Predominant intraepithelial localization of primed T cells and immunoglobulin-producing lymphocytes in Graves' disease

R. Paschke¹, N. Brückner, R. Schmeidl, P. Pfiester² and K. H. Usadel³

Institut für Reproduktionsmedizin, Universität Münster¹
II. Medizinische Klinik und Pathologisches Institut², Klinikum Mannheim der Universität Heidelberg, Mannheim, Germany
Zentrum der Inneren Medizin, Universität Frankfurt³

Abstract. It has been proposed that intrathyroidal lymphocytes, localized in specific anatomical sites might have distinct, pathophysiologically relevant functions in Graves' disease. However, most studies of intrathyroidal lymphocytes were restricted to two lymphocyte locations and used semiquantitative methods. Therefore we used seven anatomically different lymphoid compartments to classify and evaluate by quantitative representative methods the total intrathyroidal lymphocytic infiltration and the staining indexes for immunoglobulin-producing plasmocytes and primed T cells (CD45RO), which provide maximum help to pokeweed mitogen-stimulated immunoglobulin synthesis in 36 thyroid glands from patients with Graves' disease. We found only 3.4% of all intrathyroidal lymphocytes intraepithelia. However, only intraepithelial lymphocytes showed a significantly higher staining index for primed T cells compared with several other compartments. There was also a high staining index for immunoglobulin-producing lymphocytes in this compartment. Kappa- and lambda-positive plasmocytes were found in a polyclonal distribution (kappa:lambda=64.1:35.9) in all compartments. This increased incidence of CD45RO-positive T lymphocytes and of immunoglobulin-producing lymphocytes among the intraepithelial lymphocytes suggests a distinct pathophysiological function of lymphocytes in peripolesis in Graves' disease. Furthermore, there is a polyclonal intrathyroidal immunoglobulin synthesis.

Immunohistological studies of intrathyroidal lymphocytes formerly compared staining indexes of lymphocyte subpopulations between two arbitrarily chosen compartments such as intraepithelial versus interstitial (1-3), large lymphoid infiltrates versus interfollicular spaces (4), and lymphocytes infiltrating between thyroid follicles versus clusters (5). Although intraepithelial B lymphocytes might be attracted through a homing error (6) it is not known whether this or any other lymphocyte compartment so far chosen for immunohistological investigations has a specific function in Graves' disease. This previous restriction of intrathyroidal lymphocyte analyses (1-5) will therefore limit the possibilities for comparison and may neglect other possibly relevant lymphocyte compartments. Furthermore, most of the studies used semiquantitative methods to classify the degree of lymphocytic infiltration (1,3,4). Consequently there is no standardized, representative study of the total thyroidal lymphocytic infiltration. Therefore we tried to evaluate representatively the total lymphocytic infiltration of the thyroid gland in Graves' disease in seven different compartments.

Despite extrathyroidal sources (7,8) B lymphocytes localized in the thyroid seems to be the major source of TSH receptor antibodies in Graves' disease (9,10). It has been suggested that regulatory T cells and the lymphocytes producing TSH receptor antibodies are located in close proximity to thyroid epithelial cells, whereas the immune response takes place in the lymphocyte aggregates (10). However, immunohistological evaluations of intrathyroidal B-lymphocytes in Graves' disease with anti IgD (5), anti human delta
chain (2) and RFB7 (CD20) (3) antibodies showed a near absence (3,5) as well as 5.8 to 12.1% (2) of B cells among lymphocytes located intraepithelially, interstitially or in clusters. Furthermore, an exclusive monoclonality of TSH receptor antibodies opposed to a polyclonal nature of thyroglobulin and microsomal antibodies (11), and a reduction of lambda-positive plasma cells after carbimazole treatment have been reported (12). Therefore, and since thyroid autoantibody-producing B cells are likely to be immunoglobulin-producing plasma cells (plasmocytes), we chose to evaluate intrathyroidal plasma cells by cytoplasmic staining with anti-kappa and anti-lambda antibodies.

