Distribution of T-lymphocytes in follicular lymphomas as revealed by acid α-naphthol acetate esterase

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SUMMARY Twelve cases of follicular centroblastic/centrocytic malignant lymphoma were studied for acid non-specific esterase. The majority of the lymphocytes in interfollicular areas showed a pattern of enzymatic activity consistent with a T-cell nature. Variable numbers of lymphocytes with a similar enzymatic pattern were also seen among the negative centroblasts and centrocytes in neoplastic follicles.

Follicular malignant lymphomas are now considered to be of germinal centre origin (Lennert, 1973; Jaffe et al., 1974; Leech et al., 1975; Lennert 1978; Lukes and Collins, 1975; Stein et al., 1978a). However, in studies of cell surface markers and T-lymphocyte antigen, they appear to contain significant numbers of T-cells (Aisenberg and Long, 1975; Brouet et al., 1975; Jaffe et al., 1975; Leech et al., 1975). Their distribution in lymphomatous tissue has as yet been poorly defined. As EAC-rosette studies on frozen sections (Jaffe et al., 1974; Stein et al., 1978b) indicate, it seems possible that the majority of the lymphoid cells in internodular areas are T-cells.

Acid non-specific esterase, introduced as a T-cell marker in experimental animals by Müller et al. (1975), has also been proved to be quite valid in human reactive and neoplastic tissues (Kulenkampff et al., 1977).

We have studied the distribution of lymphoid cells showing an acid non-specific esterase activity identical with that shown by T-cells in histological sections from 12 cases of nodular germinal centre cell lymphomas.

Material and methods

Fresh biopsy material from 12 cases of follicular centroblastic-centrocytic malignant lymphoma was cut into thin slices and fixed immediately in buffered formol-sucrose (distilled water, 450 ml; 40% formaldehyde, 50 ml; 37.5 g sucrose; NaH₂PO₄, H₂O, 2 g; Na₂HPO₄ 3·25 g) at 4°C for 24 hours. They were kept in Holt's solution (sucrose 30 g; gum arabic 1 g; distilled water 100 ml) for another 24 hours. Frozen sections of 5 μm thickness were mounted onto slides pretreated with formol-gelatin. They were left to dry at room temperature for 1 hour and stained for non-specific acid esterase (Müller et al., 1975) as follows:

Solution A: 1 g pararosaniline, acridine-free, CI No. 42500 (Chroma, Stuttgart-Untertürkheim, Federal Republic of Germany) is dissolved in 20 ml distilled water, and 4 ml concentrated HCl is added. After gentle warming, cooling, and filtration store in the dark at 4°C.

Solution B: Freshly prepared 4% aqueous solution of sodium nitrite.

Mix equal parts of solutions A and B. Shake for a few seconds until the colour becomes amber. Dissolve 10 mg α-naphthyl acetate (Sigma, USA) in 0·4 ml acetone. Mix 40 ml 0·067 M phosphate buffer, pH 5, and 2·4 ml of hexazotised pararosaniline. Add substrate and adjust working solution to pH 5·8 using 2 N NaOH.

Incubate sections in working solution at room temperature for 2 hours. Wash in distilled water, counterstain slightly with methylene blue, dehydrate, and mount in Eukitt.

Another piece of each biopsy material was routinely processed for histological study. Paraffin sections were stained with haematoxylin and eosin, Giemsa stain, PAS-reaction, and Gomori's silver stain.
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Results

All cases showed histologically a well-developed nodular pattern. Interfollicular areas (Figs 1 and 2) were more or less prominent. They consisted mainly of small and medium-sized lymphocytes. Among them small numbers of plasma cells were found. In places venules of an epithelioid type were rec-

Fig. 1 A case of follicular lymphoma. Interfollicular area with small lymphocytes is moderately developed. Haematoxylin and eosin × 125.

Fig. 2 Another case of follicular lymphoma. Interfollicular area appears to be hyperplastic. Additionally, small lymphocytes are easily found inside the neoplastic follicles. H and E × 125.
ognized. In four cases, hyperplasia of postcapillary venules was apparent. Neoplastic nodules consisted mainly of variable proportions of cells showing the features of centroblasts and centrocytes. In all cases among the neoplastic cells, small- and medium-sized lymphocytes resembling those of the interfollicular area were found. Their numbers varied from case to case and were greater at the periphery of the nodules and near small vessels (Fig. 2). In three cases, considerable numbers of basophilic cells showing a plasmacytoid configuration were found in neoplastic nodules. In one of the three cases such plasmacytoid cells were abundant at the periphery of and around the neoplastic nodules. In this particular case the number of small lymphocytes in interfollicular spaces was small. On the other hand, cells with a centrocytic appearance were present. Postcapillary venules were very scarce.

