The distribution of immunoglobulin-containing cells in human autoimmune thyroiditis

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Abstract. The peroxidase-antiperoxidase (PAP) staining method was used to identify immunoglobulin-containing cells in the lymphoid infiltrates of thyroids removed from patients with Hashimoto's autoimmune thyroiditis. A composite picture of the distribution of such cells in the thyroid infiltrate was obtained by carefully superimposing cell-distribution maps from serial histological sections, each stained for a different immunoglobulin class. Ig-containing cells were present to varying extents in all areas of the gland, but were most dense in areas of epithelial 'invasion'. IgG was the commonest immunoglobulin, but there were more cells containing IgD and IgE than IgA or IgM. Both \( \lambda \) and \( K \) light chains were identified in cells occupying the follicle centres. Lymphoid follicle structure was strikingly similar to that described previously for gut-associated lymphoid tissue (GALT), and the theory is advanced that autoimmune thyroiditis represents a form of ectopic GALT in which B-cells, which normally home on markers in the gut, are attracted instead to antigenically active sites in the thyroid.

Since the discovery of antithyroid antibodies by Roitt et al. (1956) and the production of experimental autoimmune thyroiditis by Rose & Witebsky (1956), it has been generally accepted that chronic lymphocytic (Hashimoto's) thyroiditis is an autoimmune disease. Considerable effort has been spent on characterising the antibodies, and recent evidence suggests they are predominantly of thyroid rather that peripheral origin, produced by plasma cells within the lymphoid infiltrate (Weetman et al. 1982).

Why the thyroid gland should be the target for plasma cells which go on to produce thyroid specific antibodies is unclear, but the infiltration is certainly not haphazard. It is organised and apparently carefully orchestrated with the development of architecturally complex germinal centres and 'invasion zones' which penetrate progressively into areas of healthy thyroid (Söderstrom & Björklund 1974).

Immunofluorescence has long been used to study the functionally varied cells which comprise the lymphoid infiltration (Ortega & Mellors 1957). Immunohistochemical methods, however, have several advantages over immunofluorescence (Petts & Roitt 1971) principal among them being permanence of the preparation and the facility to study the architecture of surrounding tissue as well as the stained cells. Cell types and subsets can be distinguished immunohistochemically by the staining of surface antigens. Although these antigens are destroyed by conventional fixatives, the immunoglobulin content of the plasma cell is not, so that after mild treatment with trypsin, the class and if necessary subclass of the immunoglobulins secreted by individual plasma cells can be revealed and studied in the context of surrounding thyroid tissue.

Past immunohistochemical studies of the thyroid infiltrate have been limited to the identification of IgA, IgG and IgM-producing cells (Yagi 1981; Knecht et al. 1981). Efforts have similarly been made to classify the circulating lymphocyte sub-populations in thyroiditis (Podleski 1971; Urbaniak et al. 1973; McGregor et al. 1979). Although the histological studies have confirmed the very ordered distribution of Ig-containing cells within the thyroid infiltrate, questions remain. It is not settled for example, whether germinal centres are monoclonal, as some have claimed (Mellors & Korngold...
1963; Burtin & Butte 1967), or polyclonal (Curran & Jones 1977). The plasma cell population of the thyroiditis germinal centre has not been described in detail, nor the distribution of IgD and IgE-containing cells in the infiltrate.

By superimposing carefully drawn cell-distribution maps from serial, and therefore virtually identical, sections from thyroiditis glands, and staining immunochemically for the various immunoglobulin class specificities, we have been able to present in this report a composite picture of the distribution of plasma cells in the thyroid infiltrate, and of the antibody class they secrete.

Materials and Methods

Tissue

Paraffin blocks of formalin-fixed thyroid tissue taken from 5 females with Hashimoto’s disease were selected on the basis of tissue suitability and preservation, age range (20–60 years) and block size. Five micrometre sections were cut on a rotary microtome in ribbons, floated out on water, and mounted sequentially on clean numbered slides, in order to ensure that the sections were arranged in consecutive order. This order was carefully checked during drying, storage and use, to ensure that the sections used for immunoperoxidase staining were as close together as possible. Spare or odd sections were used for the staining of reticulin and collagen.

A) Staining

Immunoperoxidase staining was carried out according to the peroxidase-antiperoxidase method of Curran & Gregory (1978), with modifications. Sections were initially stained with polyclonal rabbit anti human immunoglobulins, in order to determine those with good positive cell populations. They were then tested for IgG, IgM, IgA, IgD, IgE and K and λ light chains.

