Infectious Mononucleosis: Sequential Immunologic, Cytochemical, and Cytokinetic Studies on Single Lymphoid Cells in Peripheral Blood

By A. Hirt, P. Imbach, A. Morell, and H. P. Wagner

Sequential immunologic, cytochemical, and cytokinetic studies were done on single lymphoid cells in the peripheral blood of 6 children with infectious mononucleosis (IM) and 1 child with toxoplasmosis 1–2 wk after onset of symptomatology. The absolute number of AET-SRBC rosetting (E') cells was increased in all patients; the absolute number of cells with surface IgM (slgM') was increased in 3 of 7 patients. On the average, 46% of all lymphoid cells were E' la slgM', 35% E' la slgM', and 5% E' la slgM'. Of the E' la slgM' cells, only 7% were shown to have a dot-like esterase reaction pattern. Of the E' la slgM' cells, 17% were esterase positive. The mean labeling index of the former was 18.2%, the mean labeling index of the latter 4.4%. No proliferating slgM' cells were observed in the 6 children with IM. The esterase reactions and the labeling indices of suppressor-cytotoxic and inducer-helper T cells were similar to those of E' la slgM' and E' la slgM' cells, respectively. These results suggest that the vast majority of the atypical cells in IM corresponds to activated suppressor cells, while a minority appears to be proliferating helper cells.

Infectious Mononucleosis (IM) is a self-limited lymphoproliferative disorder caused by the Epstein-Barr virus (EBV).

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MATERIALS AND METHODS

Patients

Age, sex, diagnosis, serologic tests, time interval between onset of clinical symptoms and study, and white blood cell count are shown in Table I. Prior to the present illness, all patients were healthy. The first 6 children presented with classical IM symptomatology, i.e., pharyngeal inflammation, lymphadenopathy, hepatosplenomegaly, fever, and malaise. Based on results of serologic investigations, patient A.S. was considered to have acquired toxoplasmosis. She presented with lymphadenopathy, hepatosplenomegaly, and jaundice. All patients recovered without complications.

Methods

Approximately 5 ml of freshly drawn venous blood were anticogulated with EDTA and incubated in vitro for 30 min, at 37°C, with tritiated thymidine (3HdT; New England Nuclear, specific activity 6.7 Ci/mM; final concentration: 2 μCi/ml of anticoagulated blood). Thereafter, mononuclear cells were isolated with Ficoll-Rompacon and processed as described elsewhere. Briefly, approximately 5 x 10⁶ mononuclear cells were incubated simultaneously with a TRITC-conjugated F(ab')² anti-IgM and a monoclonal mouse antibody reactive with human la-like antigens (Ia).* Subsequently, the cells were washed and reacted with a FITC-conjugated goat anti-mouse Ig serum (Nordic Laboratories, Tilburg, The Netherlands). After further washing, AET-treated SRBC were added at 4°C according to Kaplan and Clark. Mono
cuclear cells of patient G.S. were incubated either with monoclonal mouse anti-human Ia and subsequently exposed to AET-treated SRBC as described above or with monoclonal anti-human Leu-2a (for suppressor-cytotoxic T cells) or anti-human Leu-3a (for inducer-helper T cells) (Becton Dickinson, Sunnyvale, Calif.) in an indirect immunofluorescence assay. Brush smears were made and fixed in formaldehdy vapors. Approximately 500 cells per patient were mapped by use of a computer (PDP-12) controlled fluorescence microscope with Nomarski interference. Surface immunofluorescence and AET-SRBC rosetting as well as morphology were

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RESULTS

Absolute numbers of all lymphoid cells, of AET-SRBC rosetting (E⁺) cells, and of cells with surface IgM (sIgM⁺) are shown in Table 2.

All patients presented with an elevated number of lymphocytes and of E⁺ cells, and in 3 of 7 patients the number of sIgM⁺ cells was elevated. In 4 patients examined (3 with IM and the 1 with toxoplasmosis), the percentage of lymphoid cells reacting with anti-T serum was found to vary between 80% and 89%, the percentage of E⁺ cells between 79% and 86%. If the lymphoid cells of these patients were incubated first with the anti-T serum and second with AET-SRBC, the relative number of E⁺ cells was reduced by only 20%-40%. If lymphoid blood cells of normal individuals were treated similarly, rosetting was inhibited almost completely.

The relative frequency, the percentage of ANAE⁺ cells, and the labeling index of 6 subpopulations defined by 3 surface markers (sIgM, Ia-like antigens, AET-SRBC rosetting) are shown in Table 3. The largest subpopulation was E⁺Ia⁻sIgM⁻ (46.4%), the second E⁺Ia⁻sIgM⁻ (35.3%), and the third E⁺Ia⁻sIgM⁻ (10.3%). Small subpopulations were E⁺Ia⁺sIgM⁻ (4.8%) and E⁺Ia⁺sIgM⁻ (3.1%). E⁺Ia⁺sIgM⁻ cells were not found in all patients.

