Immunocytological studies of lymph nodes in rheumatoid arthritis and malignant lymphoma

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SUMMARY  Lymph nodes obtained from 9 patients with active rheumatoid arthritis were stained by immunological techniques permitting identification of B and T cell areas within the nodes. The lymph nodes were also compared with those obtained from patients with lymphoma. The rheumatoid lymph nodes showed prominent B cell staining in the follicular centres by the EAC and fluoresceinated immunoglobulin techniques. Interfollicular areas were intensely stained with anti-theta globulin. In contrast, lymphoma nodes stained less intensely with fluoresceinated immunoglobulins but stained like benign nodes with EAC. The difference in staining may reflect the degree of disorganisation of the neoplastic follicle and/or the different sensitivity of these 2 methods in the detection of B cells. The differences in staining are helpful in distinguishing the benign changes associated with chronic inflammation from neoplastic B cell proliferative changes seen in lymph nodes.

Some patients with rheumatoid arthritis (RA) have lymphadenopathy, and several histological studies have been published on the lymph nodes from such patients.1–5 The histological similarity of nodes from rheumatoid arthritis and nodal lymphoma has occasionally led to incorrect diagnosis and inappropriate therapy.2,4 The present study compared lymph nodes, and in some instances splenic tissues, from patients with RA and from patients with malignant lymphoma, as well as normal lymph nodes. Particular attention was directed at identification of relative patterns of lymph node architecture as well as the types of lymphoid cells present as identified by immunological techniques developed for use with frozen tissue sections.6 7

Materials and methods

PATIENTS STUDIED
Axillary lymph nodes were obtained from 9 patients with active rheumatoid arthritis. Patients were classified as definite or classical RA on the basis of ARA criteria.8 An outline of age, sex, and clinical status of all studied patients is given in Table 1.

For comparison lymph nodes or spleen were obtained from 3 patients with lymphoma, and normal lymph nodes were obtained from 3 subjects during surgical procedures for nonmalignant conditions. Mediastinal thymoma was obtained from 1 patient for study.

Table 1
Characterisation of patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of disease (years)</th>
<th>ESR (mm/h)</th>
<th>RF</th>
<th>ANA</th>
<th>Therapy</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>17</td>
<td>1:64</td>
<td>P</td>
<td>Gold*</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>76</td>
<td>1:5120</td>
<td>N</td>
<td>Steroids</td>
</tr>
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<td>3</td>
<td>21</td>
<td>17</td>
<td>1:320</td>
<td>P</td>
<td>Gold</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>6</td>
<td>1:2080</td>
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<td>Gold, steroids</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>62</td>
<td>1:1280</td>
<td>N</td>
<td>Cytoxan</td>
</tr>
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<td>3</td>
<td>62</td>
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<tr>
<td>8</td>
<td>7</td>
<td>18</td>
<td>1:512</td>
<td>P</td>
<td>Gold</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>16</td>
<td>1:5120</td>
<td>P</td>
<td>Gold, steroids</td>
</tr>
</tbody>
</table>

*P-positive, N-negative.

All patients were on salicylates or another nonsteroidal anti-inflammatory drug in addition to drug(s) listed.
IMMUNOHISTOLOGICAL STUDIES

Tissue specimens were quick-frozen in dry ice/acetone immediately after removal. Frozen tissue was stored at $-70^\circ$C, and later sections (4–6 $\mu$m) were cut by cryostat and used for B and T cell studies. Parallel sections were fixed in formalin for routine haematoxylin and eosin (H and E) staining.

TISSUE DETECTION OF B CELLS

Two methods for B cell determination were used. First, sheep erythrocytes (EA) sensitised with the 19S IgM fraction of rabbit antisheep erythrocyte haemolysin, obtained by Sephadex G–200 column chromatography, were incubated with fresh normal mouse serum as a source of complement (C) and used to detect cells binding such EAC in unfixed frozen sections. Fluorescein labelled rabbit antisheep F(ab')$_2$ was also used to identify B lymphocytes with surface Ig and to detect plasma cells with cytoplasmic Ig in tissues. (H) Fluoresceinated antiserum to IgG, IgM, and IgA (Meloy Laboratories, Springfield, Virginia) were also used to check immunoglobulin class specificity of staining.

IDENTIFICATION OF TISSUE T CELLS

T cells in tissue sections were identified by means of an extensively absorbed rabbit antihuman erythrocyte antiserum prepared for indirect immunofluorescence as previously described. Absorptions of this antiserum included: 8 serial absorptions for 30–60 minutes at $37^\circ$C with B cells (5 X 10$^7$ cells/ml) from patients with chronic lymphocytic leukaemia; 3 serial absorptions with rhesus-positive red cells (5 X 10$^8$/ml) from normal blood donors for 30 minutes at $37^\circ$C; 2 serial absorptions for 30 minutes at $37^\circ$C with polymorphonuclear leucocytes and monocytes (15 X 10$^6$/ml) isolated during in-vitro assays for chemotaxis; 2 absorptions with an equal volume of insolubilised normal serum; and finally absorption with an insolubilised homogenate of normal human liver. Specificity for T cells was confirmed by the staining of thymic-dependent areas of lymphocytes in both normal human spleen and lymph nodes. No staining of smears of plasma cells prepared from marrows of patients with multiple myeloma was noted. Absorbed anti-T cell antiserum stained an average of 65–70% of peripheral blood lymphocytes.