Moreover, studies of intrathyroidal T cells in Graves' disease mainly concentrated on helper and suppressor T cells, since an antigen specific defect and an imbalance of these cells are presumed to be etiologically relevant (13). However, functional suppressor cells have been demonstrated in Graves' disease (14) and a definite and direct proof of the existence of T suppressor lymphocytes has not yet been produced (15). Therefore we chose to evaluate intrathyroidal primed T cells (CD45RO), which provide maximum help to pokeweed mitogen stimulated immunoglobulin synthesis (16,17) and with polyclonal antibodies for lambda- and kappa-immunoglobulin light chains (21). The indirect avidin-biotin method was used with all 3 primary antibodies. Control slides in each run were incubated with normal mouse (UCHL1) and rabbit (kappa, lambda) serum instead of the primary antibody. These slides showed no specific staining.

The intensity of lymphocytic infiltration expressed as volume percent was determined by the point counting method in 100 randomly chosen visual fields per thyroid (X 500) with the Integrationsplatte 1 (Fa. Zeiss). Because of the morphological heterogeneity of the thyroid gland and in order to test the representativeness of this method, two additional cross-sections of the upper and lower poles of 3 thyroids were embedded. The intensity of lymphocytic infiltration (volume percent) was subsequently determined in 3 cross-sections for those 3 thyroid glands. Furthermore the total lymphocytic infiltration was classified as follows:

1. lymphocytes in aggregates (no germinal centre), located interstitially between thyroid follicles; 2. lymphoid follicles with germinal centers; 3. lymphocytes between thyroid follicle cells in the follicular wall (intraepithelial); 4. lymphocytes in the colloid; 5. scattered lymphocytes found interstitially between thyroid follicles; 6. lymphocytes in aggregates (no germinal center), located in the connective tissue, and 7. scattered lymphocytes in the connective tissue.

The total number of lymphocytes as well as the number and mean percentages of stained lymphocytes were each counted in 51 randomly chosen visual fields (X 500). Subsequently the distribution of all lymphocytes counted in 153 (3×51) visual fields per thyroid among the 7 compartments classified and the percentage of kappa-, lambda- and UCHL1-positive lymphocytes within each of these compartments were determined. The counting of UCHL1-, kappa- and lambda-positive cells was done in serial sections. Three slides per thyroid were stained with each antibody.

Mean values (X) and standard error of the mean (sem) for absolute numbers and percent values were calculated after testing for normal distribution. Correlations were calculated according to Pearson. Differences between groups and subgroups were evaluated with the U-test according to Wilcoxon, Mann, Whitney for independent groups and the Wilcoxon test for pair differences, respectively (18).
Results

The extent of lymphocytic infiltration (volume percent) determined in 3 different cross-sections in 3 thyroid glands did not show significant differences within the same thyroid gland. The intensity of lymphocytic infiltration as determined by the point counting technique showed a strong correlation (r=0.81, p<0.0001) with the total number of lymphocytes counted in 51 visual fields (volume percent). Differences of lymphocytic infiltration (point counting method and number of lymphocytes in 51 visual fields) between the 29 thyroids treated with thioureylene antithyroid drugs and the 7 thyroids treated with propanolol (Table 1) were not significant (U-test, p>0.05), whereas both groups showed significant differences to control thyroid tissue. The subgroups treated with carbimazole, iodine and propylthiouracil among the 29 patients treated with thioureylene antithyroid drugs showed nearly identical distributions of these cells (Table 1).

