The prevalence of immunological abnormalities in endemic simple goitre

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Abstract. Thyroid growth stimulating immunoglobulins microsomal antibodies and antibodies against thyroglobulin were determined in patients with simple goitre (n = 20) and controls (n = 6) living in an iodine deficient area. In addition, lymphocytic infiltration of thyroid tissue, the amount of the various lymphocyte subsets (Leu 4+, Leu 3a+, and Leu 2a+ T-cells as well as B1+ B cells) in the thyroid gland, as well as the expression of the histocompatibility antigen HLA-DR on thyocytes and intrathyroidal T-lymphocytes were examined. Goitrous patients were subdivided into two groups according to their individual iodine supply estimated by iodine excretion values, and immunological parameters were compared between patients with low (group A, iodine excretion < 70 µg/24 h) and with higher (group B, iodine excretion > 100 µg/24 h) iodine supply. Thyroid growth stimulating immunoglobulins and antithyroid antibodies were equally prevalent in the two patient groups, but were absent in controls. Lymphocytic infiltration of thyroid tissue was present to a comparable extent in patients of groups A and B, but to a distinctly lower degree in control persons. Intrathyroidal T-lymphocyte subsets did not differ between patients and controls. B-lymphocytes, germinal centres as well as DR+ thyocytes were detected in goitrous patients of both groups, but never in control persons. Thus, immunological abnormalities frequently occur in patients with simple goitre and do not depend upon individual iodine supply.

The importance of iodine deficiency for the development of endemic simple goitre is generally recognized (Ermans 1978). However, epidemiologic data clearly show that an insufficient iodine supply does not necessarily result in goitrogenesis (Choufleur et al. 1963; Delange et al. 1971; Roche 1959). Thus additional pathogenetic mechanisms seem to be of relevance (Chopra et al. 1975; Ermans 1978). In view of the frequent finding of a lymphocytic infiltration of the thyroid gland correlating with the occurrence of thyroglobulin and antimircoosomal antibodies (Bastenie et al. 1972; Schade et al. 1960) as well as the recent demonstration of thyroid growth stimulating immunoglobulins in patients with simple goitre (Chiovato et al. 1983; Drexhage et al. 1980; McMullan & Smyth 1984; Schatz et al. 1983; Van der Gaag et al. 1985) a disturbance of the immune system seems possible in this context. It was the aim of the present study 1) to determine the prevalence of immunological abnormalities in patients with endemic simple goitre, and 2) to investigate whether the occurrence of such abnormalities was dependent on the state of iodine supply.

Materials and Methods

Patients

Twenty patients with long-standing multinodular nontoxic goitre (3 men, 17 women, aged 44 ± 12, range 17–69 years) were studied. In all patients radionuclide thyroid scans with ⁹⁹ᵐTc showed an irregular patchy uptake. Patients were divided into two groups on the basis of iodine excretion values: A) patients with extremely low iodine supply (iodine excretion < 70 µg/
24 h, mean: 52.9 ± 15.8 µg/24 h, range 28.5–68 µg/24 h, n = 10); B) patients with higher iodine supply (iodine excretion > 100 µg/24 h, mean: 367 ± 448, range 101–1420 µg/24 h, n = 10). Iodine excretion was determined on three different occasions, and mean values were calculated. However, day to day variations were negligible in all patients investigated. Serum total T₄ (TT₄) and T₃ (TT₃) were within the normal range in all patients. TT₄ was 7.1 ± 1.5 µg/100 ml in group A and 8.3 ± 1.9 µg/100 ml in group B. Serum TT₃ was 145 ± 27 ng/ml in group A and 121 ± 19 ng/ml in group B. Stimulation of plasma TSH by TRH (400 µg TRH iv) was suppressed in two patients of each group, but normal in all the others. Antibodies against thyroglobulin (TGA), microsomal antibodies (MSA) as well as thyroid growth stimulating immunoglobulins (TGI) were determined. None of the patients received any thyroid hormone or antithyroid drug treatment. All patients underwent thyroidectomy and were classified as nodular goitre on the basis of histology. Tissue specimens from three different areas of thyroid glands were collected for immunological investigation. The extent of lymphocytic infiltration, intrathyroidal lymphocytic subsets (Leu 4⁺ = total T, Leu 3a⁺ = helper/inducer and Leu 2a⁺ = suppressor/cytotoxic T-cells and B1⁺ B-cells) as well as the expression of the histocompatibility antigen HLA-DR on thyrocytes, intrathyroidal T-lymphocytes and macrophages were studied.

