in it (Fig. 2, *a*). These changes were detected not in all follicles, but just in small ones, primarily in samples containing at least solitary oncocytes. In some cases we observed keratin expression in epithelial cells, which presumably attested differentiation disorders. Keratin expression was detected in 78.6% TG with AIP, and in some cases this expression was pronounced.

The expression of thyroglobulin in TG with cellular infiltration depended on its intensity in most cases. Expression of thyroglobulin in TG with minor cellular infiltration did not differ from its expression in normal TG, its intensity varying from minor to moderate.

The immunohistochemical profile of TG with small foci of lymphoid cells could differ significantly from that in normal TG. More intensive apoptosis was seen in foci of epithelium destruction and disorganization, adjacent to the focus of cellular infiltration (Fig. 2, *b*).

Restructuring of TG in AIP was paralleled by enhanced vascularization of the stroma. Angiogenesis in foci of restructuring and cellular infiltration was interpreted on the basis of PEGF detection. Vascular "buds" appeared even in small foci of cellular infiltration, near which the follicles underwent oncocytic transformation (Fig. 2, *c*). The

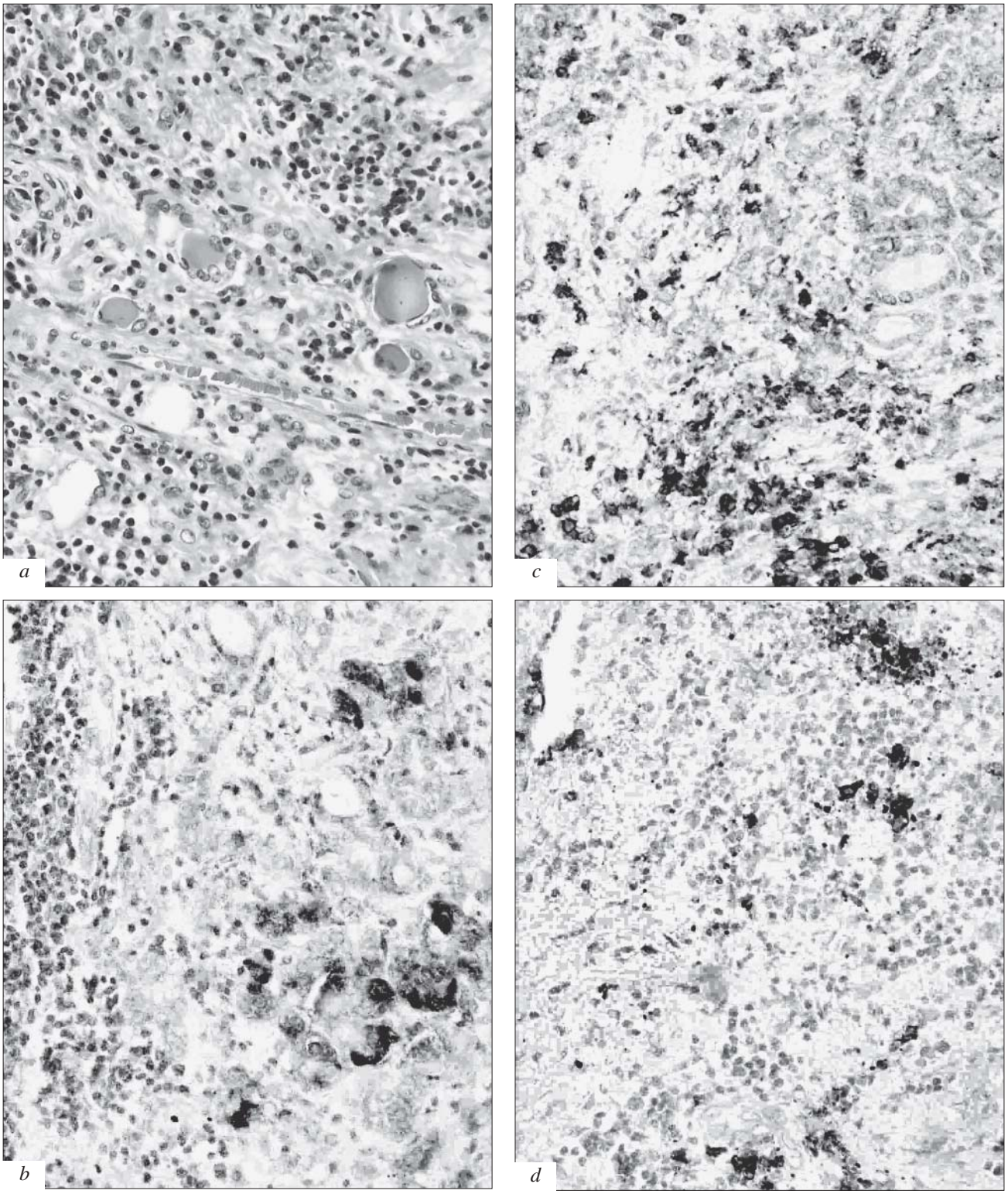


**Fig. 1.** Pathomorphological changes and immunohistochemical characterization of AIP in TG (the same site of the same organ), ×140. *a*) cellular infiltration and restructuring of the parenchyma. Hematoxylin and eosin staining; *b*) focal hyperplasia of C-cells. Reaction with chromogranin A; *c*) T cells (CD3); *d*) B cells (CB20).

presence of oncocytes in massive accumulations of lymphocytes also determined the formation of vascular "buds" among the infiltration cells and among oncocytes. The absence of oncocytes inhibited the formation of new vessels even in the presence of pronounced fibrosis (Fig. 2, *d*).

