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Intrathyroidal Dendritic Cells, Epitheloid Cells, and Giant Cells in Iodine Deficient Goiter

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Immunohistochemistry and immunofluorescence were performed on thyroid sections of 44 consecutive patients undergoing thyroid surgery for goiter due to iodine deficiency. Sections were compared with specimens from ten individuals without goiters from the same endemic area, with specimens from ten sporadic nontoxic goiter patients, and with specimens from an area with sufficient iodine supply from nine healthy subjects. Cells were characterized using monoclonal antibodies to the CR3 receptor (CD11b) and the p150/95 antigen (CD11c) present on macrophages, to HLA-DR, to antigen presenting cells (RFD1), to T helper (CD4) and to T suppressor/cytotoxic cells (CD8), and with a polyclonal antibody to human cytokeratin. In iodine deficient goiters, focal aggregates were found of RFD1-positive dendritic cells. Furthermore, RFD1-positive epitheloid cells were seen. In 27% of cases, these epitheloid cells completely filled the thyroid follicles. Within the epitheloid cell clusters, multinucleated giant cells could be detected that carried the macrophage markers. Dendritic cells, epitheloid cells, and giant cells were strongly HLA-DR positive. In nongoitrous thyroids from the endemic area such aggregates could also be seen but they were more sparse and were RFD1 negative. Giant cells were absent there. In normal thyroids with sufficient iodine supply, only a few isolated dendritic cells were seen. All except RFD1, which was negative, showed the same marker pattern. In sporadic nontoxic goiters from an area with sufficient iodine supply, dendritic cells occurred in much higher numbers than in the normal thyroids from that area, and they were RFD1 positive. They never aggregated as in iodine deficiency, and giant cells were not observed. These observations on iodine deficient goiter strongly suggest involvement of active antigen-presenting cells in this disorder. However, the immunohistologic difference between this disease and sporadic goiter suggests different underlying mechanisms. (Am J Pathol 1989, 135: 219–225)

Iodine deficiency per se is only considered part of the cause of formation of goiters in an iodine deficient area.[1] We studied the involvement of the immune system in iodine deficiency by immunohistologic techniques comparing goiters due to iodine deficiency with sporadic nontoxic goiters that are believed to represent an autoimmune disease.[2] Our study focused on dendritic cells. Dendritic cells belong to the group of accessory cells in immune responses, as do the Langerhans' cells in the epidermis and dermis, the veiled cells in lymph fluid, and the interdigitating cells in the T cell areas of the thymus, spleen, and lymph nodes.[3] These cells are of crucial importance in the presentation of antigen during the elicitation of primary immune responses.[4] They are of monocyte origin[5] (see also Kabel PJ, manuscript in preparation) and are characterized by their dendritic shape and strong membrane-bound class II MHC positivity combined with little or no cytoplasmic acid phosphatase activity. Like macrophages they are positive for the CR3 receptor and the adhesion molecule p150/95. They differ from macrophages in that they strongly bind RFD1, a monoclonal antibody that is believed to react with a molecule functionally

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relevant for antigen presentation and is probably related to MHC class II molecules.[6] Recently we reported that such dendritic cells were scarcely present in normal thyroids, but occurred in high numbers in Graves' and Hashimoto's disease, and to a lesser extent also in sporadic nontoxic goiters.[7] They are believed to travel from the thyroid to the draining lymph node, triggering the autoimmune response.[8,9]

Materials and Methods

Patients and Control Subjects

Thyroid tissue was obtained from 44 consecutive euthyroid patients operated on for goiter due to iodine deficiency (37 women and seven men with a mean age of 51 years; range, 24 to 71 years). Preoperative thyrostatic treatment consisted of thiamizol alone (seven patients) or in combination with iodine treatment (37 patients). Indications for operation were a very large or rapidly growing goiter or a multinodular goiter suspicious for the presence of adenoma. Thyroid specimens from ten individuals not having a goiter, but living in the same iodine-deficient area as the patients with goiters, were studied (five thyroids were obtained at laryngectomy for carcinoma of the larynx without preoperative chemotherapy or radiation therapy and 5 specimens were obtained at autopsy from four women and six men with a mean age of 58 years, range, 39 to 82 years). Nine thyroid specimens from an area with sufficient iodine supply (Amsterdam, the Netherlands) were studied (three obtained at autopsy and six at laryngectomy from four women and five men with a mean age 68 years; range, 49 to 89 years). Furthermore, we studied thyroid tissue from ten patients with sporadic nontoxic goiters living in Amsterdam (eight women and two men with a mean age of 50 years; range, 24 to 88 years).