Controls
Six persons with normal thyroid glands (all men aged 63 ± 7 years) served as controls. All suffered from carcinoma of the larynx, and thyroid tissue specimens were obtained at laryngectomy. Peripheral as well as tissue investigations were performed as described above for goitrous patients. Serum TT₄, TT₃ and basal as well as TRH stimulated TSH plasma concentrations were within the normal range in all control persons.

Laboratory method
TT₄, TT₃ and TSH were determined by RIA, TGA and MSA were measured with the tanned cell haemagglutination technique (Burrough-Wellcome, Beckenham, UK). TGI were assayed in IgG concentrates (Shewring & Smith 1982) using a highly sensitive cytochemical bioassay (CBA) based on the measurement of changes in glucose-6-phosphate dehydrogenase (G6PD) activity in guinea pig thyroid follicular cells (Drexhage et al. 1982; McMullan & Smyth 1984). In short, segments of guinea pig thyroid were incubated for 5 h at 37°C with IgG concentrates (50 and 500 µg/ml) prepared from patients plasma. After incubation segments were snap frozen at −70°C, 10 µm frozen sections cut on a cryostat and reacted for glucose-6-phosphate dehydrogenase (G6PD) activity which was quantitated in thyroid follicular cells by scanning and integrating microdensitometry as integrated extinction (IE) units X 100. Results for TGI were expressed as the maximum % increase in G6PD activity in thyroid follicular cells from segments incubated with IgG concentrate compared to the activity in cells from segments incubated with medium alone: TGI values > 8% were termed positive.

Lymphocyte and HLA studies on thyroid tissue were performed as follows: tissue was cut into small blocks, was frozen in liquid nitrogen immediately after thyroidectomy and was stored at −80°C. Four µm frozen cryostat sections were cut from each tissue sample. The staining of intrathyroidal lymphocytes was performed by the immunoperoxidase technique (Stein et al. 1980) using monoclonal antibodies against Leu 4⁺, Leu 3a⁺ and Leu 2a⁺ T-lymphocytes (Becton Dickinson) as well as against B1⁺ B-lymphocytes (Coulter Electronics Inc, Hialeah, FL). The expression of the histocompatibility antigen HLA-DR on thyroid follicular cells, intrathyroidal lymphocytes and macrophages also was examined by immunoperoxidase technique (Stein et al. 1980) using the monoclonal antibody anti HLA-DR (Becton Dickinson) specific to the non-polymorphic region of the DR molecule. Lymphocytic infiltration was classified per high power field as diffuse and focal. Diffusely infiltrating lymphocytes were enumerated within 20 high power fields (X 40). The numbers of Leu 4⁺, Leu 3a⁺ and Leu 2a⁺ T- and B1⁺ B-lymphocytes were counted. The Leu 3a⁺/Leu 2a⁺ cell ratio was evaluated. Lymphocytic foci were counted within 10 high power fields (X 10). The numbers of the various lymphocyte subpopulations within foci was evaluated semiquantitatively (−) absence, (+) few cells, (+++) numerous and (++++) numerous. Scattered lymphocytes as well as lymphocytic foci were determined in each tissue specimen (3 specimens/gland), and data given present mean values of three locally distinct tissue areas of one gland. The occurrence of lymphoid germinal centres as well as of HLA-DR⁺ thyrocytes, DR⁺ T-lymphocytes and of macrophages was noted. The numbers of DR⁺ cells were evaluated semiquantitatively as described for local lymphocytes. Data in text and figures are presented as mean ± SD. Student’s t-test and correlation analysis were used for statistical evaluation.