Of all E⁺ cells, only 11.3% ± 2.7% were ANAE⁺. Of E⁺Ia⁻sIgM⁻ cells, 16.9% and of E⁺Ia⁺sIgM⁻ cells, 7.4% were ANAE⁺. The difference in ANAE positivity of the two latter subsets was significant (p < 0.01). The mean labeling index of all lymphoid cells (10.3%) was much higher than that of lymphoid blood cells of normal individuals (≤0.2%).³⁹² By far the highest mean labeling index was found in E⁺Ia⁺sIgM⁻ cells (18.2%). E⁺Ia⁻sIgM⁻ and E⁺Ia⁺sIgM⁻ cells had lower mean labeling indices (4.4% and 4.6%, respectively). Proliferating E⁺Ia⁺sIgM⁻ and E⁺Ia⁺sIgM⁻ cells were found in all, proliferating E⁺Ia⁺sIgM⁻ cells only in 3 patients. In these, 5.3%, 10.0%, and 12.4% of E⁺Ia⁺sIgM⁻ cells were labeled. Labelled E⁺Ia⁺sIgM⁻ cells were found exclusively in the patient with toxoplasmosis. In patient St.J. with IM, a total of 210 sIgM⁺ cells were selectively mapped. None was labeled.
Table 3. Frequency, Acid Esterase, and Labeling Index (Mean ± 1 SD in Percent)

<table>
<thead>
<tr>
<th>Subpopulation (Range)</th>
<th>Frequency (Range)</th>
<th>Esterase Positive Cells (Range)</th>
<th>Labeling Index (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E Ia slgM</td>
<td>35.3 ± 14.5</td>
<td>16.9 ± 4.7</td>
<td>4.4 ± 2.8</td>
</tr>
<tr>
<td>(10.0–52.7)</td>
<td>(10–22)</td>
<td>(1–8.2)</td>
<td></td>
</tr>
<tr>
<td>E Ia slgM</td>
<td>46.4 ± 17.6</td>
<td>7.4 ± 2.4</td>
<td>18.2 ± 3.5</td>
</tr>
<tr>
<td>(30.5–79.0)</td>
<td>(5–10)</td>
<td>(12.7–22.2)</td>
<td></td>
</tr>
<tr>
<td>E Ia slgM</td>
<td>4.8 ± 3.2</td>
<td>10.0 ± 7.1</td>
<td>0.4 ± 0.9</td>
</tr>
<tr>
<td>(1.4–11.2)</td>
<td>(0–21)</td>
<td>(0–2.1)</td>
<td></td>
</tr>
<tr>
<td>E Ia slgM</td>
<td>3.1 ± 1.3</td>
<td>5.4 ± 5.5</td>
<td>4.6 ± 5.5</td>
</tr>
<tr>
<td>(1.4–4.6)</td>
<td>(0–12)</td>
<td>(0–12.4)</td>
<td></td>
</tr>
<tr>
<td>E Ia slgM</td>
<td>10.3 ± 4.1</td>
<td>25.7 ± 7.4</td>
<td>2.0 ± 2.4</td>
</tr>
<tr>
<td>(5.0–18.2)</td>
<td>(19–36)</td>
<td>(0–5.6)</td>
<td></td>
</tr>
<tr>
<td>E Ia slgM</td>
<td>0.1 ± 0.2</td>
<td>*</td>
<td>*†</td>
</tr>
<tr>
<td>(0–0.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All lymphoid cells</td>
<td>100</td>
<td>12.7 ± 2.5</td>
<td>10.3 ± 2.9</td>
</tr>
<tr>
<td>(9–17)</td>
<td></td>
<td>(7.0–15.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Cell number too small.
†All cells unlabeled.

Cytophotometric determinations of nuclear DNA content in single E 'Ia'slgM and E 'Ia'slgM cells revealed that Ia-like antigen-related fluorescence could be detected in cells of all phases of the cell cycle (Fig. 1).

A comparison of the esterase reaction and the labeling index in 4 lymphoid cell populations of patient G.S. is shown in Table 4. It will be noted that Leu-2a' and E 'Ia'slgM as well as Leu-3a' and E 'Ia'slgM cells had similar esterase reactions and labeling indices.

Fig. 1. Nuclear DNA content in arbitrary units (AU) of E 'Ia'slgM (upper diagram) and E 'Ia'slgM cells (lower diagram). Open bars: unlabeled cells; black bars: labeled cells.

DISCUSSION

Possible pitfalls in sequential marker studies on single cells have been discussed elsewhere. In order to eliminate unspecific fluorescence without in vitro incubation at 37°C in serum-free medium, all sequential marker studies were done with a TRITC-conjugated F(ab')2 anti-IgM. B cells with sIgG or sIgA were therefore registered as E Ia'slgM.