Antibodies

The following monoclonal antibodies (MoAb) were used: Leu M5 (CD11c) directed to the p150/95 antigen, Leu 2a (CD8, T suppressor/cytotoxic cells), Leu 3a (CD4, T helper cells), (Becton and Dickinson, Mountain View, CA), FK24 (CD 11b, Sanbio) directed to the CR3 receptor, Okla to class II MHC antigens (Orthodiagnostics, Raritan, NY), RFD1 specific for antigen-presenting cells and B cells,[6] and polyclonal rabbit anti-human cytokeratin antibody.

Controls were: a) the second reagent only, b) normal mouse and rabbit serum instead of the specific antibodies. These controls were negative.

Immunohistochemistry

Small tissue parts of the removed glands were frozen and stored in liquid nitrogen. Serial sections of 6 μm were mounted on slides, air dried, and fixed in acetone. The slides were incubated with the first antibodies for 1 hour at room temperature. Thereafter two different immunohistochemical techniques were used.

Peroxidase Labeling

After three washes in PBS the preparations were incubated for 30 minutes in a horseradish peroxidase conjugate solution (Dako, Copenhagen, Denmark). Subsequently, sections were rinsed three times in PBS and stained for peroxidase activity with 3.3′-diaminobenzidine-tetra-HCL (Sigma, St. Louis, MO) in PBS containing 0.01% H_2O_2. In addition, section were examined for acid phosphatase activity according to the method of Burnstone, with naphtol AS-BI phosphate as substrate and hexazotized pararosaniline as diazonium salt.[10]

Alkaline Phosphatase: Anti-alkaline Phosphatase Complex Labeling (APAAP)

After three washes in PBS the slides were incubated for 30 minutes in rabbit anti-mouse IgG diluted 1:30 in 20% human plasma/PBS (Dako, Denmark). After washing in PBS the sections were incubated with an APAAP complex ready for use (diluted 1:100 in PBS; Dako, Copenhagen, Denmark). APAAP activity was visualized using a Fast Red/Naphtol AS-BI solution diluted in a veronal-acetate-buffer by addition of 100 mg levamisol (Sigma, St. Louis, MO) per 50 ml of the mixture for 45 minutes at room temperature.

Double-labeling Immunofluorescence

Serial cryostat sections of 6 μm were air dried and fixed in acetone. Sections were labeled with Leu M5 for 30 minutes and washed three times in PBS. Thereafter they were incubated with Texas red-conjugated sheep anti-mouse IgG (Amersham, Buckinghamshire, England) for 30 minutes washed three times, and incubated with anti-cytokeratin antibody. After three washings, the slides were incubated with the FITC-conjugated goat anti-rabbit IgG$(Fab)_2$ (Jackson Immuno Research Lab, West Grove, California).

The conjugates were used in 1:50 dilutions. After three more washings and dehydration in pure alcohol the slides were mounted in Mowiol (Hoechst, Frankfurt, West Germany).

Positive controls for double-labeling immunofluorescence were cryostat sections of lymph nodes incubated with Leu 1 (CD5), followed by Leu 3a (CD3). Both monoclonals were consecutively treated with Texas Red-conjugated sheep anti-mouse IgG or FITC-conjugated goat anti-rabbit IgG(Fab)$_2$. In the paracortical or interfollicular areas, approximately 75% of the Leu 1 positive cells coexpressed membrane fluorescence with Leu 3a. The choice of conjugated anti-mouse IgG did not influence results.