Results
Antibodies against thyroglobulin were present in 35% of the goitrous patients (5/10 patients of group A and 2/10 patients of group B), but in none of the control persons (Table 1). Titres ranged from 1:320–1:2560 in group A and were 1:160 and 1:1280, respectively, in group B. Microsomal antibodies were found in 50% of the goitrous patients (6/10 patients of group A and 4/10 patients of group B) and in one (16.7%, titre
1:100) of the controls. Titres ranging from 1:100–1:1600 in group A and from 1:50–1:400 in group B did not differ between goitrous patients with low and high iodine supply ($P > 0.05$).

TGI were detectable in 12/20 (60%) of the goitrous patients, but in none of the control persons. Peak responses for G6PD activity occurred at an IgG-concentration of 50 µg/ml in 5 patients (range 25%–70% stimulation) and at an IgG concentration of 500 µg/ml in 7 (range 10%–28% stimulation). In each group 6/10 patients presented with positive TGI titres, in group A 3/10 and in group B 2/10 at an IgG concentration of 50 µg/ml. TGI titres did not differ between the groups ($P > 0.05$).

Lymphocytic infiltration was present in all thyroid tissue samples investigated (Table 2). Scattered lymphocytes were found to a comparable extent in goitrous tissue of patients of group A and B as well as in control specimens obtained at laryngectomies. Neither the number of the various T-lymphocyte subsets nor the Leu 3a+/Leu 2a+-ratio in the thyroid tissue differed between patients and control persons ($P > 0.05$).

Lymphocytic foci were detected in 18/20 (90%) of the goitrous patients (9/10 patients of group A and B). Among these patients the number of foci did not differ ($P > 0.05$). In two of the 6 control persons (33.3%) small lymphocytic foci (4 and 2 foci/10 fields, respectively) were found.

Germinal centres were noted in 6/20 (30%) of the goitrous patients (4/10 in group A and 2/10 in group B), but never in control persons. B1+ cells occurred only in germinal centres. Semiquanti-
tative analysis of T-lymphocyte subsets within lymphocytic foci did not reveal the pre-dominance of one subpopulation. Thyrocytes of 16/20 (80%) of the goitrous patients (7 patients of group A and 9 patients of group B), but of none of the control persons stained strongly with anti HLA-DR. HLA-DR staining always occurred in patches. DR+ activated T-lymphocytes were found in the thyroid tissue of 14/20 (70%) of the goitrous patients (6 patients of group A and 8 patients of group B) and in one of the control persons. Large amounts of DR+ macrophages were present in 16/20 (80%) of the goitrous patients (7 patients of group A and 9 patients of group B), but in none of the control persons.

Table 3 demonstrates the semiquantitative evaluation of the numbers of DR+ cells in the individual goitrous patients of both groups. It is evident that similar amounts of DR+ thyrocytes and T-lymphocytes were found in patients of group A and of group B. DR+ thyrocytes were always adjacent to DR+ macrophages and adjacent to activated T-lymphocytes in 14 cases.

None of the parameters investigated differed between male and female patients. In the individual patients there was no significant correlation between the various immunological parameters studied (antibody titres versus number of lymphocytic foci or number of DR+ cells, respectively). However, the two patients in whom no lymphocytic infiltration of thyroid tissue was found (one in group A and one in group B) did neither present with aberrant DR expression on thyrocytes nor with antibodies against thyroglobulin or microsomal antigen. TGI were negative in one and a borderline titre (14%) was detected in the other one.

