RAPID COMMUNICATION

Expression of Interleukin-1 in Reed–Sternberg Cells and Neoplastic Cells From True Histiocytic Malignancies

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INTERLEUKIN-1 (IL-1), a low-molecular-weight peptide produced by monocytes/histiocytes and other antigen-presenting cells, plays an important role in the regulation of immune function. In this study, we examined the production of interleukin-1 in 115 patients with a variety of human lymphomas by using a rabbit anti-interleukin-1 antibody and the immunoperoxidase technique. Interleukin-1 was detected in Reed–Sternberg cells from 20 patients with Hodgkin's disease as well as in neoplastic cells from 9 patients with true histiocytic lymphoma or malignant histiocytosis. In the other 86 cases, which included T- and B-cell lymphomas, no interleukin-1 could be detected. This result indicates a close relationship between Hodgkin's disease and true histiocytic malignancies and provides additional evidence to support our hypothesis that Reed–Sternberg cells are related to interdigitating reticulum cells. (Am J Pathol 1986, 125:221–225)

Materials and Methods

Anti-Interleukin-1 Antibody, Interleukin-1, and Interleukin-2

Rabbit anti-human IL-1 was obtained from Genzyme Co. (Boston, Mass). The antibody binds to human IL-1 from natural as well as recombinant sources, and also to the IL-1 precursor molecule. The specificity of this antibody has been documented previously.兔

Human monocyte-derived IL-1 was provided by Genzyme Co. and by Dr. L. B. Lachman (M. D. Anderson Cancer Hospital, Houston, Tex). The isolation, purification, and biologic properties of IL-1 have been reported. In addition, recombinant human interleukin-2 (IL-2) was also obtained from Genzyme Co.

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Cell Culture

Cells of the human lymphoma/leukemia cell lines U-937, THP-1, CTV-2, Ramos, and Molt-4 were cultured in RPMI 1640 medium as previously described. The monoblastic/histiocytic cell lines U-937 and THP-1 were known to exhibit IL-1 activity. In contrast, the third monoblastic cell line, CTV-2, and Molt-4 (T-cell) and Ramos (B-cell) are not stained by anti-IL-1.

Tissues and Immunoperoxidase Staining

We prepared 115 lymphoma tissue blocks for immunoperoxidase study (Table 1). All but two of these specimens were fixed in B5 fixative. One specimen from a patient with THL and one from a patient with MH were fixed in 10% buffered formalin. The diagnosis of all of these lymphomas was confirmed by extensive monoclonal antibody (MAb) study. We used the avidin–biotin complex (ABC) immunoperoxidase procedure as previously described to examine the expression of IL-1 in the lymphoma cells. The anti-IL-1 was diluted to 1:200.

The specificity of anti-IL-1 staining was evaluated by absorption of the antibody with lysates of IL-1-producing (U-937 and THP-1) and IL-1-nonproducing cells (CTV-2, Molt-4, and Ramos) at a concentration of 1 x 10^6 cells/ml. In addition, purified IL-1 or recombinant IL-2 (200 U/ml, Genzyme Co., Boston, Mass) was used for absorption prior to immunostaining. The antibody (1:200) was incubated with purified IL-1 (1:200), IL-2 (50 U/ml), or lysates (1:20) for 30 minutes at room temperature before being applied for tissue staining.

Table 1—Reaction with Anti-Interleukin-1 in Lymphomas

<table>
<thead>
<tr>
<th>Histologic classification</th>
<th>Number of cases</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-cell lymphomas</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Well-differentiated lymphocytic</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Intermediate differentiated</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Hairy-cell leukemia</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>Nodular poorly differentiated</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Nodular mixed</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Nodular histiocytic</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Diffuse histiocytic</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>Burkitt’s</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Lymphoblastic</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Immunoblastic</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>T-cell lymphomas</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Peripheral T-cell</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>Lymphoblastic</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Nodular sclerosis</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>Mixed cellularity</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Lymphocyte-predominant</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>Lymphocyte-depletion</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>True histiocytic malignancies</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>True histiocytic lymphoma</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Malignant histiocytosis</td>
<td>4</td>
<td>+</td>
</tr>
</tbody>
</table>

–, negative; +, positive.

Double-Immunohistochemical and Histochemical Staining

In addition to its reactivity with lymphoma cells from patients with HD, THL, and MH, anti-IL-1 reacts with some normal or reactive lymphoreticular cells in tissues. Some appear to be histiocytes, and others, the interdigitating reticulum (IR) cells in the T-cell zone. We used a double staining technique, as previously described, to determine the nature of these cells. Briefly, we used a hexazotized acid phosphatase method to stain IR cells and histiocytes. This was followed by anti-IL-1 immunostaining. For this purpose, we prepared frozen sections of normal lymphoid tissues and of lymph nodes from patients with dermatopathic lymphadenitis. The latter tissue is known to contain numerous IR cells.

Results

Among the 115 specimens tested, anti-IL-1 stained the neoplastic cells from HD, THL, and MH. No staining was observed in the B- or T-cell lymphomas. In all 20 cases of HD, anti-IL-1 reacted with the cytoplasm of H-RS cells. The percentages of cells positively stained ranged from 40% to 80%. The staining intensity varied from case to case (Figure 1A–C); this may be attributable in part to differences in the duration of fixation.