The low number of total (x=0.1 volume percent, range 0-0.3) and especially stained lymphocytes in normal thyroid tissue did not permit calculation of meaningful staining indexes. Regarding the 29 thyroids treated with thioureylene antithyroid drugs, differences in the UCHL1-staining index between all lymphocyte compartments were not significant (p>0.05, Wilcoxon test) except for a significantly higher staining index of intraepithelial lymphocytes compared with interstitial lymphocytes, with aggregates in connective tissue, and with scattered lymphocytes in connective tissue. Aggregates in connective tissue was the only other compartment to show one significantly (p<0.05) higher staining index compared with interstitial lymphocytes as the only compartment. For the thyroids from the 7 propanolol-treated Graves’ patients significant (Wilcoxon test) differences in UCHL1 staining indexes could only be observed between the intracol loidal and the intraepithelial compartments. The differences in kappa- and lambda-staining indexes between the different compartments could not be tested for significance by the Wilcoxon test, since too few patients showed kappa- and lambda-positive lymphocytes in all the 7 compartments.

There was no consistent evidence that the mode of antithyroid treatment in Group 1 could have influenced the staining indexes for kappa-, lambda- and UCHL1-positive lymphocytes in the 7 compartments. Only the intraepithelial percentage of kappa-positive plasma lymphocytes differed between iodine-treated and the 29 Graves’ patients (31.6 vs 22.5%) as did the percentage of UCHL1-positive lymphocytes in follicles with germinal centers (29.5 vs 40.1%) and in the small compartment with intracolloidal lymphocytes (15.6 vs 23.3%). In all thyroids from the propanolol-treated Graves’ patients, a ratio of kappa > lambda was found. Whereas in the thyroids from 29 Graves’ patients treated with

Table 1.
kappa-, lambda- and UCHL1-positive intrathyroidal lymphocytes (x±range) each counted in 51 visual fields (X 500) for thioureylene-, carbimazole-, propylthiouracil-, iodine-, and propanolol-treated patients, ratio of kappa- to lambda-positive plasma cells, and volume percent (x±range) of lymphocytic infiltration determined by the point counting method.

<table>
<thead>
<tr>
<th></th>
<th>Thioureylene antithyroid drugs N=29</th>
<th>Carbimazole N=20</th>
<th>Iodine preoperatively N=10</th>
<th>Propylthiouracil N=7</th>
<th>Propanolol N=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% lambda + lymphocytes</td>
<td>6.5 (1.2-15.2)</td>
<td>5.8 (1.2-9.6)</td>
<td>8.3 (3.6-15.4)</td>
<td>6.9 (3.2-12.7)</td>
<td>7.9 (1.1-21.1)</td>
</tr>
<tr>
<td>% kappa + lymphocytes</td>
<td>11.6 (0.6-26.6)</td>
<td>11.6 (3.3-25.8)</td>
<td>13.1 (5.9-26.6)</td>
<td>11.8 (0.6-26.6)</td>
<td>15.7 (3.5-38.5)</td>
</tr>
<tr>
<td>% UCHL1 + lymphocytes</td>
<td>27.8 (11.8-25.4)</td>
<td>27.5 (11.8-65.4)</td>
<td>26.2 (12.5-38)</td>
<td>26.1 (15.8-38.0)</td>
<td>32.7 (3.3-57.8)</td>
</tr>
<tr>
<td>Lymphocytic infiltration (volume percent) Ratio kappa:lambda</td>
<td>4.5 (0.1-28.5)</td>
<td>5.3 (0.1-28.5)</td>
<td>3.48 (0.6-12.3)</td>
<td>2.5 (1.1-6.8)</td>
<td>8.25 (0.2-22.1)</td>
</tr>
</tbody>
</table>

632

Downloaded from Bioscientifica.com at 11/20/2018 06:50:53PM via free access
thioureylene antithyroid drugs a ratio of kappa > lambda was found in 24, kappa= lambda in 2, and kappa < lambda in 3.

Discussion

The representativeness of a single random section within a thyroid lobe, which was previously demonstrated with regard to follicle radii (19), could be confirmed by our determination of lymphocytic infiltration by the point counting method. The intensity of lymphocytic infiltration as determined by the established point counting method (20,21) showed a reliable correlation (r=0.81, p<0.0001) with counting lymphocytes in 51 randomly chosen visual fields (500X), thus confirming this method for the quantitative determination of lymphocytic infiltration intensity. Only the latter method allowed a representative evaluation of labelled cells, small lymphocytic infiltrations and interstitial, intraepithelial or scattered intrathyroidal lymphocytes.