Discussion

Our findings of a lymphocytic infiltration of the thyroid gland and of positive autoantibody titres in the majority of goitrous patients agree with earlier reports demonstrating focal lymphocytic thyroiditis associated with circulating antibodies to thyroglobulin as well as to thyroid microsoma in a high percentage of patients with non-toxic nodular goitre (Bastenie et al. 1972; Schade et al. 1960). These results as well as the detection of growth stimulating immunoglobulins in 60% of our goitrous patients seem to suggest that autoimmune processes may be relevant in the pathogenesis of simple goitre. The percentage of positive TGI results in patients with simple goitre is comparable to data by Van der Gaag et al. (1985), who used a similar bioassay system, but higher than in comparable studies in which [H]thymidine incorporation assays of less sensitivity were applied (Chiovato et al. 1983; Schatz et al. 1983; Valente et al. 1983). This discrepancy may be explained by the sensitivity of the respective assays used. The finding that peak increases in G6PD activity were observed at relatively high IgG con-

Table 3.

Semiquantitative evaluation of HLA-DR expressing thyrocytes, intrathyroidal T-lymphocytes and macrophages in patients with simple goitre.

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centrations (500 μg/ml) in the majority of goitrous patients further suggests that growth stimulators, when present, are of minor potency.

The aberrant expression of the histocompatibility antigen HLA-DR on thyroid epithelial cells and the infiltration of thyroid tissue with activated T-lymphocytes were first described in Hashimoto's thyroiditis and Graves' disease (Hanafusa et al. 1983) and postulated to be markers of autoimmunogenic activity of the thyroid gland (Bottazzo et al. 1983). Recently it has been demonstrated that DR+ thryocytes can present antigen (Londei et al. 1984) and are recognized by autologous T-lymocytes (Londei et al. 1985) in Graves' disease. The finding of HLA-DR+ thryocytes in the majority of the patients with simple goitre studied is in accordance with earlier data obtained in patients with autonomously functioning thyroid nodules (Grubeck-Loebenstein et al. 1985). It seems further to underline the theory that autoimmune mechanisms may be of relevance in the pathogenesis of these disorders. However, the coexistence of multinodular goitre and possibly secondary autoimmune thyroid disease cannot be ruled out. In view of the close local relationship between DR+ thryocytes and macrophages observed, similar evidence as obtained in Graves' disease will be needed to prove that aberrant DR-expression on thryocytes also implies autoantigen presentation in thyroid diseases sofar generally classified as non-autoimmune.

There was no statistically significant correlation between the various immunological parameters studied within the individual patients. This may, however, be due to the relatively small numbers of patients in the various groups. The simultaneous occurrence of thyroid autoantibodies and lymphocytic tissue infiltration in the majority of patients and the lack of both in two patients are nevertheless further in favour of ongoing autoimmunity.

No relevant immunological abnormalities were found in the six control persons investigated in this study. The relatively high number of scattered intrathyroidal lymphocytes in controls may be due to the fact that these persons were suffering from larynx carcinoma and that local inflammatory reactions may have extended to the thyroid glands. Thus the absence of difference in lymphocytic subpopulations between goitrous patients and controls, must not be overestimated.

All goitrous patients described in this study came from an area with endemic iodine deficiency (Galvan et al. 1982; Grubeck-Loebenstein et al. 1982; Grubeck-Loebenstein & Waldhäusl 1985), showing that immunological abnormalities are not restricted to sporadic, non-iodine deficient goitres. The similar incidence of immunological abnormalities in goitrous patients with lower and higher iodine supply seems to be in contrast to data by Bagchi et al. (1985) who demonstrated that dietary iodine induces autoimmune thyroiditis in genetically susceptible chickens and that iodine depletion has an ameliorative effect. However, it has to be pointed out, that such dramatic alterations of immunoregulation are only likely to occur as the result of excessive iodine loading. Iodine deficiency is only moderate in Austria, and no extreme iodine excretion values were observed in either of the groups investigated.

It can be summarized that immunological abnormalities do frequently occur in multinodular non-toxic goitre. However, whether they present obligatory cofactors in the pathogenesis of the disease or are just evidence of coexisting autoimmune processes remains to be elucidated.

References