In THL, staining with anti-IL-1 was readily detectable in tissues in which the tumor cells showed a noncohesive growth pattern and in which reactive lymphocytes were numerous. In these cases, the majority of the tumor cells (>70%) were stained (Figure 1D). In contrast, in THL with cohesive growth, staining was usually restricted to small numbers of cells (20–40%), and the staining intensity was weak.

In MH, only a small number of tumor cells (10–30%) were stained by anti-IL-1. The staining intensity was generally weak.

The staining by anti-IL-1 in HD, THL, and MH was specific; it could be inhibited by absorption with IL-1 or with lysates from cells producing IL-1, but not with IL-2 or with lysates from cells not producing IL-1. In the two formalin-fixed samples, the anti-IL-1 staining was weak.

Anti-IL-1 stained approximately 20–30% of the histiocytes in the germinal centers, interfollicular zones, and sinuses of the lymphoid tissues. Very few histio-
INTERLEUKIN-1 IN LYMPHOMAS

Figure 1—Anti-IL-1 staining in paraffin-embedded sections of lymph nodes from patients with Hodgkin's disease (A-C) and true histiocytic lymphoma (D). In A–C, arrows indicate the Hodgkin's mononuclear cells and Reed–Sternberg cells that are stained by anti-IL-1. Numerous histiocytes and interdigitating reticulum cells in the background are also stained. The nature of these cells was confirmed by double enzyme histochemical staining. In D, anti-IL-1 staining in a case of noncohesive THL is illustrated.

cytes (<5%) in the red pulp of the spleen were positive for anti-IL-1. Anti-IL-1 stained IR cells; this was confirmed by double immunoperoxidase and enzyme histochemical staining in dermatopathic nodes.17,18 The anti-IL-1 staining in histiocytes and IR cells served as a built-in control in tissues from patients with B- or T-cell lymphomas. In all of the samples of normal tissues, anti-IL-1 did not stain B or T lymphocytes or other cells such as endothelial cells or fibroblasts.

Discussion

The expression of IL-1 in malignant cells from patients with THL and MH was not unexpected, because both are related to histiocytes. However, the expression of IL-1 in H-RS cells is of interest. The presence of IL-1- or IL-1-like activity in the supernatant from H-RS cells after short-term culture has also been reported by others.19

In normal lymphoid tissues, anti-IL-1 stained histiocytes and IR cells, but not B or T lymphocytes. Interdigitating reticulum cells have long been regarded as antigen-presenting cells; they are related to fixed histiocytes, as shown by the great similarity in their marker expression.20–22 Furthermore, the expression of IL-1 in IR cells indicates that histiocytes and IR cells may be functionally similar, despite the fact that IR cells do not phagocytose.18

The expression of IL-1 in H-RS cells suggests that HD is related to histiocytes or IR cells. We have previously proposed that H-RS cells may be derived from IR cells, rather than histiocytes, for several reasons.17,21,23–25 The markers expressed by H-RS cells and IR cells are similar and include Leu-M1, 2H9, and Leu-1. In addition, H-RS cells may express a marker
called IRAc, which is normally observed in activated IR cells in dermatopathic nodes.\textsuperscript{23} IR cells are localized in the T-cell zone; H-RS cells also tend to involve the T-cell zone.\textsuperscript{27} The expression of IL-1 in H-RS cells in the present study provides further support for their IR-cell origin.

However, IL-1 or IL-1-like activity can also be detected in a variety of other cells, including keratinocytes, astrocytes, mesangial cells, corneal cells, endothelial cells, large granular lymphocytes, and Epstein–Barr virus–transformed B lymphocytes.\textsuperscript{1} Recently, it has also been detected in B cells stimulated \textit{in vitro} by anti-immunoglobulin or lipopolysaccharide.\textsuperscript{28,29} In the present study, nevertheless, we observed anti-IL-1 staining neither in normal B cells nor in lymphoma cells of B-cell origin from 54 patients. Furthermore, we have been unable to now to detect B-cell markers in H-RS cells in more than 50 cases tested.\textsuperscript{17} Phorbol ester-induced H-RS cells also consistently failed to express B-cell antigens.\textsuperscript{10} This indicates that a B-cell origin for H-RS cells is quite unlikely.

Although IL-1 can be detected in all lymphomas derived from IR cells/histiocytes, its expression is more prominent in HD and noncohesive-type THL than in MH and the cohesive type of THL. The former two types of lymphomas are characterized by the presence of abundant lymphocytes, mostly T-cell, in the tissues.\textsuperscript{30} In a recent study, we noted that the noncohesive type of THL generally had a nonaggressive clinical course.\textsuperscript{30} This lymphoma may wax and wane for several years without treatment. Determining whether IL-1 produced by these tumor cells affects the clinical and histopathologic manifestations of HD and noncohesive THL will require further study. IL-1 can also induce the synthesis of acute-phase reactants and the proliferation of fibroblasts, and it is pyrogenic.\textsuperscript{1,5,6,31} Interestingly, patients with HD usually have a low-grade fever, increased acute-phase reactants are present in the serum, and the tissues involved by H-RS cells may show a significant degree of T-cell infiltration and fibrosis.

References

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Acknowledgment

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