In Situ Immunologic Characterization of Cellular Constituents in Lymph Nodes and Spleens Involved by Hodgkin’s Disease


The cellular constituents in lymph nodes and spleens of patients with Hodgkin’s disease were studied with a series of monoclonal antibodies directed against human thymocyte, peripheral T-cell, and Ia antigens. Utilizing both an immunoperoxidase technique on frozen tissue sections and indirect immunofluorescence on cell suspensions, we found that a majority of lymphocytes were T cells, since they stained with anti-T1 and anti-T3 antibodies, which react with all peripheral T cells. In addition, most of these cells were reactive with anti-T4 antibody, which defines the helper/inducer T-cell population, whereas only a minority of cells stained with anti-T5 and anti-T8 antibodies, which are reactive with suppressor/cytotoxic T cells. Moreover, a large proportion of T cells expressed T10 antigen, which is found on activated T cells. A minority of the T cells also expressed Ia antigen(s), again suggesting that some of the T cells are activated. In contrast, the Reed-Sternberg cells did not react with any of these anti-T-cell antibodies or with anti-IgM antiserum, but displayed strong membrane and cytoplasmic staining with anti-la antibody. Taken together, these findings suggest that Reed-Sternberg cells are not of T-cell lineage but may be derived from antigen-presenting reticulum cells in the thymus-dependent areas of lymphoid tissues; these cells are normally associated with T4+ cells.

The nature of Reed-Sternberg cells and the role of associated lymphocytes in organs involved by Hodgkin’s disease have remained an enigma despite a considerable number of immunologic and cell culture studies. T cells might play an important role in the pathogenesis of Hodgkin’s disease, as cell-mediated immunity is impaired during the course of the disease and early histologic involvement has been noted in T-dependent areas of lymph nodes and spleens. In addition, there seems to be a relation between a predominance of lymphocytes in affected tissues and the prognosis of the disease, as evidenced by the different clinical course of the subtypes of Hodgkin’s disease. Studies of cell suspensions have indicated a high percentage of T lymphocytes (as shown by their ability to form rosettes with sheep red blood cells) in tissues involved by Hodgkin’s disease, but such studies are subject to errors of sampling and do not provide information regarding the location and the interrelation of the different cell types. A previous study using tissue sections indicated a preponderance of B lymphocytes in the lymph nodes showing lymphocyte predominance and a relative increase of T lymphocytes in those showing mixed cellularity and nodular sclerosis.

The development of monoclonal antibodies reactive with antigens of thymocytes and peripheral T cells has made it possible to define specifically individual T-cell subsets in suspensions and in tissue sections. In the present study these antibodies have been used to characterize by immunoperoxidase and immunofluorescence techniques the lymphocytes and Reed-Sternberg cells in lymph nodes and spleens involved by Hodgkin’s disease.

MATERIALS AND METHODS

Source of Tissue

Lymph nodes and spleens used for the study were obtained by surgical procedures performed for diagnostic purposes at the Massachusetts General Hospital and at the Sidney Farber Cancer Institute. Representative blocks of fresh tissue were snap frozen in OCT compound (Ames Co., Division of Miles Laboratories, Inc., Elkhart, Ind.) and stored at −70°C. Frozen tissue sections from 11 lymph nodes and 6 spleens from patients with untreated Hodgkin’s disease were studied. In addition, in 4 cases, cytocentrifuge smears prepared from cell suspensions obtained from lymph nodes or spleens were used. Cytofluorographic analysis was performed on cell suspensions obtained from four involved lymph nodes.

Description of Antibodies

A series of monoclonal antibodies that are reactive with thymocyte and peripheral T-lymphocyte surface antigens were employed in the present study. The antibodies were obtained in the form of ascitic fluid from mice injected with hybridoma cells. Control ascitic fluid was obtained from animals injected with nonsecreting hybridoma cells. The methods of production and characterization of the antibodies have been reported. In brief, anti-T1 and anti-T3 antibodies react with all peripheral E-rosette-positive T cells and 10% of thymocytes, while anti-T6 reacts with 70% of thymocytes and not with peripheral T cells. Anti-T4 antibody, which defines helper/inducer T cells, reacts with approximately 55% of peripheral T cells. Anti-T5 and anti-T8 antibodies, which define suppressor/cytotoxic T cells, react with approximately 20%–30% of peripheral T cells. Anti-T10 antibody reacts with 90% of thymocytes, a significant proportion of activated T cells and only a minority of resting peripheral T cells, B cells, and monocytes.

In addition, an anti-la monoclonal antibody (anti-11), reactive with a nonpolymorphic region of the human Ia-like antigens, and a...
rabbit anti-human IgM antiserum (Dakopatts, Copenhagen, Denmark) were used.