In addition, a sample of a mediastinal thymoma stained diffusely in all lymphoid elements with the anti-T cell antiserum. This tumour showed no elements identified as B cells by surface Ig fluorescence or with EAC. Fluoresceinated pepsin F(ab')$_2$ was used as second layer.

Peripheral blood lymphocytes were separated by Ficoll-Hypaque gradient as previously described. These lymphocytes were evaluated for B cell markers by immunofluorescence and EAC techniques and for T cell markers by spontaneous rosette formation with sheep erythrocyte (E). Pieces of lymph nodes from the rheumatoid patients were minced and passed through a fine metal sieve into RPMI-1640 (Grand Island Biological Co.) culture media. The suspensions were washed with RPMI-1640 and brought up to a volume of 2 X 10$^8$ cells. These suspensions of lymphocytes were then evaluated for B and T cells in a manner similar to the handling of the peripheral blood lymphocytes.

Results

LYMPH NODES FROM PATIENTS WITH RA

Lymph nodes from the 9 patients with rheumatoid arthritis generally showed intense binding of EAC to follicular or germinal centre areas (Fig. 1). By contrast there was either weak or no binding of EAC to interfollicular areas. While the germinal centres that bound EAC in a given case were strong, the numbers of germinal centres with EAC binding varied from 20% to 100% as determined by visual estimation (Table 2). In general those centres that did not bind EAC appeared in the H and E stained sections with prominent mitoses and tingible bodies (macrophages containing phagocytosed material). Neither EAC staining nor hyperplastic appearance on H and E sections correlated with seropositivity or seronegativity as determined by concurrent latex fixation titres.

![Fig. 1 Dark field microphotograph (× 50) of EAC staining of lymph node from patient with rheumatoid arthritis, showing intense follicular binding of red cells.](http://ard.bmj.com/.../group.bmj.com)
Immunocytological studies of lymph nodes in rheumatoid arthritis and malignant lymphoma

Table 2  Lymph nodes; frozen sections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Follicular Inter-</th>
<th>% Follicular</th>
<th>Node</th>
<th>PBL</th>
<th>EAC</th>
<th>EAC</th>
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<tr>
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<td>follicular centres</td>
<td></td>
<td>suspension</td>
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<tr>
<td>RA</td>
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<td>3+</td>
<td>2+</td>
<td>50</td>
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<tr>
<td>Lymphoma</td>
<td>10</td>
<td>±</td>
<td>N</td>
<td>50</td>
<td>41</td>
<td>72</td>
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<tr>
<td></td>
<td>11**</td>
<td>2+</td>
<td>N</td>
<td>15</td>
<td>15</td>
<td>58</td>
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<tr>
<td>(Spleen)</td>
<td>12***</td>
<td>4+</td>
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<td>20</td>
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<td>ND</td>
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<tr>
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</tbody>
</table>

N=Negative, ND=Not done. *Visual estimation. **Pathology showed residual and nodular lymphoma with predominantly diffuse pattern in EAC staining. ***Histiocytic lymphoma.

Germinal centre activity as defined by EAC staining correlated well with intensity of direct immunofluorescence staining for cellular IgG and IgM in these areas (Fig. 2). In addition plasma cells were easily identified in the sections at the margins of germinal centres and in the interfollicular zones by their uniform distribution of cytoplasmic immunofluorescence. Direct immunofluorescence staining showed prominently IgG, but also IgM within lymphoid follicles and germinal centres, while IgA was less frequently seen. Collections of T cells were also localised mainly to the interfollicular and partly to the peripheral parts of the follicular areas. No staining for T cells was observed in germinal centres. Examples of these findings are shown in Fig. 3.

LYMPHOMAS AND OTHER CONTROL LYMPHOID TISSUES

Examination of H and E stained sections showed that the lymphoma cases were represented by a variety of cellular types, namely, lymphocytic as well as histiocytic (large follicular cell type), and both nodular (follicular) and diffuse types. The direct

Fig. 2  Microphotograph (x 50) of rheumatoid lymph node germinal centre stained with FITC labelled goat antihuman-IgM, showing intense staining of follicular stroma and occasional plasma cells at the margins of these centres.

