AN ENZYME HISTOCHEMICAL STUDY OF NON-HODGKIN'S LYMPHOMA AND ALLIED DISEASE

BY

Ryuichi KAMIYAMA*1 and Reiko SHIMOKAWA*2

ABSTRACT

The enzyme activity for a-naphthyl-acetate esterase, naphthol-AS-acetate esterase, naphthol-AS-D-chloroacetate esterase, acid phosphatase, L(+-)-tartrate-resistant acid phosphatase, adenosine triphosphatase, and 5'-nucleotidase was examined on the neoplastic cells of giant follicular lymphoblastoma, the so-called reticulum cell sarcoma and Sézary syndrome. The neoplastic cells of giant follicular lymphoblastoma showed distinct activity for adenosine triphosphatase and 5'-nucleotidase, and those of the so-called reticulum cell sarcoma had no characteristic nature of the reticulum cells or histiocytes enzyme histochemically. These findings suggest that these neoplastic cells may be derived from the B-cell system. In Sézary syndrome, acid phosphatase activity was localized in a small paranuclear area in Sézary cells, which were considered to have a T-cell nature. It is thought that these enzyme histochemical methods are easy and useful in differentiating the B- or T-cell nature and the classification of non-Hodgkin's lymphomas.

INTRODUCTION

In recent years, non-Hodgkin's lymphomas have been studied from the immunological aspect,1-5 and whether these neoplastic cells are of B- or T-cell nature has been clarified by the application of surface markers. Consequently, a number of new classification of non-Hodgkin's lymphomas have been presented by introducing immunological concepts and immunohistochemical analyses,6-7

On the other hand, there are a few studies on enzyme histochemical characteristics of these lymphomas and allied diseases,8-11 although non-neoplastic lymphatic tissues were investigated considerably.11-15

We examined the enzyme histochemical behavior of neoplastic cells in non-Hodgkin's lymphomas and allied disease, and its results are reported in this paper with some discussions, considering the recent progress in immunology.

MATERIALS AND METHODS

In this investigation, the lymph nodes or peripheral blood from 2 patients of giant follicular lymphoblastoma, 3 of the so-called reticulum cell sarcoma, and 2 of Sézary syndrome were studied by enzyme histochemical methods (Table 1). For controls, we used the lymph nodes obtained at surgery from 5 patients with gastric carcinoma, in which metastasis was not recognized.

*1 神山隆一, Department of Pathology (Chief: Prof. S. HATAKAWA), School of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
*2 下川治子, Department of Pathology (Chief: Prof. M. ARIYOSHI), Medical Research Institute, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).

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Table 1. List of the cases examined

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Source</th>
<th>Pathological diagnosis</th>
<th>E-rosette (%)</th>
<th>EAC-rosette (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>M</td>
<td>Lymph node</td>
<td>Giant follicular lymphoblastoma</td>
<td>15.8</td>
<td>81.5</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>F</td>
<td>Lymph node</td>
<td>Giant follicular lymphoblastoma</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>F</td>
<td>Lymph node</td>
<td>Reticulum cell sarcoma</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>M</td>
<td>Lymph node</td>
<td>Reticulum cell sarcoma</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>F</td>
<td>Lymph node</td>
<td>Reticulum cell sarcoma</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>F</td>
<td>Peripheral blood</td>
<td>Sézary syndrome</td>
<td>94.0</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>M</td>
<td>Lymph node skin Peripheral blood</td>
<td>Sézary syndrome</td>
<td>89.6</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Tissues for enzyme histochemistry were sectioned in 6–8 μm thickness with a cryostat. α-naphthyl-acetate esterase by Leder,16 naphthol-AS-acetate esterase by Stüte,14 naphthol-AS-D-chloroacetate esterase and acid phosphatase by Leder,16 L(+)-tartrate-resistant acid phosphatase by Yam et al.,17 and adenosine triphosphatase and 5’-nucleotidase according to the method of Wachstein and Meisel18 with modification by Müller-Hermelink,13 were investigated on these sections. Tissue imprints as well as smears from peripheral blood were also available for enzyme histochemistry. Methods of the enzyme histochemistry used are presented in Table 2.

Table 2. Methods of enzyme histochemistry used

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Coupling agent</th>
<th>pH</th>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-naphthyl-acetate esterase</td>
<td>α-naphthyl-acetate</td>
<td>Hexazonium pararosaniline</td>
<td>7.0 (7.5)</td>
<td>5–30 (30–60)</td>
</tr>
<tr>
<td>Naphthol-AS-acetate esterase</td>
<td>Naphthol-AS-acetate</td>
<td>Fast blue BB salt</td>
<td>6.8</td>
<td>30, two times</td>
</tr>
<tr>
<td>Naphthol-AS-D-chloroacetate esterase</td>
<td>Naphthol-AS-D-chloroacetate</td>
<td>Hexazonium pararosaniline</td>
<td>6.3</td>
<td>30–60</td>
</tr>
<tr>
<td>Acid phosphatase and L(+)-tartrate-resistant acid phosphatase*</td>
<td>Naphthol-AS-BI phosphatase</td>
<td>Hexazonium pararosaniline</td>
<td>5.0–5.1</td>
<td>30–60 (240–300)</td>
</tr>
<tr>
<td>Adenosine triphosphatase</td>
<td>Adenosine 5'-triphosphate</td>
<td></td>
<td>7.2</td>
<td>60,90 (45,60)</td>
</tr>
<tr>
<td>5’-nucleotidase</td>
<td>Adenosine 5'-monophosphoric acid</td>
<td></td>
<td>7.2</td>
<td>60,90 (45,60)</td>
</tr>
</tbody>
</table>

* Final concentration of L(+)-tartrate acid is 0.05 M.
Figures in parentheses indicate pH and incubation time for imprints or smears.