Five micron paraffin sections were deparaffinised in xylene and taken to 100% alcohol (74 OP). The sections were then subjected to the following procedure:

a) Incubation in fresh 0.5% hydrogen peroxide in methanol (Streefkerk 1972) for 10 min at room temperature (to inhibit endogenous peroxidase activity).

b) Rinse in running tap water 2–3 min.

c) Controlled trypsin digestion (Mepham et al. 1979; Curran & Gregory 1977): slides were warmed in distilled water at 37°C, then transferred to a solution of 0.1% trypsin (Sigma, Cat. No. T8182) in 0.1% CaCl₂ at pH 7.8 (adjusted with 0.1 N NaOH) at 37°C for various times.

These times were established by experimentation in IgG alone to determine the correct digestion time for each section.

d) Rinse in running tap water 2–3 min.

e) Rinse in Tris-HCl buffered saline pH 7.6 (TBS) 10 min at room temperature. Repeat.

f) Incubation in rabbit anti human Ig antigen diluted in TBS for 30 min at room temperature. The same titres were used on each section, having been determined by prior experimentation as follows: Polyclonal Ig 1/1500 (Nordic Immunology 14-177), IgG 1/1000 (DAKO A090), IgM 1/1000 (DAKO A091), IgA 1/500 (Miles-Yeda 65-065), IgD 1/200 (Behringer Institut (Hoechst) OTND 05), IgE 1/200 (Miles-Yeda 65-062). K 1/500 (Behringer Institut (Hoechst) OTNI 05), λ 1/1000 (Behringer Institut (Hoechst) OTNM 05).

g) Wash in TBS 10 min. three times. Drain.

h) Incubate in swine anti rabbit Ig antigen (DAKO Z196) at 1:400, diluted in TBS for 30 min at room temperature.

i) Repeat step (g).

j) Incubate in peroxidase antiperoxidase complex (DAKO Z113) at 1:400, diluted in TBS for 30 min.

k) Repeat step (g).

l) Incubate in fresh 0.05% DAB oxidase solution for 10 min (5 mg 3,3-diaminobenzidine tetrahydrochloride (DAB Sigma D5637) in 10 ml of Tris-HCl buffer pH 7.6 + 100 µL fresh 1% hydrogen peroxide).

m) Rinse in TBS, stand in running tap water for 5 min.

n) Counterstain with Mavers’ haematoxylin for 1–2 min, differentiate (about 10 seconds) and blue in running tap water.

o) Dehydrate, clear and mount in D.P.X.

B) Histochemical stains

Sections from each block were stained with Harris’ haematoxylin and eosin, to show their basic structure, and with Gordon and Sweet’s reticulin stain and Van Geisow’s collagen stain to show the supporting structure in each section (Cook 1974).

Cell-counts

Detailed cell counts were not performed. Instead, a representative area (containing up to 6 germinal centres) was chosen, and identified on each serial section of the series from a block. The area was reproduced on acetate for each stain specificity by means of a drawing attachment to the microscope, and individual plasma cells were recorded. The + to +++++ classification for cell counts was based on a visual impression of cell density within the designated area.

Results

Lymphoid follicle structure

Lymphoid follicles are the hallmark of Hashimoto’s thyroiditis (Figs. 1, 2, 3). Assuming that the follicles
Fig. 1.
Neighbouring lymphoid follicles in thyroiditis gland to illustrate contrast in appearance resulting from different planes of section. × 80 PAP staining of fixed tissue for IgM.

Fig. 2.
Lymphoid follicle in autoimmune thyroiditis showing 'polarity' of structure. An arteriole (arrowed) enters zone A. Zone B is characterised by a higher density of Ig-containing material. Zone C comprises the mantle layer of small lymphocytes. × 160 PAP staining of fixed tissue for IgM.
Fig. 3.
Margin of lymphoid follicle in thyroiditis gland showing (apparent) discrete transfer of Ig-containing cells between follicle centre and mantle (C) zone. × 200 PAP staining of fixed tissue for IgG.

Fig. 4.
Tracking of Ig-containing cells along interlobular space in thyroiditis gland. × 320 PAP staining of fixed tissue for IgG.
were orientated randomly in three dimensions, they would have been sectioned for histological study in a variety of planes. Thus the contrasting appearances in neighbouring germinal follicles seen in Fig. 1 could be due either to real differences in structure or more likely to a heterogeneous tissue viewed in different planes. The latter seems the more likely explanation when considering

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Fig. 2 in which both the appearances of Fig. 1 are seen side by side in a single follicle.

