T Lymphocytes Expressing Human Ia-Like Antigens in Infectious Mononucleosis (IM)

By Eiji Tatsumi, Kaori Kimura, Yasuko Takiuchi, Shiro Fukuhara, Shigeru Shirakawa, Haruto Uchino, Shigeru Morikawa, and Jun Minowada

In six patients with Epstein-Barr virus (EBV) induced infectious mononucleosis (IM) and in two patients with IM-like syndrome, peripheral blood lymphocytes in the acute and convalescent phase were tested for human Ia-like antigens as well as other cell surface markers. The major population of T lymphocytes in the acute phase showed Ia-like antigens, as detected by indirect immunofluorescence with heteroantisera, and the number of Ia-like antigen-positive T lymphocytes decreased with convalescence. The crossabsorption study indicated that the amount of Ia-like antigen on the surface of IM T cells was less than that of Raji cells.

In primary Epstein-Barr virus (EBV) infection, which constitutes the disease known as infectious mononucleosis (IM), T-cell lymphocytosis with the appearance of atypical lymphocytes is an essential clinical feature among several laboratory findings and physical manifestations, and is thought to be a result of immunologic responses against B lymphocytes antigenically modified by EBV. Reactive states, almost wholly or only partly similar to IM, which are termed IM-like syndrome, are known to occur in response to various etiologic agents, including viruses other than EBV, toxoplasma, rickettsias, and drugs, although the mechanisms of T-cell lymphocytosis are not so well understood as in the case of EBV-induced IM, where T cells are thought to be mobilized through immunologic reactions against EBV-infected B cells.

The immunobiologic implications of I-region gene-associated (Ia) antigens have been extensively investigated in mice. The investigations of human Ia-like antigens with the use of heteroantisera prepared against human B-cell-specific antigens revealed T-B disparity with regard to Ia antigens with normal and some malignant lymphocytes and cultured cell lines derived from lymphoid malignancies. However, as the results in murine T cells indicate, human T cells also are expected to acquire Ia antigens in some functional states and such is indeed the case with T cells sensitized in mixed lymphocyte culture and cultured T cells maintained by mitogenic factor and certain antigenic stimulations. Therefore, we expected to find human Ia-like antigens on T lymphocytes in IM or IM-like syndrome.

MATERIALS AND METHODS

Patients

The age, sex, EBV serology, heterophile antibody, and diagnosis of eight patients are listed in Table 1. Patients 1–6 had EBV-induced IM, confirmed by EBV serology and had compatible clinical features such as tonsillar swelling followed by neck or systemic lymphadenopathy and serum enzyme alterations indicating moderate hepatic dysfunction. Heterophile antibody titer was elevated sufficiently in patients 1–5, but only slightly in patient 6. In patients 4 and 6, the time of the virus entry was determined on 11/26/79 and 1/7/80, respectively, as these patients stated that they had had oral contact.

Patient 7, a 52-yr-old woman, had transient lymphocytosis and systemic eruption probably due to isoniazid, which was prescribed during the hospitalization for mild fever of unknown origin. Although a provocative test was not performed, the apparent absence of other causative agents and the disappearance of the lymphocytosis and the eruption immediately after the discontinuation suggested that isoniazid was the factor involved. Patient 8, a 38-yr-old man, exhibited lymphocytosis and eruption after the 16-day administration of primidone, which was prescribed because of epileptic attacks after surgery for a right parietal meningioma. The lymphocytosis and the eruption subsided immediately after the discontinuation of the drug. In either patient, 7 or 8, the initial sera at the time of lymphocytosis and the follow-up sera showed no elevation of heterophile antibody titer and the pattern of stable seropositive state regarding EBV; stable positive titer of anti-viral capsid antigen (VCA), no elevation of anti-early antigen (EA) and stable sufficient titer of anti-EBV determined nuclear antigen (EBNA). Therefore, EBV was ruled out as the cause of their disorders.

Epstein-Barr Virus (EBV) Serology

The viral capsid antigen (VCA) of EBV was determined by a modification of the method of Henle and Henle, in which P,HR-1 cells were cultured at 32°C for 3 wk in arginine-deficient MEM (Nissui, Tokyo, Japan) supplemented with 10% calf serum (GIBCO, Grand Island, N.Y.) and fixed in acetone for 10 min. The early antigen (EA) was detected by the method of Glaser and Nonoyama. Raji cells were cultured in the presence of IUdR (25 μg/ml) for 3 days and then in fresh medium for 3 days, and fixed in acetone for 10 min. For EBV-associated nuclear antigen (EBNA), Raji cells were fixed in 50% methanol and 50% acetone at −20°C for 10 min, according to the method of Reedman and Klein.