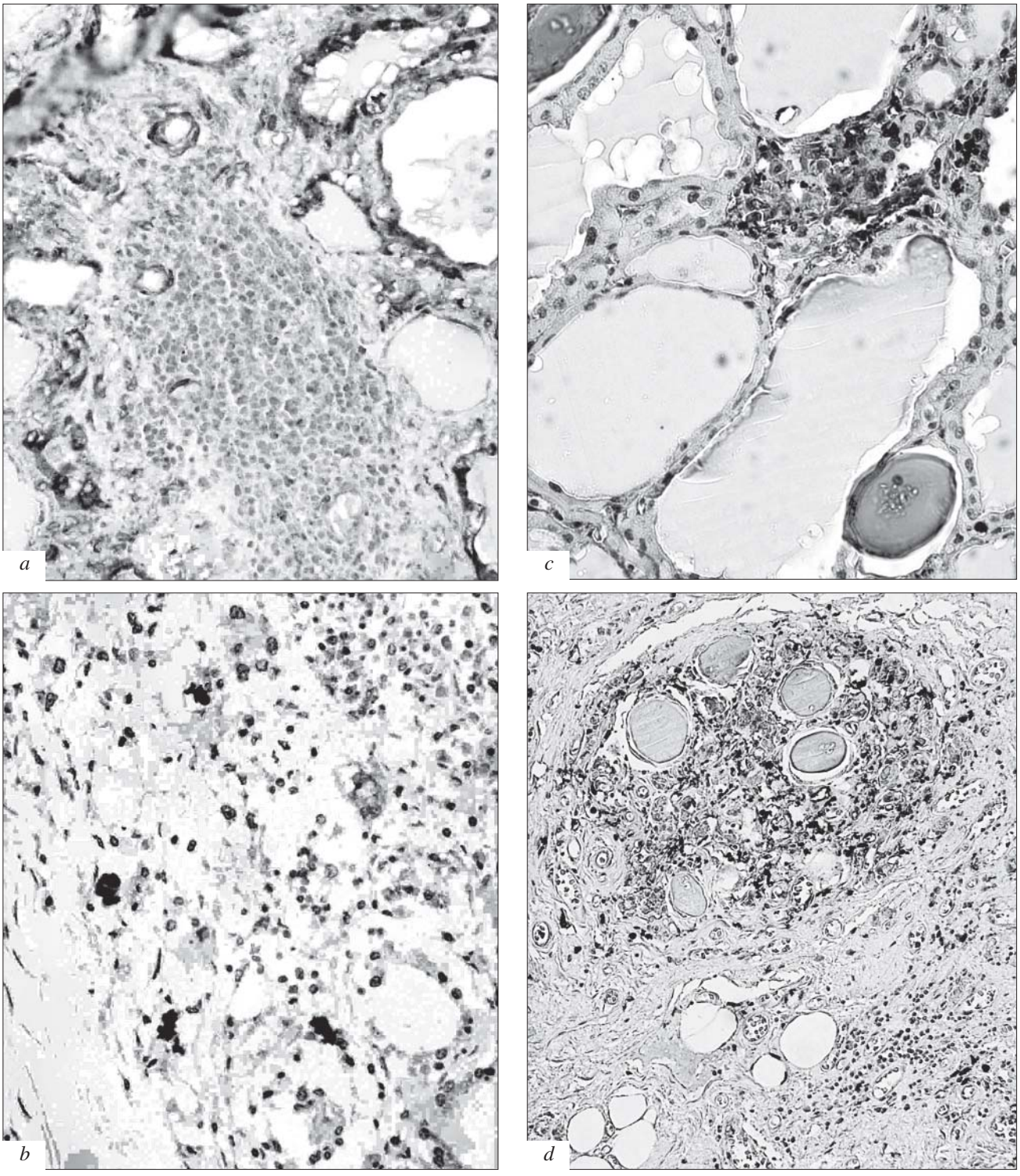


**Fig. 2.** Immunohistochemical characterization of AIP in TG. *a*) expression of parathyroid hormone in follicular epithelium, ×140; *b*) apoptosis in the zone of parenchyma restructuring, ×140; *c*) new vascular "buds" in a small focus of parenchyma restructuring. Reaction with PEGF, ×200; *d*) no PEGF expression in a site of cellular infiltration without parenchyma restructuring (upper right area on the picture). Numerous vascular "buds" in the restrutured nodule, ×63.

Hence, AIP in TG, irrespective of the morphogenesis stage, was paralleled by dysregeneratory disorders in the follicular epithelium, which in many cases acquired immunohistochemical characteristics of the main parathyroid cells. Structural reorganization was paralleled by changes in cell

composition of the stroma, focal hyperplasia of oncocytes and C-cells, more intensive angiogenesis. This indicates a relationship of changes in the parenchyma and stroma in TG during AIP and manifestation of this relationship at the earliest stages of the process. The progress of cellular infiltration in TG leads to reorganization of the parenchyma and appearance of oncocytes in the follicular epithelium. The formation of lymphoid follicles indicates progressing increase in the content of B cells, this process correlating with restructuring and neoangiogenesis processes. It seems that these processes, starting from the appearance of solitary lymphocytes and eventuating in manifest morphological changes, indicate a succession of autoimmune thyroiditis stages. Hashimoto's thyroiditis is the final stage of AIP in TG.

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