Results

Iodine Deficient Goiters

With the used monoclonal antibodies, iodine deficient goiters showed a remarkable immunohistochemical picture that has not been described. Dendritic cells were seen in the interstitium where they clustered in focal aggregates (Figure 1). Such aggregates were seen in 53% of these goiters. The cells were strongly positive for HLA-DR, for the p150/95 antigen, and for the CR3 receptor. Most of the dendritic cells possessed the RFD1 marker molecules. The cells were negative for acid phosphatase. Dendritic cells seemed to infiltrate thyroid follicles from the interstitium as is shown in Figure 1. Inside follicles, epitheloid cell clusters (Figure 2) were found in 39% of the cases, and in 27% completely filled the thyroid follicles (Figure 2A). The epitheloid cells had the same marker pattern as the dendritic cells but showed a weak acid phosphatase activity. Cells lining the thyroid follicle occasionally showed a cytoplasmic reaction for RFD1 (Figure 1B), for the p150/95 antigen, and for the CR3 receptor (Figures 2C and 2D). To examine whether or not these cells were of epithelial origin, we performed double-labeling immunofluorescence with the monoclonal antibody to the p150/95 antigen and anticytokeratin antibody. This showed no double-labeled cells, thus indicating that cells belonging to the monocyte–macrophage lineage had replaced the epithelial thyrocytes. The described marker pattern of the dendritic and the epitheloid cells strongly suggests their activity in antigen handling and presentation.

A further remarkable finding was the presence of multinucleated giant cells in 25% of goiters (Figures 2B, 2C, and 2D). These cells had the morphology of foreign body giant cells and showed two to six nuclei. They located in the interstitium as well as in the thyroid follicles, and were clearly positive for HLA-DR, the p150/95 antigen, and the CR3 receptor and weakly positive for acid phosphatase. No RFD1-positive giant cells were observed. Lymphocytic infiltrates around the described cell aggregates were either absent or very scarce. Four specimens did contain lymphocytic infiltrates and in two cases even showed a follicle center reaction, but their localization was not related to the cell clusters described above. The lymphocytic infiltrates without follicle center reaction contained mainly T cells, with a 1:1 distribution of Th:Ts/c cells. Follicle center reactions, when present, contained mainly B cells positive for RFD1. Very large dendritic cells positive for the p150/95 antigen and the CR3 receptor and strongly acid phosphatase positive were observed between the B cells. The lymphocytes surrounding the follicle center were mainly T cells with a 2:1 distribution of Th:Ts/c cells. Between this rim of T cells there were dendritic cells strongly positive for the p150/95 antigen and the CR3 receptor, as well as for RFD1. These cells were only weakly positive for acid phosphatase. In connection with the lymphocytic infiltrations, HLA-DR-positive thyrocytes were observed coexpressing positivity for the used anticytokeratinantibody in double-labeling immunofluorescence.

Nongoitrous Thyroids from the Iodine Deficient Area

Non-goitrous thyroids from the same iodine deficient area also showed dendritic cell aggregates in three of ten cases and epitheloid intrafollicular cell clusters in two of ten cases. However, they were more scarce as was the case with the goitrous specimens. The cells showed the same reactivity for HLA-DR, the p150/95 antigens, and the CR3 receptor. The difference between goitrous and nongoitrous tissues consisted of the strong expression of the RFD1 molecule on dendritic and epitheloid cells in the goitrous thyroids and its absence in the nongoitrous ones. This suggests that in the latter cases these cells had no or very little activity in antigen presentation. Giant cells were not observed in nongoitrous tissues.

Sporadic Nontoxic Goiters

We compared the observed thyroid morphology in iodine deficiency with sporadic nontoxic goiters. As described before,[7] there were many dendritic cells scattered throughout the interstitium in these thyroids. They never built aggregates as in iodine deficiency, and epitheloid cells or giant cells were not observed. The dendritic cells showed the same marker pattern as that seen in iodine deficient goiters.