Fig. 3  Microphotographs of paracortical lymph nodes from patients with rheumatoid arthritis stained with rabbit antihuman thymocyte antiserum showing uniform rim staining of T cell membranes in (a) with less evidence for T cells towards the more central areas of the node at the bottom of the photograph (x 300). Fig. 3 (b) shows intense membrane T cell staining in peripheral portion of lymph node from patient with rheumatoid arthritis (x 430).
immunofluorescence technique revealed a distinct difference in the intensity of staining for immunoglobulins in the neoplastic follicles of patients with nodular (follicular) lymphomas as compared with follicles from patients with rheumatoid arthritis. While the hyperplastic follicular centres in patients with RA had stained intensely with antisera to both IgG and IgM, the lymphomatous follicles stained only weakly (Fig. 4). In contrast the neoplastic follicles in lymphoma with nodular pattern showed similar intense adherence of EAC to that seen in germinal centres of rheumatoid nodes. T cells were present in a rather diffuse distribution in the lymphoma tissues examined.

In normal control lymph nodes the fluoresceinated anti-F(ab')2 antiserum stained the cells in germinal centres showing both surface and cytoplasmic staining in addition to peripheral staining of lymphocytes localised to the follicles. Cells in the interfollicular areas were readily identified by the anti-T cell antiserum. The number of germinal centres was clearly lower when compared to the RA nodes. Most germinal centres and follicles in normal lymph nodes also bound EAC.

Results of the distribution of B and T cells from peripheral blood lymphocytes were generally in the normal range (Table 2). There was also a good correlation between the peripheral blood B and T cells and the numbers of these cells counted in the suspensions made from the minced rheumatoid nodes.

**Discussion**

The present study has focused on the lymph node morphology and subpopulations of mononuclear cells making up germinal centres, cortical areas, and paracortical areas in patients with active rheumatoid arthritis. From the uniformity of the immunocytochemical pattern observed in this disease it seems clear that most nodes were involved in an active immune response. Evidence supporting such an interpretation included the common finding of numerous germinal centres in the RA nodes examined. Of interest was the finding that numerous lymph node germinal centres were frequently associated with B cell hyperactivity even in patients who were seronegative for the presence of rheumatoid factor. It would therefore seem to be of considerable importance next to examine such nodes for evidence of possible primary antigens—viral or otherwise—in an attempt to define what it is that may be generating such an active cellular pattern or profile of immune response. Immunofluorescence studies to this end are in progress with a panel of antisera to various viral antigens.

It is apparent from the results with both the EAC and immunofluorescence techniques for cells with surface or cytoplasmic Ig that the germinal centres of many RA lymph nodes showed high B cell activity. Although such hyperactive B cell germinal centres also stained prominently for both IgM and IgG, it was difficult to ascertain if either of the latter showed rheumatoid factor activity by using fluoresceinated aggregates of IgG, since this reagent also bonds to Fc receptors on lymphocytes, monocytes, polymorphonuclear leucocytes, and macrophages. A similar overlap between the above mentioned cell population has also been noted when fluoresceinated aggregates of IgG react with Fc receptors. Accordingly, fluorescein labelled rabbit antihuman F (ab')2 was also used to identify B lymphocytes with surface Ig or plasma cells with cytoplasmic Ig in tissues.

The failure of neoplastic follicular centres to stain with the same intensity as hyperplastic germinal centres has been previously noted. This difference may reflect the degree of disorganisation of the neoplastic follicular centre cell or alternatively blockade of lymphocyte transformation in the neoplastic as compared to the normal follicular centre B cell. Further studies on nodular 'histoctic' (large transformed germinal centre cell) lymphoma will be of interest in this connection.

Agreement is still lacking on what constitutes the ideal surface marker for B cells. From several recent studies it seems clear that cells binding fluoresceinated anti-Ig alone cannot be utilised as
the single criterion for B cells, particularly in view of binding of whole Ig reagents to Fc receptors. It was for this reason that pepsin-digested anti-F(ab')2 reagents were used in the current study. Most groups of investigators appear to relate the presence of C3 receptors or EAC binding cells to the B cell population. It is also clear that many B cells may contain surface Ig as well as C receptors. The discrepancy found with negative staining for Ig as contrasted with the intense binding of EAC in germinal centres of lymphomatous nodes is therefore not clarified but may be explained by different sensitivity of the 2 techniques or by dissociation of B cell markers in various types of lymphomatous tissues. On the other hand concordance between surface Ig and Fc receptors on B cells appears to be either nil or extremely low. The heterogeneity of various B cell subpopulations has been previously reported. As yet no final definition of the various B cell subpopulations is possible on examinations of tissue section by immunofluorescence. Clearly of fundamental importance is the question whether cells bearing a variety of B cell markers—including C receptors surface Ig, or Fc receptors—constitute subpopulations with important differences in functional capacities.

By contrast to the immunocytological findings in lymph nodes from patients with rheumatoid arthritis the lymph nodes involved with lymphomatous processes showed various degrees of structural disorganisation and in some instances evidence of an infiltrative T cell process. These findings marked the nodes of patients with lymphoma as clearly different from those of RA. No extensive analysis of the changes in malignant lymphoma is given here because a much larger material will be needed before this is possible. Several recent reports have already dealt in some detail with immunocytological characteristics of some classes of lymphoma.

References


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