**Immunoperoxidase Staining**

Frozen tissue sections, 4 μm thick, were air-dried, fixed in acetone for 10 min, and stained by a four-step peroxidase-antiperoxidase (PAP) method as described previously. Sections were incubated with a 1:500 dilution of ascitic fluid containing monoclonal antibodies for 60 min, followed by 30-min incubations with rabbit anti-mouse IgG (Cappel Laboratories, Inc., Cochranville, Pa), swine anti-rabbit Ig, and finally with rabbit PAP reagent (Dakopatts, Copenhagen, Denmark), all diluted 1:40. Each incubation was followed by repeated washing in phosphate-buffered saline (PBS). Control sections were incubated with PBS or control ascitic fluid and some sections were incubated with a 1:200 dilution of control ascitic fluid. Subsequently, specific staining was determined for an individual sample by subtracting the staining obtained with a given monoclonal antibody, as previously described. Briefly, the lymph node was minced and passed through a 50-μm nylon screen. The lymphocytes were washed and incubated with fluorescein-conjugated goat anti-mouse IgG antiserum (Cappel) and fluorescein-conjugated rabbit anti-goat IgG antiserum (Cappel), with 10-min washings in PBS after each step.

**Immunofluorescence Staining**

Cytocentrifuge preparations were fixed in acetone for 10 min and stained with an indirect immunofluorescence method as previously reported. In short, the smears were incubated with a 1:50 dilution of the monoclonal antibodies, followed by 1:40 dilutions of fluorescein-conjugated goat anti-mouse IgG antiserum (Cappel) and fluorescein-conjugated rabbit anti-goat IgG antiserum (Cappel), with 10-min washings in PBS after each step.

**Cytocentrifugography**

Single cell suspensions were prepared and analyzed as previously described. Briefly, the lymph node was minced and passed through steel mesh. One to two x 10^9 cells were pelleted and incubated with 0.15 ml of a given monoclonal antibody for 30 min at 4°C. The cells were washed and incubated with fluorescein-conjugated goat anti-mouse IgG for 30 min at 4°C and washed. Analysis was then performed on a fluorescence activated cell sorter (FAC-I) (Becton-Dickinson, Mountain View, Calif.). The intensity of fluorescence per cell was recorded on a pulse-height analyzer and a histogram was generated. Percentage of background staining was determined by reacting cells with control ascitic fluid. Subsequently, specific staining was determined for an individual sample by subtracting the percentage of background reactivity on mononuclear cells from that obtained with a given monoclonal antibody, as previously described.

**RESULTS**

Tissue from all lymph nodes and two spleens included in the study were involved by Hodgkin's disease. Tissue from four other spleens frozen for the study did not include involved areas, although in two cases the spleen showed focal involvement by Hodgkin's disease as judged by routine sections taken for diagnostic purposes. The histologic subclassification and the staining characteristics of the lymph nodes and spleens involved by Hodgkin's disease are listed in Table 1. The anti-IgM antiserum stained most of the small lymphocytes in primary follicles and mantle zones of secondary follicles in a peripheral pattern consistent with membrane staining. Germinal centers showed intercellular staining in addition to peripheral and/or cytoplasmic staining of large lymphocytes. In areas containing Reed-Sternberg cells, cells reacting with anti-IgM represented a minority of the cell population in most cases and were scattered among unstained lymphocytes. In contrast, in a majority of the cases, most lymphocytes in involved areas stained with anti-T1 and anti-T3 antibodies, indicating a predominance of T cells (Fig. 1). Anti-T6 antibody, which reacts with a majority of thymocytes but not with mature peripheral T cells, did not stain any lymphocytes.

The nature of the T cells in involved areas was next studied with subset-specific monoclonal antibodies. Anti-T4 antibody, which defines helper-inducer T cells, stained a majority of T lymphocytes in all cases, while anti-T8 and anti-T5 antibodies, which react with cytotoxic/suppressor T cells, stained only a minority of cells in involved areas. T8^+ cells were more intensely stained and were more numerous than T5^+ cells. Although, in general, T8^+ cells were only scattered throughout the lesions, in cases of nodular sclerosis type of Hodgkin's disease, a high proportion of T8^+ cells were present at the periphery of nodules and in the fibrotic bands (Fig. 2).

Lymphocytes reactive with anti-T10 antibody were present in involved areas (Fig. 3) in all cases. In 6 cases, lymphocytes expressing T10 antigen and formed a majority of the cells that were also reactive with anti-T1, -T3, and -T4 antibodies. The reactivity of anti-T10 monoclonal antibody is not restricted to cells of T-cell lineage. In hyperplastic lymph nodes, germinal center cells and plasma cells react with anti-T10 antibody. The intensity of staining achieved with the anti-Ia antibody varied considerably from case to case. Although a majority of lymphocytes in involved areas were not reactive, the number of small lymphocytes staining for Ia exceeded that staining for IgM. This would indicate that some of the T cells also expressed Ia antigens. In addition, large cells with cytoplasmic extensions scattered in between the lymphocytes were stained diffusely.