Formalin-fixed tissues were routinely stained with Hematoxylin-Eosin, Giemsa, periodic acid-Schiff, and silver impregnation from paraffin sections. Some cases were also studied by the surface markers and electron microscopic observation. Electron microscopic study will be presented in a subsequent publication.

**RESULTS**

1) Giant follicular lymphoblastoma (Fig. 1): Adenosine triphosphatase and 5’-
nucleotidase activities were demonstrated on the cytoplasmic membrane of almost all of the neoplastic cells (Fig. 2). Three esterases and acid phosphatase reactions were negative in the neoplastic cells.

2) So-called reticulum cell sarcoma (Fig. 3): In all cases of the so-called reticulum cell sarcoma, almost all of the neoplastic cells were negative or only a few were weakly positive for α-naphthyl-acetate esterase, naphthol-AS-acetate esterase, and acid phosphatase stainings, while reactive, stromal reticulum cells or histiocytes intervening between the neoplastic cells were strongly positive for these enzyme reactions (Figs. 4 and 5). It was worthy of note that the neoplastic cells in one case (Case 5) showed positive adenosine triphosphatase activity (Fig. 6). On the other hand, naphthol-AS-D-chloroacetate esterase reaction was negative and L(+) tartrate-resistant acid phosphatase activity was not demonstrated in the neoplastic cells.

3) Sézary syndrome (Fig. 7): Sézary cells showed no enzyme activity except that acid phosphatase reaction was strongly positive. Acid phosphatase activity was localized in a small paranuclear area in the majority of Sézary cells (Fig. 8a); in 89% of Sézary cells in Case 6 and in 99% in Case 7. L(+) tartrate-resistant acid phosphatase activity was not observed within the cytoplasm of Sézary cells (Fig. 8b). In contrast with acid phosphatase reaction, periodic acid-Schiff staining was weakly positive in a smaller proportion, i.e., 4% in Case 6 (Fig. 8c).

These enzyme histochemical findings are summarized in Table 3.

4) Control series: The reticulum cells or histiocytes showed a strong activity for α-naphthyl-acetate esterase and acid phosphatase reactions (Figs. 9 and 10) and slight to moderate for naphthol-AS-acetate esterase, while no activity for naphthol-AS-D-chloroacetate esterase. The lymphocytes of the B-cell regions, namely, primary follicles and mantle of germinal centers, were positive, and the germinal center cells showed weakly positive activity for adenosine triphosphatase and 5'-nucleotidase reactions (Figs. 11 and 12).

**Table 3.** Enzyme histochemical findings of the neoplastic cells in non-Hodgkin's lymphomas and allied disease

<table>
<thead>
<tr>
<th>Case No.</th>
<th>α-naphthyl-acetate esterase</th>
<th>Naphthol-AS-acetate esterase</th>
<th>Naphthol-AS-D-chloroacetate esterase</th>
<th>Acid phosphatase</th>
<th>L(+) tartrate-resistant acid phosphatase</th>
<th>Adenosine triphosphatase</th>
<th>5'-nucleotidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>~+(a few)</td>
<td>~+(a few)</td>
<td>-</td>
<td>~+(a few)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>~±(a few)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Discussion**

It is pointed out that the lymphocytes of primary follicles and mantle of germinal centers which correspond to the B-cell regions show adenosine triphosphatase and 5'-nucleotidase activities, while the lymphocytes of the T-cell regions, i.e., the paracortical areas, are always negative for both enzymes.10) Braunstein et al.12) reported
that the germinal center cells in reactive lymph nodes had 5' nucleotidase activity. Our enzyme histochemical study also showed similar results that the lymphocytes of primary follicles and mantle of germinal centers as well as the germinal center cells in our control series were positive for adenosine triphosphatase and 5' nucleotidase reactions.

It is noteworthy that the neoplastic cells in giant follicular lymphoblastoma are positive for adenosine triphosphatase and 5' nucleotidase reactions. The result of our enzyme histochemistry as well as the surface marker in one case suggests that giant follicular lymphoblastoma may be derived from the B-cell system, as speculated by electron microscopic\cite{19-20} and immunological\cite{1} approach.