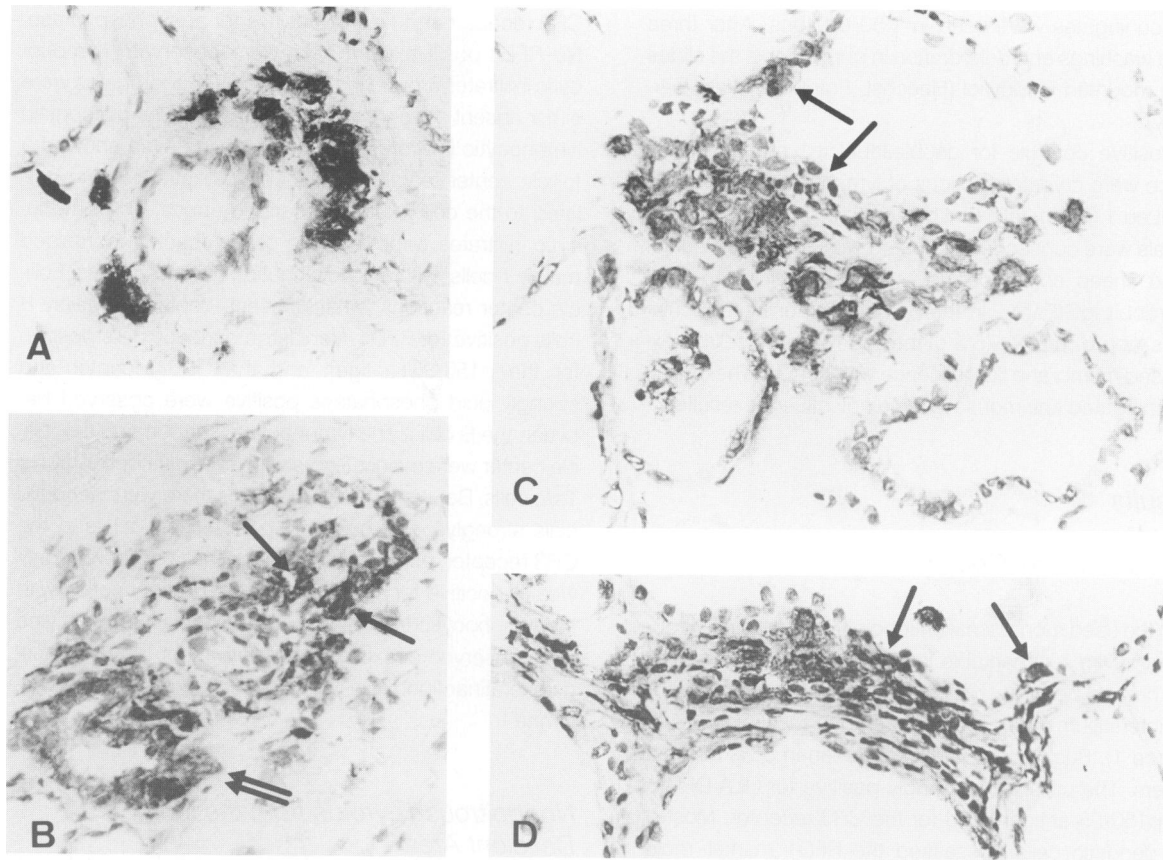

Figure 1. *Indirect immunoperoxidase staining of interstitial aggregates of dendritic cells in iodine deficient goiter.* **A:** *Dendritic cells strongly positive for the p150/95 antigen (Leu M5, CD11c) in the interstitium surrounding a thyroid follicle (×400).* **B:** *Interstitial dendritic cells (arrows) and cells lining the thyroid follicle (double arrow) positive for RFD1 (×400).* **C:** *Intersitial RFD1-positive dendritic cells form aggregates and infiltrate the thyroid epithelium (arrows) (×250).* **D:** *The same as in C, but stained with the monoclonal antibody to the p150/95 antigen (Leu M5, CD11c) (×400).*

Normal Thyroids with Sufficient Iodine Supply

In normal thyroids from an area with sufficient iodine supply dendritic cells were also clearly present but very scarce, and were isolated in the interstitium. Again they showed the described marker pattern, except RFD1, which was not detectable in these thyroids. In areas with sufficient iodine, the same difference regarding RFD1 expression was found between goitrous and nongoitrous thyroids as was found under iodine-deficient conditions. Table 1 gives an overview of the described findings.

Discussion

This study describes RFD1-positive dendritic cells in iodine-deficient goiters and their presence in focal clusters, RFD1 positive epitheloid cells, and multinucleated giant cells filling thyroid follicles. Occasionally it was observed that dendritic cells and epitheloid cells were located between the cells lining the follicles, suggesting that the follicles were infiltrated from the interstitium. On the basis of this light microscopic study it is difficult to say whether intermediate forms of dendritic and epitheloid cells were present. Dendritic cells may be the precursors of epitheloid cells but it is also possible that epitheloid cells infiltrate the thyroid separately. They are both very likely to be involved in antigen presentation because they carry RFD1 molecules, which are characteristic for antigen-presenting interdigitating cells in secondary lymphoid organs.[6] On some occasions the thyroid follicle lining consisted of cells bearing markers of the monocyte–macrophage lineage, and epithelial thyrocytes seemed to be replaced by them.