It was not always possible to recognize Reed-Sternberg cells readily in frozen sections. However, in some sections, they were clearly identifiable and were seen to be stained diffusely with anti-Ia antibody (Fig. 4A) but not with anti-T-cell antibodies. This pattern of reactivity was confirmed by immunofluorescence staining of cytocentrifuge preparations of cell suspensions prepared from involved lymph nodes and spleen (Fig. 4B). The majority of lymphocytes surrounding Reed-Sternberg cells in both tissue sections (Fig. 3) and cytocentrifuge preparations were T1^+, T3^+, T4^+,
Table 1. Nature of Lymphocyte Populations in Lymph Nodes and Spleens Involved by Hodgkin’s Disease*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Location of Tissue</th>
<th>Diagnosis</th>
<th>Antibodies</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-T1,T3/Anti-IgM Anti-T4/Anti-T5,T8 Anti-T10</td>
</tr>
<tr>
<td>1</td>
<td>Axillary lymph node</td>
<td>Nodular sclerosis</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; irregular aggregates of IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>2</td>
<td>Cervical lymph node</td>
<td>Nodular sclerosis</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; small aggregates of IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>3</td>
<td>Cervical lymph node</td>
<td>Nodular sclerosis</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; residual germinal centers with IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>4</td>
<td>Inguinal lymph node</td>
<td>Nodular sclerosis</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; small aggregates of IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>5</td>
<td>Supraclavicular lymph node</td>
<td>Nodular sclerosis</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; scattered IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>6</td>
<td>Supraclavicular lymph node</td>
<td>Nodular sclerosis</td>
<td>Large minority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; majority of IgM&lt;sup&gt;+&lt;/sup&gt; cells in nodules</td>
</tr>
<tr>
<td>7</td>
<td>Cervical lymph node</td>
<td>Mixed cellularity</td>
<td>Large minority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; majority of IgM&lt;sup&gt;+&lt;/sup&gt; cells in nodules</td>
</tr>
<tr>
<td>8</td>
<td>Cervical lymph node</td>
<td>Mixed cellularity</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; residual follicles with IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>9</td>
<td>Abdominal lymph node</td>
<td>Mixed cellularity</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; residual follicles with IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
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<tr>
<td>10</td>
<td>Cervical lymph node</td>
<td>Mixed cellularity</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; few scattered IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
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<td>11</td>
<td>Parotid lymph node</td>
<td>Mixed cellularity</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; large irregular aggregates of IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>12</td>
<td>Involved spleen*</td>
<td>Mixed cellularity</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; large irregular aggregates of IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>13</td>
<td>Involved spleen*</td>
<td>Nodular sclerosis</td>
<td>Majority of T1&lt;sup&gt;+&lt;/sup&gt;, T3&lt;sup&gt;+&lt;/sup&gt; cells; irregular aggregates of IgM&lt;sup&gt;+&lt;/sup&gt; cells</td>
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*In lymph nodes with follicular and diffuse hyperplasia, a majority of cells in the paracortical area (T-cell area) are T1<sup>+</sup>, T3<sup>+</sup>, and T4<sup>+</sup>, whereas only a minority of cells are T8<sup>+</sup>. Most of the cells in the primary and secondary follicles (B-cell area) are IgM<sup>+</sup>. In addition, a small number of T1<sup>+</sup> and T3<sup>+</sup> cells are also seen in both primary and secondary follicles. Most of these cells are T4<sup>+</sup> as well. Anti-T10 antibody reacts with germinal center cells of secondary follicles and plasma cells, whereas T cells in the paracortical area are not stained. The malpighian follicles of spleen show similar reactivity to that seen in the lymph nodes.

†Since it is not possible to accurately estimate the percentage of reactive cells in tissue sections, the approximate range of reactivity observed has been tabulated as: majority, >60%; large minority, 30%-40%, and minority, <20%.

‡Same patient as number 8.
and T10⁺ and unreactive with anti-T5, anti-T6, and anti-T8 antibodies.

In areas of spleens from patients with Hodgkin's disease not involved by the disease, some of the periarteriolar sheaths of lymphocytes (PALS) were enlarged as compared to PALS of 5 spleens that were removed because of traumatic rupture or for the treatment of idiopathic thrombocytopenia. Most of the cells were T1⁺, T3⁺, and T4⁺. Some of the T1⁺, T3⁺, and T4⁺ cells in markedly enlarged PALS were also stained with anti-T10 antibody. These areas did not contain Reed-Sternberg cells and were therefore not considered to be involved by Hodgkin's disease.