The observed wide variations of the extent of lymphoid infiltration in thyroids from patients with Graves' disease (range=0.1-28.5 volume percent) are in accordance with previous studies (22,23). We found most intrathyroidal lymphocytes in Graves' disease located interstitially. Compared with normal thyroid tissue, the site of maximal lymphocytic infiltration has shifted from scattered lymphocytes in connective tissue to interstitial lymphocytes (Fig. 1). Only small percentages of the total lymphocytic infiltration are located in the intraepithelial compartment and as aggregates in the connective tissue (Fig. 1).

In the intraepithelial compartment we found the highest staining index for UCHL1 (CD45RO)-positive T cells. For the 29 thyroid glands treated with thioureylene drugs, this intraepithelial compartment is the only one with a significantly higher UCHL1 staining index compared with 3 other compartments. The intraepithelial compartment was also the one with the highest staining indexes for kappa- and lambda-positive plasmocytes. Because of these findings and the low staining index for kappa- and lambda-positive plasmocytes in germinal centers it should be further elucidated whether these results support the hypothesis of thyroid autoantibody production in close proximity to its targets, the thyroid cells (10).

A higher semiquantitative (CD45RO) staining index for interstitial than for intraepithelial lymphocytes has previously been described in 5 thyroids from patients with Graves' disease. Furthermore, no B lymphocytes (CD20) could be found in the intraepithelial compartment (1). Our results are at variance with these findings. Especially CD45RO-positive lymphocytes showed a significantly higher staining index in the intraepithelial than in the interstitial compartment. Moreover, plasmocytes could easily be detected in the intraepithelial compartment. In group 1 there was an even higher staining index in the intraepithelial as opposed to the interstitial compartment. The quantitative evaluation in our study opposed to the previously used semiquantitative determinations of lymphocytic infiltration and our larger number of patients are possible explanations for these differences. Furthermore, different B cell populations

![Fig. 1.](image)

Quantitative distribution of all lymphocytes counted in 153 visual fields among the 7 compartments classified, expressed as x of percentages of total lymphocytic infiltration ± SEM for 29 patients treated with thioureylene antithyroid drugs (Group 1), 7 patients treated with propanolol (Group 2), and 10 normal thyroid glands (Group 3).
might have been evaluated since the CD20 antigen and surface IgG staining are lost some time before the plasma cell stage, whereas we determined immunoglobulin-producing plasma cells by positive staining of immunoglobulin-light chains in the cytoplasm with anti-kappa and anti-lambda antibodies. This was done to identify B lymphocytes with the capability to produce and possibly secrete thyroid autoantibodies, e.g. plasmocytes. The functional capacity of the intraepithelial kappa- and lambda-positive lymphocytes remains to be investigated since intraepithelial lymphocytes were repeatedly shown to be predominantly OKT8 or Leu 2a positive but mainly CD3 negative, thus not classic T cells (1-4). The lack of a definite and direct proof of the existence of T suppressor cells (15) further illustrates the need for a functional characterisation of intraepithelial lymphocytes. We found the highest staining index for CD45RO in this compartment. Therefore, a significant proportion of intraepithelial T cells might function as memory cells (16,17). Furthermore, UCHL1-positive T cells have also been shown to provide maximum help for pokeweed mitogen-stimulated immunoglobulin synthesis by B cells (16).