It seems that the neoplastic cells in all our cases, considered as the so-called reticulum cell sarcoma on a light microscopic level, have no distinct characteristic nature of reticulum cells or histiocytes, because almost all of the neoplastic cells show negative reaction for naphthyl acetate esterase, naphthol-AS acetate esterase, and acid phosphatase, and reticulum cells or histiocytes in our control series as well as in the reports on non-neoplastic lymphatic tissues\cite{11,12,14} are positive for these enzymes. In addition, it is of interest that adenosine triphosphatase activity was revealed on the cytoplasmic membrane of the neoplastic cells in one case of the so-called reticulum cell sarcoma. Although the surface marker was not examined in this case, these neoplastic cells from the enzyme activity may show the B-cell nature, such as described as B-cell-immunoblastic sarcoma\cite{17} or malignant lymphoma, immunoblastic.\cite{6} In other words, it seems that these neoplastic cells have the lymphatic character rather than the reticulum cell or histiocytic one. It is thought that this problem in the so-called reticulum cell sarcoma should be studied further, including the immunological approach.

Tamaoki and Essner\cite{15} demonstrated that the lymphocytes in the T-cell regions had a localized activity for acid phosphatase and \( \beta \)-glucuronidase, and those in the B-cell regions were negative for both enzyme reactions. Furthermore, Catoovsky et al.\cite{8} reported that acid phosphatase and periodic acid-Schiff reactions showed a different pattern of positivity in the cells of lymphoproliferative disorders according to their B- or T-cell nature. They pointed out that acid phosphatase reaction in the T-cell type was positive in a small paranuclear area in the majority of cells, while periodic acid-Schiff reaction was positive only in a minority; and a small proportion of lymphocytes in the B-cell type gave a weakly or moderately positive reaction for acid phosphatase reaction. These findings show that the localized activity for acid phosphatase is thought to be the marker enzyme for T-lymphocytes.

Concerning Sézary syndrome, it is said that the neoplastic cells are a variant of the thymus-derived lymphocytes by immunological study.\cite{21} Flandrin and Daniel\cite{9} also pointed out that Sézary cells were of T-cell nature according to the pattern of \( \beta \)-glucuronidase activity. In our two cases of Sézary syndrome, acid phosphatase activity localized to a small paranuclear area in the majority of Sézary cells. L(+)-tartrate-resistant acid phosphatase as observed in hairy cell leukemia\cite{37} was not present in Sézary cells. This result confirms that Sézary cell is thought to be the T-cell nature, as shown also by the surface marker of the present study.

It is of interest that there are distinct differences for acid phosphatase and \( \beta \)-
glucuronidase activity as the lysosomal enzyme, and adenosine triphosphatase and 5'-nucleotidase as the cytomembrane enzyme between the B- and T-cell nature, as mentioned above. These findings may indicate the difference of functional properties on the B- or T-cell system. In addition, it is thought that these enzyme histochemical methods are useful for the investigation and classification of non-Hodgkin's lymphomas or lymphoproliferative disorders, especially when cell suspensions cannot easily be obtained for immunological studies.

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REFERENCES


EXPLANATION OF FIGURES

Plate 1.

Fig. 1. Neoplastic cells of giant follicular lymphoblastoma (Case 1). Giemsa. ×500.

Fig. 2. Positive 5′-nucleotidase reaction of the neoplastic cells (arrows) in giant follicular lymphoblastoma (Case 1). ×500.

Fig. 3. Neoplastic cells of the so-called reticulum cell sarcoma (Case 3). Giemsa, ×500.

Fig. 4. Neoplastic cells of the so-called reticulum cell sarcoma (Case 3) are negative (arrows) for α-naphthyl-acetate esterase, while reactive, stromal reticulum cells (R) are positive. ×500.

Plate 2.

Fig. 5. No acid phosphatase activity (arrows) in the neoplastic cells of the so-called reticulum cell sarcoma (Case 3). R: Reactive, stromal reticulum cell. ×1,200.

Fig. 6. The cytoplasmic membrane of neoplastic cells in the so-called reticulum cell sarcoma (Case 5) shows distinct activity (arrows) for adenosine triphosphatase reaction. ×1,200.

Fig. 7. Sézary cells showing cerebriform nuclei (arrows). Peripheral blood of Case 7. Giemsa. ×1,200.

Fig. 8a. Acid phosphatase activity localized to a small paranuclear area (arrow) of Sézary cells. Peripheral blood of Case 7. ×1,200.

Fig. 8b. No L(+)-tartrate-resistant acid phosphatase activity in Sézary cell. Peripheral blood of Case 7. ×1,200.

Fig. 8c. Some Sézary cells are weakly positive for periodic acid-Schiff reaction (arrow). Peripheral blood of Case 6. ×1,200.

Plate 3.

Fig. 9. α-naphthyl-acetate esterase reaction of the lymph node in control series. Reticulum cells or histiocytes show strong activity (arrows). GC: Germinat center. ×120.

Fig. 10. Strong acid phosphatase activity of reticulum cells or histiocytes (arrows) in the lymph node of control series. GC: Germinat center. ×120.

Fig. 11. Adenosine triphosphatase reaction of the lymph node in control series. The lymphocytes of mantle (M) of germinal center and the germinal center cells (GC) show positive enzyme activity. ×120.

Fig. 12. Higher magnification of mantle (M) of germinal center in Fig. 11. ×500.