In frozen sections stained for acid non-specific esterase many lymphocytes in interfollicular areas showed a focal 'dot-like' positivity for the enzyme (Figs 3 and 4). Their relative numbers were usually more than 70% of the whole lymphocytic population in such areas (Table). In the single case with abundant plasmacytoid cells around the neoplastic nodules, lymphocytes with an analogous enzymatic reaction were rare. They were more abundant around the epithelioid venules which were occasionally found. In neoplastic nodules centroblastic and centrocytic cells were negative for acid non-specific esterase (Fig. 5). Among them, however, variable numbers of lymphocytes, corresponding apparently to those described in paraffin sections, showed a pattern of enzymatic reaction similar to that found in interfollicular lymphocytes (Fig. 6).

Discussion

Jaffe et al. (1974) found that in follicular lymphomas the areas that are mainly positive for EAC-rosettes are neoplastic nodules. Stein et al. (1978b), using in EAC-rosettes the subtypes C3d and C3b, have shown that neoplastic follicles are C3d and C3b positive while in interfollicular areas cells positive for C3b only were found. However, such studies cannot offer direct evidence for the presence of T-cells. T-cell markers studied in cell suspensions

Table  Frequency of interfollicular lymphocytes with a 'dot-like' enzyme positivity in the 12 follicular lymphomas

<table>
<thead>
<tr>
<th>Percent of positive lymphocytes</th>
<th>&lt;30</th>
<th>30-50</th>
<th>50-70</th>
<th>&gt;70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 3 'Dot-like' positivity of lymphocytes for non-specific esterase in interfollicular area of a follicular lymphoma. $\times$ 500.
have shown that in some cases of follicular lymphoma the number of T-cells is as high as 82% of the whole cell population (Aisenberg and Long, 1975). Such observations support the view that nodular lymphomas, although rarely, could be of T-cell origin. This view, however, is debatable so long as the nodular pattern could be attributed to follicular centre rests inside a T-cell neoplasm (Stein, 1978). As well as epithelioid venules, Kaiserling (1976) has found electron microscopically in

Fig. 4 The same enzyme pattern as in Fig. 3 found in interfollicular lymphocytes of another follicular lymphoma. × 300.

Fig. 5 Neoplastic follicular centre cells are negative for non-specific esterase. Follicular area of the same case as in Fig. 3. × 500.
interfollicular areas of follicular lymphomas large numbers of interdigitating reticulum cells and so-called T-associated plasma cells. Accordingly, he assumes that this area represents a T-zone and the lymphocytes found there are presumably T-cells.

Our present series includes follicular lymphomas with the typical cytology of centroblastic/centrocytic malignant lymphomas (Lennert, 1978). Centroblasts and centrocytes were negative for acid non-specific esterase, a fact consistent with their B-cell nature. As the majority of the lymphocytes in interfollicular areas were focally positive for acid non-specific esterase, they must be considered to be of a T-cell nature. Accordingly, the areas containing them should represent a T-zone, as proposed electron microscopically by Kaiserling (1976). Such areas could generally be accepted as lymphatic tissue rests compressed between expanding neoplastic nodules. In four cases, however, they showed a true active hyperplasia. Whether T-zone hyperplasia represents an immunological reaction against neoplastic tissue or a non-neoplastic T-cell hyperplasia paralleling B-cell neoplasia cannot be stated at present. Ree and Leone (1978), however, have shown that in follicular lymphomas survival was closely related to the prominence of interfollicular areas.

Our finding of lymphocytes inside the neoplastic nodules showing an enzymatic activity consistent with a T-cell nature is of great importance and has not been described previously. It could indicate that the possible normal interaction between follicular centre cells and T-cells is preserved in neoplastic nodules also. Weissman et al. (1976), using anti-B-cell and anti-T-cell sera, have shown that in primary and secondary germinal centers a number of T-cells is always present. Additionally, Kelly and Wolstencroft (1974) have shown that germinal centre reaction is enhanced by T-cell extracts.

References


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