In an attempt to standardise terminology (Curran & Gregory 1978), we have referred to the right-hand (pale) zone in Fig. 2 as zone A, and to the left as zone B. Zone B contained a large number of Ig-containing cells, whereas zone A had relatively few. Both zones were enclosed (in three dimensions, encapsulated) by a mantle of small lymphocytes, designated zone C.

The vasculature of lymphoid follicles is difficult to identify accurately without special techniques, but where the plane was appropriate, as in Fig. 2, an arteriole could be identified entering the germinal centre. When we found such arterioles, they were always situated in relation to zone A.

Immunoglobulin-containing cells were seldom found in zone C, but when present were often grouped together, sometimes in apparent continuity with those of zone B (Fig. 3).

**Plasma cell distribution**

Ig-containing cells were present to varying extents in all areas of the thyroiditis gland – germinal centre zones, mantle (C) zone, parenchyma and occasionally colloid space. Trails of cells, mainly IgG-containing, were frequently seen tracking along the interlobular spaces (identified by the presence of both reticulin and collagen) (Fig. 4). Alternatively, dense areas of plasma cells, closely related to a germinal centre, could be seen ‘invading’ the thyroid parenchyma. In this case the fine reticulin structure of the healthy thyroid (Fig. 5a) was found to be fragmented, compressed and destroyed by the ‘invasion zone’ (Fig. 5b). In some instances, the integrity of the basement membrane surrounding individual thyroid follicles was clearly breached by Ig-containing (IgG) cells (Fig. 6).

The overall plasma cell distribution, class by class, and thyroid by thyroid, is summarised in Table 1. Both λ and K light chains were present in cells occupying the germinal centres. While γ heavy chains were predominant overall, there were more cells containing δ and ε than α and μ chains, and this was particularly true of the dense parenchymal infiltrates outside the germinal centres. γ heavy chains were particularly strongly represented in the plasma cells adjacent to the germinal centres and in the invasion zones.

**Fig. 6.**

Thyroid follicles from a thyroiditis gland showing infiltration of epithelial lining by Ig-containing cells. 
× 1000 PAP staining for IgG.
Distribution of immunoglobulin classes (defined by light and heavy chains) in the Ig-containing cells of thyroids from 5 patients with Hashimoto's thyroiditis. GC = germinal centre. A, B and C zones are explained in the text. 'Adj GC' refers to plasma cells contiguous with germinal centre, 'invasion zone' to plasma cells infiltrating healthy thyroid tissue and 'focal' to plasma cells in dense areas of focal thyroiditis where germinal centres are absent.

### Table 1.

<table>
<thead>
<tr>
<th>Ig chain</th>
<th>(\gamma)</th>
<th>(\mu)</th>
<th>(\alpha)</th>
<th>(\delta)</th>
<th>(\varepsilon)</th>
<th>(\lambda)</th>
<th>(K)</th>
</tr>
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<tr>
<td>Gland No.</td>
<td>1 2 3 4 5</td>
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A + B zones

| + | + | + | + | + | + | + | + |

C zone

| + | + | + | + | + | + | + | + |

Adjacent GC

| + | + | + | + | + | + | + | + |

Invasion zone

| + | + | + | + | + | + | + | + |

Focal

| + | + | + | + | + | + | + | + |

Discussion

According to Salabé et al. (1974), the immunoglobulin content of the Hashimoto thyroid is ten times that of normal. It seems important to localise the source of thyroid antibodies in Hashimoto's disease as they may be a cause of the thyroiditis. Although we have no proof that the immunoglobulins identified in this study were thyroid-directed autoantibodies, circumstantial evidence is strong. Weetman et al. (1982), for example, have demonstrated that lymphocytes teased from thyroiditis glands produce thyroglobulin (Tg) antibody in vitro. Indeed, in contrast to peripheral blood lymphocytes, thyroiditis-derived cells formed Tg antibody specific plaques without the need for mitogenic stimulation, suggesting they were already activated by local antigen (Weetman et al. 1982).

The use of immunoglobulin class-specific staining has enabled us to map the frequency-distribution of the various Ig-producing cells within the Hashimoto thyroid gland. Contrary to some previous reports (Mellors & Korngold 1963; Burtin & Buffe 1967), we have unequivocal evidence against the monoclonality of germinal centres, and although it remains possible that each centre generates a single clone of cells, others becoming...
enmeshed during a process of recirculation, the antibodies produced in a germinal centre are polyclonal, represented by λ and K light chains and a variety of heavy chains.