In iodine deficient goiters, HLA-DR expression was found on dendritic cells, epitheloid cells, and giant cells. MHC Class II expression was recently reported to be present on thyroid follicular cells in 16% of nonautoimmune thyroid glands,[11] and was mostly associated with lymphocytic infiltration. This focal thyroiditis is a common finding

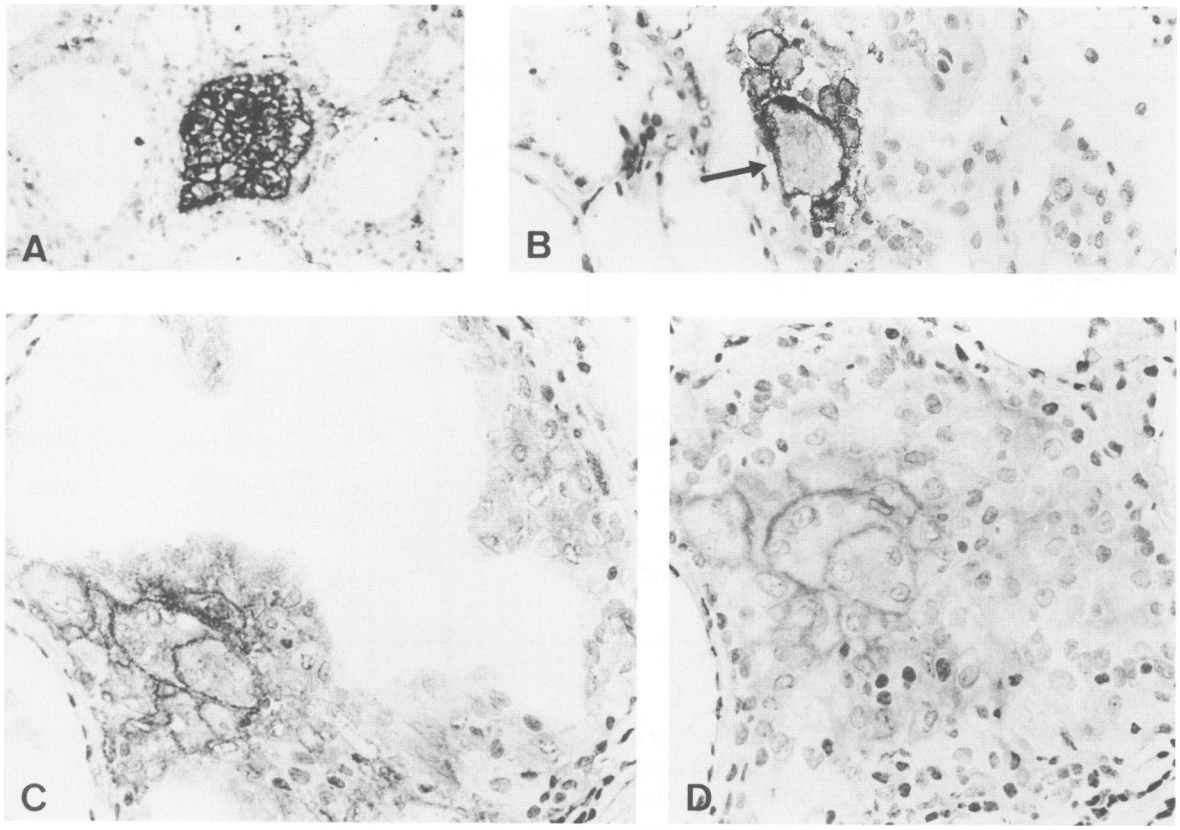

Figure 2. *Indirect immunoperoxidase staining of intrafollicular epitheloid cells and giant cells in iodine deficient goiter.* **A:** *Thyroid follicle completely filled with epitheloid cells, positive for the p150/95 antigen (Leu M5, CD11c) (×160).* **B:** *Multinucleated giant cell (arrow), positive for the p150/95 antigen (Leu M5, CD11c).* **C:** *Multinucleated cells with strong membrane-bound positivity for the CR3 receptor (FK24, CD11b). Note the slight cytoplasmic positivity for this marker of the cells lining thyroid follicles.* **D:** *The same as in C. The thyroid follicle is completely filled with large epitheloid cells and giant cells positive for the CR3 receptor (FK 24, CD11c) (B, C, and D, ×400).*

when normal or nodular thyroids are examined. We also found lymphocytic infiltrates in 10% of the iodine deficient goiters and can confirm the relationship between these infiltrates and HLA-DR expression on thyrocytes. However, HLA-DR expression on dendritic, epitheloid, and giant cells occurred without lymphocytic infiltrations.