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Sera were diluted with phosphate-buffered saline (PBS) (pH 7.2) to 1/40, 1/160, and 1/2560 for anti-VCA; 1/10, 1/40, 1/160, and 1/640 for anti-EA and with PBS (containing Ca
d+ and Mg2+) to 1/4 for anti-EBNA. Indirect immunofluorescence using fluorescein-conjugated rabbit anti-human IgG (Hoechst, Marburg, Germany) was used for anti-VCA or EA, and a three-step method using fivefold diluted serum preserved at -70°C obtained from patients with EBV-induced IM and known to be anti-EBNA negative as complement and fluorescein-conjugated rabbit anti-human p28 globulin (Hoechst, Marburg, Germany) was used for anti-EBNA. The antibody titer (reciprocal of dilution) of 20 seropositive healthy adults (year range, 22-30) was 40-640 (geometrical mean value, 74) for anti-VCA, <10 for anti-EA, and >4 for anti-EBNA. Primary EBV infection was confirmed by negative (<4) anti-EBNA and positive (>40) anti-VCA with the initial serum and by transient elevation of anti-EA, gradual elevation of anti-VCA, and positive seroconversion of anti-EBNA with the follow-up sera.1

**Cell Surface Markers and Human Ia-like Antigens**

Mononuclear cells were isolated from the heparinized venous blood of patients or healthy individuals by Ficoll-Hypaque-Conray discontinuous gradient centrifugation.21 Spontaneous rosette formation with sheep erythrocytes (E-rosettes) was performed as previously described.22 Receptors for Fc portion of IgG were tested by rosette formation with ox erythrocytes (Exo) precoated with IgG rabbit anti-Eox (EoxG rosette) by the method of Moretta et al.22 Receptors for complement were detected with rosette formation using zymosan beads (Zymosan A, Sigma Chemical Company, St. Louis, Mo.) preincubated in human serum (ZHC-rosette) or in AKR mouse serum (ZMC-rosette) as described by Huber and Wizell.24 Cell surface immunoglobulin was detected by direct immunofluorescence using fluorescein-conjugated rabbit anti-human whole γ globulin (Hoechst, Marburg, Germany) as previously described.22

Human Ia-like antigens were detected by indirect immunofluorescence with two sets of rabbit anti-human Ia-like antigens and fluorescein-conjugated goat anti-rabbit γ globulin. One was prepared at the Rosewell Park Memorial Institute,25 and the specificity directed to the glycoproteins p28, 33 had been confirmed.23

The other, which was prepared according to the method of Billing et al.,26 was purchased from Alpha Gamma Labs (Sierra Madre, Calif.). Washed and pelleted 10⁶ cells were suspended in 50 μl of the first reagent (rabbit anti-human Ia-like antigen serum), incubated for 30 min at room temperature, washed 3 times in PBS containing 0.1% NaN₃, and suspended in 50 μl of 20-fold diluted fluorescein-conjugated goat anti-rabbit globulin (Hoechst, Berlin, Germany). After the second incubation for 30 min at room temperature, the cells were washed 3 times and observed under a Olympus (Tokyo, Japan) fluorescence microscope equipped with a Osram (Germany) mercury lamp, HB 200W.

**Crossabsorption**

Fifty microliter of the rabbit anti-human Ia-like antigen antibody (prepared at Rosewell Park Memorial Institute, x 100 diluted) was absorbed with 10⁶, 4 x 10⁶, or 16 x 10⁶ Raji cells or with 10⁶, 4 x 10⁶, 16 x 10⁶, or 64 x 10⁶ IM T cells (patient 6, 1/22/80) for 1 h at 4°C. These preabsorbed sera were applied for staining 10⁶ Raji cells or IM T cells.

**Isolation of T lymphocytes**

Formed E-rosettes were suspended, and E-rosetting and non-E-rosetting cells were separated by Ficoll-Hypaque-Conray discontinuous gradient centrifugation.21 Sheep erythrocytes were lysed in ammonium chloride Tris buffer (pH 7.65) at 37°C for 3 min. From 85% to 95% of the isolated T cells formed E-rosette. Since the mononuclear cells from the IM patients in the acute phase originally contained a high percentage of T lymphocytes (76%-90%), the effect of enrichment of E-rosetting cells by this procedure was minimal. The proportion of Ia-like antigen-positive cells among isolated E-rosetting cells from healthy individuals ranged from 2% to 4%.

**RESULTS AND DISCUSSION**

The WBC and the percentages of E, ZHC, ZMC, and EoxG rosetting cells and human Ia-like antigen-positive cells in the mononuclear cells from 8 patients with IM or IM-like syndrome are shown in Table 2.