The pathophysiological significance of intraepithelial lymphocytes (lymphocytes protruding between thyroid follicular epithelial cells, a process called peripolesis) has previously been investigated in Hashimoto's thyroiditis (24). From these and other immunohistological studies (2) it is known that intraepithelial lymphocytes in Hashimoto's thyroiditis can frequently be identified as helper/inducer lymphocytes, whereas in Graves' disease suppressor lymphocytes are more numerous in this compartment. Other investigators described similar but less prominent differences (25). Our results suggest that a high proportion of immunoglobulin-producing lymphocytes in the intraepithelial compartment is a further morphological hallmark to distinguish Graves' disease from Hashimoto's thyroiditis with an almost absence of B cells (TO15) and very few plasma cells (IgG-, IgM+) in this compartment (24).

TSH receptor antibodies are predominantly produced by intrathyroidal lymphocytes (10,11). In our series of 29 patients with relapsing Graves' disease, plasmocytes producing either kappa- or lambda-light chains constituted 18% of all intrathyroidal lymphocytes with wide interindividual variations (Table 1). Previous immunohistologic studies of immunoglobulin-producing lymphocytes in thyroids from patients with Graves' disease found varying results. Whereas a near absence of mature B cells, determined by histological surface IgD staining and the point counting method has been reported (5) other investigators found a range from 5.8 to 12.1% B cells (anti-human delta chain) (2). In fine needle aspirates from patients with Graves' disease almost no plasma cells could be found (26). Determination of B cells after thyroid tissue digestion showed 20% of all lymphocytes to be B cells (27).

In thyroids from 29 patients with relapsing Graves' disease treated with carbimazole or propylthiouracil preoperatively we found a distribution of 64.1% kappa- and 35.9% lambda-immunoglobulin light chains (Table 1) by a quantitative method. Nearly identical distributions of kappa- and lambda-Ig-light chain producing plasma cells can be found in normal human lymphoid tissues (28). Moreover, the same distribution of kappa- and

Fig. 2.
Means of individual percentages of kappa- and lambda-positive lymphocytes and UCHL1-positive lymphocytes of all lymphocytes in each of the 7 compartments and ratio of kappa/lambda-positive lymphocytes in each compartment for thyroids from 2 g patients with Graves' disease treated with thioureylene antithyroid drugs and for 7 treated with propanolol preoperatively.
lambda-Ig-light chains can also be observed in normal serum (29,30). It seems unlikely that this observation is influenced by the mode of treatment, since similar distributions of kappa- and lambda-light chains could be observed after carbimazole, propylthiouracil, additional preoperative iodide and, especially, propranolol treatment (Table 1).

A recent report showed a reduction of lambda-positive plasma cells among the diffusely scattered lymphocytes in the thyroids of patients with Graves' disease after carbimazole treatment (12). Furthermore, evidence has been presented for a TSH receptor antibody monoclonality (31) and a possible restriction of its production mainly to lymphocytes closely associated with thyroid follicles (10). For immunoglobulin-producing lymphocytes we found a ratio of kappa >lambda in all 7 lymphoid compartments of the 29 Graves' patients treated with thioureylen antithyroid drugs (Fig. 2). Therefore the suggestion that kappa- or lambda-Ig-light chain mono- or oligoclonality is present in one possibly pathophysiology more relevant lymphocyte compartment (12) is unlikely. Moreover, for nearly all the thyroids evaluated, the ratio kappa- to lambda-Ig-light chain-producing plasmocytes was >1 and the ratio of the kappa to lambda mean percentages was nearly identical after carbimazole, propylthiouracil and propranolol treatment (Table 1). Therefore, the mode of treatment does not seem to influence the polyclonal nature of the intrathyroidal immunoglobulin production.

Acknowledgments

We thank Dr S. M. McLachlan, Endocrine Immunology Unit, College of Medicine, Cardiff, for providing us with tissue from patients with Graves' disease treated with propranolol. We thank C. Cheraifi for typing the manuscript.

References

11. Laing P. Both kappa and lambda light chain types are present in thyroid microsomal and thyroglobulin autoantibodies. Proceedings of the University Otago Medical School, Dunedin, New Zealand 1983;61:75-7.
17. Akbar AN, Tery L, Timms A, Beverly PCL, Janossy