Cytotoxic Effector Cells from Infectious Mononucleosis Patients in the Acute Phase Do Not Specifically Kill Epstein-Barr Virus Genome-Carrying Lymphoid Cell Lines

PRAVIN C. PATEL,1 GUÉRIN DORVAL,2 AND JOSÉ MENEZES1*

Laboratory of Immunovirology, Pediatric Research Center, and Department of Microbiology and Immunology, University of Montreal, Ste-Justine Hospital, Montreal, Quebec, Canada H3T 1C51 and Division of Clinical Immunology, Royal Victoria Hospital, and McGill University, Montreal, Quebec, Canada H3A 1A2

Received 21 January 1982/Accepted 7 June 1982

We describe a study in which we investigated the cytotoxic activities of thymus-derived (T) lymphocytes and natural killer cells against Epstein-Barr virus (EBV) genome-carrying lymphoid cell lines. Purified subpopulations of lymphocytes from eight patients with infectious mononucleosis and six healthy normal EBV-seropositive donors were tested. Enriched T-cells were obtained by passing purified whole blood lymphocyte preparations through human immunoglobulin–anti-immunoglobulin-coated glass bead columns. The cytolytic activity of effector cells was determined by the ability of these cells to lyse human target cells that were internally labeled with 51Cr. These targets included cells from both EBV genome-carrying and EBV genome-negative lymphoid lines derived from malignant tumors, as well as from lymphocytes transformed in vitro by EBV, and were chosen to represent a wide spectrum of EBV-associated membrane antigens. We found that cytotoxic T-cells from patients with infectious mononucleosis showed no EBV-related specific cell killing per se, although a trend for increased killing of cell lines derived from spontaneous in vivo growing tumors, EBV genome carrying or not, was noted; however, this trend was not observed with cell lines derived from cord blood lymphocytes after EBV infection in vitro. In addition, our data suggest that natural killer cells may play an important role in controlling EBV infection in patients with infectious mononucleosis in the acute phase of the disease, particularly since T-cells (obtained after removal on immunoglobulin–anti-immunoglobulin columns of natural killer cells presumably bearing Fc receptors) were less efficient killers than whole blood lymphocytes; furthermore, lysis by whole blood lymphocytes was also greatest against cell lines derived from malignant tumors (as opposed to in vitro EBV-transformed cord blood lymphoid lines), irrespective of whether these targets were EBV genome positive or negative.

Epstein-Barr virus (EBV) is the etiological agent of infectious mononucleosis (IM), a self-limiting lymphoproliferative disease (12), and can transform or immortalize human and simian lymphocytes in vitro. In addition, the EBV genome is regularly detectable in the cells of two types of neoplasia, African Burkitt’s lymphoma (BL) and undifferentiated nasopharyngeal carcinoma (23). Analysis of the specific EBV immune response in IM patients may provide a better understanding of the composite immune mechanisms involved in the pathogenesis of benign versus malignant virus-induced lymphoproliferative disorders. Infectious EBV particles or infected cells undergoing a productive virus-replicative cycle have not been detected in the blood of patients with acute IM (40). However, non-virus-producing but EBV genome-containing B-lymphocytes continue to be present in the circulation (4, 24), and continuous EBV-carrying B-cell lines can be established readily in vitro from the blood of IM patients (5). Thus, it has been suggested that the B-cells in IM patients are latently infected (9) and that the full cytological replacitive cycle of virus production is arrested at an early stage, with the expression of only the early virus-detected antigens. These antigens include the EBV-induced nuclear antigen (39) and the lymphocyte-detected membrane antigen (LYDMA) (45); however, it is noteworthy that the observations on the expression of LYDMA remain controversial (21, 41). It is
likely that EBV-induced nuclear antigen cannot be recognized readily by the different known lymphoid effector cells, since it is an intranuclear antigen, not a plasma membrane-associated antigen (39). In IM, it has been thought that the majority of the so-called atypical lymphocytes are actually EBV-immune T-killer cells, which are capable of arresting EBV infection by virtue of their high cytotoxic potential for LYDMA (1, 16, 33, 42, 45, 46). This mechanism was suggested by in vitro studies in which EBV-carrying cell lines were used as target cells.

In this study, we investigated the activities of cytotoxic thymus-derived (T) lymphocytes (CTLs) and natural killer (NK) cells (35) in the destruction of EBV genome-positive and EBV-negative cell lines. Enriched effector T-lymphocytes or whole blood lymphocytes (WBL) were obtained from IM patients and from normal controls. It is important that our experimental approach also included, as targets, a unique series of isogenic cell lines (i.e., BJA-B and its in vitro EBV-converted sublines), which were particularly relevant to the possibility of identifying unambiguously the viral antigen(s) required for CTL activity specific for EBV. In addition, we thought that this approach might enable us to detect any strain-specific antigenic change(s) on the cell surface caused by the use of two different strains of EBV.