As shown in Table 2, analysis of lymphocyte subpopulations, utilizing single cell suspensions and indirect immunofluorescence analysis, confirmed the results found in frozen sections. The predominant cells in lymph nodes were T3⁺ and T4⁺. In some lymph nodes, a majority of cells were T10⁺, indicating that T cells in these preparations expressed T10 antigen. Again anti-T6 antibody did not react with any lymphocytes.

DISCUSSION

In this study, lymphocytes and Reed-Sternberg cells in lymph nodes and spleens of patients with Hodgkin's disease were characterized employing a series of monoclonal antibodies directed against human thymus, peripheral T cells, and Ia antigens. The studies were mostly carried out in cell suspensions using indirect immunofluorescence technique and on frozen sections using the four-step peroxidase–antiperoxidase
Patients 14, 15, and 16 had nodular sclerosis and patient 17, lymphocyte-predominant Hodgkin’s disease. Controls included hyperplastic lymph nodes. All suspensions were made from cervical lymph nodes and analyzed by cytofluorography.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Percent Reactivity With Monoclonal Antibody</th>
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<tr>
<td></td>
<td>Anti-T3</td>
</tr>
<tr>
<td>14</td>
<td>81</td>
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<tr>
<td>15</td>
<td>81</td>
</tr>
<tr>
<td>16</td>
<td>83</td>
</tr>
<tr>
<td>17</td>
<td>78</td>
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<tr>
<td>Controls (5 patients)</td>
<td>68 ± 10</td>
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*Patients 14, 15, and 16 had nodular sclerosis and patient 17, lymphocyte-predominant Hodgkin’s disease. Controls included hyperplastic lymph nodes. All suspensions were made from cervical lymph nodes and analyzed by cytofluorography.
ture character of these cells. Moreover, the reason that T10 antigen was expressed on a large number of T cells in only some patients is unclear, but points to the heterogeneity of the T-cell population or immunologic state of activation in these individuals.

The finding that Reed-Sternberg cells were not stained with any of the anti-T-cell antibodies is in agreement with previous observations that these cells do not rosette with sheep red blood cells and are not reactive with heterologous anti-T-cell antisera. No definite conclusion can be drawn from the finding of Ia antigen in Reed-Sternberg cells, since Ia antigens may be expressed on B cells, monocytes, and activated T cells. Presence of Ia antigen in Reed-Sternberg cells has also been suggested by studies carried out with heteroantiserum directed against B cells. Although a B-cell origin of Reed-Sternberg cells has been suggested by other studies on the basis of the presence of intracytoplasmic IgG in these cells, the presence of both kappa and lambda light chains in individual Reed-Sternberg cells points to an exogenous origin of these immunoglobulins. In addition, in this study the Reed-Sternberg cells were found to be unreactive with anti-IgM antibodies. On the other hand, tissue culture studies have suggested a monocyte/macrophage origin of Reed-Sternberg cells, but definite proof of this possibility in the cultured cells to the in vivo Reed-Sternberg cells has been lacking. Moreover, Reed-Sternberg cells are not stained by heterologous antimacrophage antiserum and a monoclonal antibody (anti-MI), which reacts with circulating monocytes, natural killer cells, and granulocytes (unpublished observations). However, these observations do not rule out the possibility that Reed-Sternberg cells are of monocyte/macrophage origin, since tumor cells may express antigens that are different from those possessed by their normal counterparts. Moreover, these antibodies may not react with all cells belonging to monocytes/macrophage lineage. Spleen reticulum cells, Langerhans cells of the epidermis, and interdigitating reticulum cells, which are probably related to each other, also express Ia-antigens and therefore have to be considered as possible cells of origin of Reed-Sternberg cells.

Interdigitating reticulum cells, which have been described in several species, including rabbit, rat, and man, appear to be present exclusively in T-dependent areas. They are intimately associated with helper T lymphocytes and are thought to play a role in the presentation of antigen to T cells. The juxtaposition of T4 helper-inducer T lymphocytes and Reed-Sternberg cells suggests an additional analogy between the latter cell type and interdigitating reticulum cells. Therefore, it is plausible that Reed-Sternberg cells are derived from antigen-presenting reticulum cells in thymus-dependent areas of lymphoid tissue. The observed loss of T cells from peripheral blood in Hodgkin's disease and their apparent enrichment in lymph nodes and spleens may occur partially as a consequence of cell--cell interactions between abnormal reticulum cells and T4 lymphocytes.

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