Despite the heterogeneity of the clinical material investigated, the absolute number of lymphoid cells was clearly above the normal range in all patients. This increase was due to a higher than normal absolute T-cell number in all and to an increased absolute number of slgM+ cells in 3 of the 7 patients. In contrast to an earlier report,5 slgM+ cells always corresponded to small lymphocytes. No correlation was observed between the absolute number of slgM+ cells and the duration of clinical symptomatology.

In accordance with Asma et al.,22 the percentage of E' cells was slightly less than the percentage of cells reacting with an anti-T serum. Sequential incubation with the anti-T serum and AET-SRBC confirmed earlier observations,24 suggesting that T-cell antigens reacting with the anti-T serum may not be identical with receptors mediating AET-SRBC rosetting.

With our new method for sequential characterization of single lymphoid cells by 3 surface markers (slgM, Ia-like antigens, and AET-SRBC receptors) we could demonstrate that in the 7 patients investigated, on the average, 46% (range 31%–79%) of all lymphoid cells or 56% (range 38%–89%) of all T cells were of the phenotype E 'Ia'slgM+. These values correspond well to results reported by others.15,17,26,27

Lymphoid cells in the peripheral blood of IM patients are known to have strong suppressor activity.12-14 In addition, the majority of these cells are Ia+...
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Infectious mononucleosis, a marker phenotype corresponding to activated suppressor cells. Our E'1a'slgM^- cells might therefore well correspond to the same functional subset of lymphoid cells. This assumption is further supported by the results presented in Table 4.

A unique feature of our methodology is the possibility to assess the proliferative activity of single lymphoid cells characterized by several immunologic and cytochemical markers. With this technique we could demonstrate that the mean labeling index of E'1a'slgM^- lymphoid cells was 18.2%, a labeling index comparable only to that of some neoplastic lymphoid cell populations. Morphologically, E'1a'slgM^- cells were predominantly atypical, large and intermediate size lymphoid cells, but a few small E'1a'slgM^- lymphocytes were also found. In contrast, E'1a'slgM^- cells (10.0%-52.7% of all lymphoid cells) were predominantly small lymphocytes with fewer atypical large cells and with a much lower labeling index (Table 3). Interestingly enough, the patient with acquired toxoplasmosis showed the same proportions and the same labeling indices of E'1a'slgM^- and E'1a'slgM^- cells as the 6 patients with IM. Since the relative size of these cell populations could be influenced by variations in the expression of the 1a-like antigens during the cell cycle, it was of interest to find by cytophotometric DNA determinations on single cells, that both E'1a'slgM^- and E'1a'slgM^- cells were distributed throughout the cell cycle (Fig. 1). When the esterase pattern of these two cell populations was studied, it was found that only 7.4% of the E'1a'slgM^- and 16.9% of the E'1a'slgM^- cells were ANAE+. In patients with MLS, only 11% of all T cells had a dot-like ANAE reaction and not 70% as in normal individuals. In this context, it will be noted that the percentage of Leu-2a^- cells with positive esterase reaction was low (11%, Table 4). In addition, the relative number of esterase positive Leu-3a^- cells was higher (25%, Table 4), but still considerably lower than in Leu-3a^- cells of normal individuals (unpublished observation). It appears therefore that not only suppressor-cytotoxic but also inducer-helper T cells are activated in IM. This assumption is supported by the fact that the latter have a moderately elevated labeling index.

In all of our IM patients investigated, practically no labeled slgM^- cells were found. However, EBV-infected proliferating slgA^- or slgG^- cells, due to the methodology used, would be found in the compartment of E'1a'slgM^- cells. E'1a'slgM^- and E'1a'slgM^- cells represent heterogeneous subsets. Of the former, some may have been B lymphocytes. Monocytes were excluded by morphological criteria and the esterase reaction pattern. In some patients, large atypical lymphoid cells were observed in these compartments, more often among E'1a'slgM^- cells. Since some of these cells had one or two attached SRBC, it is conceivable that these were T cells with incomplete rosetting or rosettes destroyed when brush smears were made. The presence of atypical cells might explain why, at least in some patients, cells of these populations were labeled. As in normal individuals, a few E'slgM^- cells were found in some MLS patients. All of these were 1a^-.

In conclusion, our methodology related four markers (three immunologic, one cytochemical) with two proliferative characteristics (3HdT uptake in vitro and nuclear DNA content) of single lymphoid cells. At the time of study the majority of the atypical large lymphoid cells in the peripheral blood of children with MLS were E'1a'slgM^- cells with a high labeling index and a non-dot-like esterase reaction pattern and most probably represent activated suppressor cells. Our studies suggest, furthermore, that not only suppressor but also some helper T cells were activated. Some of the atypical cells were E'1a'slgM^-, but none were slgM+. In different subsets of lymphoid cells, the labeling index appeared to be related to the relative number of atypical large cells. It remains to be seen why the compartment of atypical cells comprises different phenotypes and what the relations between the labeling index and the phenotype mean.

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