Ig-containing cells were rarely seen in the mantle (C) zone surrounding the germinal centres. The plasma cells infiltrating the thyroid parenchyma were predominantly IgG-containing, those breaching the epithelial basement membrane virtually exclusively so.

Plasma cells have been seen to breach thyroid epithelium on previous occasions (Yagi 1981; Irvine & Muir 1962; Matsuta 1982), and the phenomenon may represent a specific and advanced stage of thyroiditis (Matsuta 1982). The process whereby the reticulin framework supporting the thyroid follicles fragments in the face of invading plasma cells is particularly striking, and distinguishes the pathological behaviour of thyroiditis from the physiological (and non-invasive) nature of lymphoid tissue in lymph nodes and gut (see below).

Thyroid antibodies in the circulation of patients with Hashimoto’s disease may be of the IgA, IgG or IgM type (Fahey & Goodman 1969). According to Torrigiani et al. (1968), some 20% of the antibodies are IgA. Podleski (1971) also found a significant elevation in circulating IgA, second only to that of IgG. Little information is available on the circulating levels of IgD and IgE in Hashimoto’s disease and none, until now, on their intrathyroidal distribution. The present observations suggest that IgD and IgE-containing cells may play a major role in thyroiditis, but further investigation is needed to clarify it. Others have nevertheless pointed out that the number of IgE-containing cells in lymphoid tissues was greater than would be predicted from the concentration of IgE in the serum, owing perhaps to its short half-life of 1½–2 days (Tada & Ishizaka 1970). Crabbe & Hermans (1967) found that IgD positive cells were sometimes more numerous than IgM in tonsil.

Söderstrom & Björklund (1974) suggested an analogy between the organisation of lymphatic tissue in thyroiditis and that of tonsil, which forms part of the specialised lymphoid elements of the gut: the gut associated lymphoid tissue (GALT). The secondary follicles of GALT comprise a follicle centre and surrounding mantle (Parrott 1976).

The follicle centre of the tonsil, according to Curran & Jones (1977), has two zones: the basal A zone contains macrophages and few plasma cells, while in the B zone are to be found numerous Ig-containing cells. The A and B zones are together surrounded by the C zone, a layer of deeply staining small lymphocytes. Distal or exterior to the C zone, a ‘front’ of plasma cells lies subjacent to the epithelial cells of the tonsillar crypts. This arrangement differs, according to Söderstrom & Björklund (1974), from that of a conventional lymph node. In the latter, the efferent lymphatic and blood vessels penetrate the follicle distally rather than basally, an afferent lymphatic (absent in GALT) replaces the efferent basally, and the plasma cell ‘front’ (medulla) is limited not by epithelium but by a terminal sinus.

We have borrowed the ‘ABC’ terminology for defining the zones of secondary follicles in lymphocytic thyroiditis in the belief that a close structural analogy may exist between thyroiditis and GALT.

Thus where the plane of the section was appropriate, we were able to show vascular pedicles entering the A zones of thyroiditis follicles. Ig-containing cells within the follicle were mostly committed to the more distal B zone. The C zone of small lymphocytes was clearly distinct and the plasma cell front of GALT was presented in thyroiditis by an invasion zone which was often very extensive, sometimes penetrating and breaching the thyroid epithelium.

There may be more fundamental reasons for seeking an analogy between thyroiditis and GALT. The thyroid is embryologically an appendage of the gut; it arises from the mid-pharyngeal floor and contains epithelium with the same endodermal origins (and surface antigens?) as that lining the gastrointestinal tract.

GALT is part of a broader category known as mucosa associated lymphoid tissue (MALT), which involves lung, salivary gland and thyroid. Some of the strongest evidence for the concept of MALT as a functional unit is the appearance and behaviour of the extra-nodal lymphomas. There are striking histological similarities between gastrointestinal lymphomas and those of the thyroid (Isaacson & Wright, in press). Furthermore the cells of these lymphomas, which circulate freely, are considered to share homing patterns characteristic of MALT-derived lymphocytes (Isaacson & Wright, in press). In the case of thyroiditis, we would speculate on a ‘homing error’ in which GALT-derived follicle centre cells are attracted to as yet unidentified markers on the thyroid’s gut-derived epithelium.
This attraction may be related to the recent demonstration of aberrant expression of HLA-Dr antigens on thyroid epithelial cells in thyroiditis, rendering them actively antigenic to their host (Hanafusa et al. 1983).

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References


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