The described findings contrasted with those in normal thyroids, sporadic nontoxic goiters, and normal thyroids from the iodine deficient area. Because the occurrence of the described cell aggregates is focal and mostly devoid of lymphocytic infiltrations, the question of their possible function arises. The total absence of these aggregates in thyroids with sufficient iodine supply strongly suggests that the iodine shortage causes their presence. It is noteworthy that a striking light microscopic similarity exists between intrafollicular cell aggregates described in this study and in a recent report on amiodarone-associated thyroid histopathology.[12] Amiodarone is an antiarrhythmic agent containing 37% iodine by weight. Iodine overloading is considered responsible for the thyroid pathology seen in patients receiving amiodarone. However, no monoclonal markers were used and no data are available on the occurrence of dendritic cells.

Giant cells can be seen in other thyroid conditions such as subacute thyroiditis (De Quervain). This condition coincides with a painful thyroid, which was not a finding in our patients. Also, other causes of follicular damage can be ruled out such as palpitation thyroiditis, first because one can assume that noniodine deficient goiters react to palpation in ways similar to noniodine deficient goiters, and second because the damage in palpitaion thyroiditis shows a different histopathology. In this latter condition the follicle lining is interrupted and colloid invades into the stromal tissues. The epithelial cells are swollen and degenerated, and lymphocytes and plasma cells invade the follicles. These are occasionally filled with foamy histiocytes. Multinucleated giant cells are only occasionally present. There may be considerable fibrosis and vascular lesions.[13] The described findings, therefore, seem to be a specific reaction of the immune system to an aberrant iodination degree, either iodine deficiency or iodine overload.

Table 1. *Table Title*

	Findings	p150/95 antigen	C3bi receptor	RFD1	Acid phosphatase
Normal iodine sufficient thyroids (N = 9)					
Dendritic cells	Isolated; scarce	+++	++/−	−	−
Epitheloid cells	Absent				
Giant cells	Absent				
Sporadic nontoxic goiters (N = 10)					
Dendritic cells	Isolated	+++	++/−	+++	−
Epitheloid cells	Absent				
Giant cells	Absent				
Iodine deficient goiters (N = 44)					
Dendritic cells	Focal aggregates in 53%	+++	++/+	+++	−
Epitheloid cells	In 39%; thyroid follicles stuffed in 27%	+++	+	++/+	Weak
Giant cells	In 25%	+++	++	−	Weak
Normal thyroids endemic area (N = 10)					
Dendritic cells	Focal aggregates in 33%	+++	++/+	−	−
Epitheloid cells	In 20%	+++	+	−	Weak
Giant cells	Absent				

Iodine therapy (Lugol's solution) is commonly used as preoperative treatment. However, we can rule out preoperative iodine overloading as a cause of the observed cell clustering because seven of our 44 iodine deficient goiter patients did not get this treatment; of these seven, three had the described immunohistochemical characteristics. The degree of iodination of thyroblobulin, whether high or low, may alter its antigenicity. It has been reported that alteration of the three-dimensional structure of thyroglobulin depends on hormone content and probably also occurs during the iodination process.[14] In the chicken, highly iodinated thyroglobulin is believed to be more immunogenic than low iodinated thyroglobulin,[15] but in mice noniodinated thyroglobulin is also highly antigenic.[16] Antigens other than thyroglobulin may also play a role. The actual lack of sufficient iodination may promote alteration of certain autoantigens or may activate dendritic cells. A reaction of the immune system to the state of iodine deficiency is furthermore suggested by reports on the presence of thyroid growth-promoting antibodies in sera of endemic goiter patients (Wilders-Truschnig et al, manuscript in preparation).[17,18] Thus, autoimmune effects are not confined to thyroid disorders such as Graves' disease, Hashimoto thyroiditis, and sporadic nontoxic goiter. The striking difference between the immunohistologic picture of sporadic and iodine deficient goiters, however, makes it likely that there are different immunologic mechanisms involved. Sporadic goiter is considered to be an autoimmune dysfunction,[2] whether or not in iodine deficiency the metabolic state or the hampered hormone production predisposes the otherwise normal immune system to react with local thyroid components or autoantigens.

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