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**Table 1. Patients, EBV Serology, Heterophile Antibody, and Diagnosis**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (Sex)</th>
<th>Date</th>
<th>Anti-VCA</th>
<th>Anti-EA</th>
<th>Anti-EBNA</th>
<th>Paul Bunnell</th>
<th>Monospot</th>
<th>EBV as Etiology</th>
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<tr>
<td>1</td>
<td>25 (M)</td>
<td>1/17/79</td>
<td>40</td>
<td>10</td>
<td>&lt;4</td>
<td>224</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>24 (M)</td>
<td>1/12/79</td>
<td>40</td>
<td>&lt;10</td>
<td>&lt;4</td>
<td>224</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>19 (M)</td>
<td>4/25/79</td>
<td>40</td>
<td>10</td>
<td>&lt;4</td>
<td>224</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
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<td>12/17/79</td>
<td>40</td>
<td>10</td>
<td>&lt;4</td>
<td>112</td>
<td>+</td>
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</tr>
<tr>
<td>5</td>
<td>29 (M)</td>
<td>12/28/79</td>
<td>160</td>
<td>10</td>
<td>&lt;4</td>
<td>112</td>
<td>NT*</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>25 (F)</td>
<td>1/30/80</td>
<td>640</td>
<td>&lt;10</td>
<td>&lt;4</td>
<td>56</td>
<td>NT</td>
<td>Yes</td>
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<tr>
<td>7</td>
<td>51 (F)</td>
<td>12/28/78</td>
<td>640</td>
<td>&lt;10</td>
<td>&gt;4</td>
<td>32</td>
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<tr>
<td>8</td>
<td>38 (M)</td>
<td>10/17/79</td>
<td>40</td>
<td>&lt;10</td>
<td>&gt;4</td>
<td>14</td>
<td>-</td>
<td>No</td>
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<tr>
<td>9</td>
<td>12/14/79</td>
<td>40</td>
<td>&lt;10</td>
<td>&gt;4</td>
<td>14</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

*Not tested.*
Detection of Ia-like antigens was performed with unfractionated mononuclear cells in patients 1 and 2 and with both unfractionated mononuclear cells and isolated E-rosetting cells in patients 3–8. These results clearly indicate that the major population (62%–82%) of IM T cells in the acute phase expressed Ia-like antigens, these findings being in sharp contrast to the evidence that at most, only 4% of the isolated E-rosetting cells from 5 healthy adults were Ia-like antigen-positive. In the isolated E-rosetting cells from acute IM patients, fluorescence was not confined to large, probably atypical lymphocytes, but was present on small cells that can probably be morphologically classified as nonatypical. Thus, the percentage of Ia-positive T cells was much higher than that of morphologically determined atypical lymphocytes. The degree of the fluorescence in IM T lymphocytes was variable, stronger than that in positive mononuclear cells from healthy individuals, and weaker than that in the malignant cells from the lymph node of a patient with histiocytic lymphoma.

Because of the presence of EoxG-rosetting T lymphocytes, which may or may not reflect some switch-on state of peripheral blood T lymphocytes in IM, a control stain using fluorescein-conjugated goat anti-rabbit γ globulin serum alone or 20-fold diluted normal rabbit serum and the conjugate was used in patient 3. Less than 0.5% of the cells were positive.

The follow-up studies in patients 3–6, particularly those in patient 6, indicated that a larger proportion of T lymphocytes expressing Ia-like antigens persisted after the disappearance of atypical lymphocytes and at least for a month after the clinical onset.

In patient 8, the proportion of Ia-like antigen-positive T cells was not prominent in the initial sample (28%) in contrast to patient 7, in whom 78% of E-rosetting cells were positive. Thus, much earlier sampling may have revealed Ia-like antigens on the major population of T cells in patient 8.

The results of the crossabsorption studies are shown in Fig. 1. The preabsorption with 4 × 10⁶ Raji cells eliminated fluorescence on either 10⁶ IM T cells or 10⁶ Raji cells. However, 16 × 10⁶ IM T cells were required to eliminate fluorescence on 10⁶ IM T cells or 10⁶ Raji cells. These findings indicate that the amount of relevant antigens on the surface of IM T cells was significantly less than that seen in the Raji cells.

Pattengale et al. were unable to demonstrate human B-lymphocyte-specific antigen on the IM T lymphocytes with their antiserum. The differences in specificity and potency of the antisera employed or in the method of assay, complement-dependent cytotoxicity versus indirect immunofluorescence, may explain the discrepancy in the results.

Since it is readily suspected that functionally activated T lymphocytes may appear in the peripheral blood in inflammatory states other than IM or IM-like antigen serum to various altered lymphocytes is required to determine the specificity of our present.
observations to IM or IM-type reactions. During our present work, Johnsen et al. pointed out the presence of HLA-DR antigen-positive T cells in acute IM. Furthermore, suppressive activity of IM T cells has been described in the system of pokeweed-mitogen-induced B-cell differentiation, and such has been also reported in Japan. We also noted the suppressive effect by EBV-induced IM T cells and those in IM-like syndrome on normal lymphocyte response to mitogens or allogeneic lymphocytes in the work, the objective of which was to determine whether or not the la-like antigens on IM T cells lead to stimulation in mixed lymphocyte culture (unpublished observation).

It is therefore feasible that the la-like antigen expression may be associated with a suppressive activity. Further “lymphocytologic” studies should elucidate underlying shared or different mechanisms regarding lymphocyte mobilization between EBV-induced IM and IM-like syndrome.

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