Our results indicate that CTLs from IM patients do not kill EBV genome-containing cell lines specifically; however, our data do show an enhanced cytotoxic activity of effector cells from IM patients against in vivo-derived target cells, irrespective of the EBV genome status of the targets.

MATERIALS AND METHODS

Clinical sampling. (i) Patients with IM. Ten students attending McGill University volunteered to give blood during the acute phase of bona fide IM. Each diagnosis was made clinically and after an IM test performed by S. A. Smith and colleagues at the University Health Service. The presence of at least 20% atypical lymphocytes and the presence of elevated levels of heterophil antibodies were confirmed in our laboratory at the time that our study was performed. Two of the subjects were excluded, one because he was already in an asymptomatic convalescent phase and the other because he did not strictly meet all of the criteria for IM. When peripheral blood from the other eight confirmed IM patients was collected, each of these individuals was reported to be symptomatic for a different period of time; the shortest of these periods was 7 days, and the longest was 20 days.

(ii) Healthy subjects. Six EBV-seropositive healthy individuals without any history of recent infection or disease were used as controls. These donors were referred to as normal controls.

Preparation of WBL. (i) Purification of lymphocytes. A 50-ml sample of venous blood was collected in heparinized syringes and incubated in the presence of 100 μg of iron carbonyl particles (3 μm; A. D. Mackay Inc., New York, N.Y.) at 37°C for 30 min with intermittent shaking. Phagocytic cells that ingested iron carbonyl and free particles were removed with a magnet (11). An equal part of phosphate-buffered saline (50 ml) containing 4 mM EDTA (6) was added to each blood sample, and the mixture was applied in 25-ml portions to 15 ml of Ficoll-Hypaque (3); the tubes were then centrifuged at 400 x g for 20 min. The lymphocyte-enriched layer at the plasma-Ficoll interface was gently aspirated. The cells were washed three times with RPMI 1640 medium (GIBCO Laboratories, Burlington, Ontario, Canada). One part of this preparation was kept as whole lymphocytes; these cells were referred to as WBL.

(ii) Purification of T-lymphocytes. Enriched T-lymphocytes were obtained after filtration of WBL through an anti-immunoglobulin-coated glass bead column, as described previously (48). This technique was chosen because it depletes not only B-cells, by virtue of absorbing surface immunoglobulin-bearing cells, but also Fc receptor-bearing cells (unpublished data); this is because the immunosorbent matrix is reacted first with human immunoglobulins and then with a xenogenic rabbit anti-immunoglobulin antiserum, thus forming complexes with CH domains that are readily available for passing Fc receptor-bearing cells (6). Removal of these cells was important because they are considered to be the effectors involved in NK activity (47) and thus could mask specific T-cell killing (13, 14). The effluent cells were referred to as enriched T-cells.

(iii) Target cells. Target cells of different origins were selected to represent a wide spectrum of EBV-associated membrane antigens. The 16 cell lines used are listed in Table 1; the origin, EBV genome status, and surface characteristics of each cell line are also summarized in Table 1. The cells were grown in tissue culture medium (RPMI 1640 medium) containing 100 μg of streptomycin per ml, 100 IU of penicillin per ml, and 10% heat-inactivated fetal bovine serum. All cultures were fed twice weekly and were maintained in stationary flasks at 37°C in a humidified CO2 incubator. For all assays, the target cells were harvested at an identical time (16 h after feeding of the cultures).

Analysis of cell surface markers. Four different approaches were used to assess the purity of the enriched T-cells and to investigate the lymphoid cell surface characteristics of the cell lines.

(i) E-rosette testing. Effector cell preparations (WBL and enriched T-lymphocytes) were monitored for their contents of cells capable of forming rosettes with sheep erythrocytes, the so-called E-rosette-forming cells, a marker for T-cells (19). The detailed technical parameters of the method used have been described elsewhere (34). For all of our tests with anti-immunoglobulin column-passed cells, the purity of the T-cell preparations exceeded 90%.

(ii) EA-rosette testing. The Fc receptor-bearing cells (erythrocyte antibody rosette-forming cells [EA-RFC]) among the effector-lymphocyte and target cell line populations were assayed for their capacity to form rosettes with ox erythrocytes sensitized with a subhemagglutinating dose of immunoglobulin G antibodies (18). The actual EA-rosette technique was performed in the absence of any extrinsic serum or
protein, using only RPMI 1640 medium; incubation was for 20 min at 37°C. Only preparations with less than 1% EA-RFC were used for enriched T-cell preparations.

(iii) EAC-rosette testing. In all cell lines, receptors for the activated C3 component of complement were assayed by using as indicator cells (EAC) human blood group A erythrocytes that we sensitized with a subhemagglutinating dose of 19S allogeneic antibodies and then reacted with fresh mouse serum from strain A/Wu Sn, which congenitally lacks the C3 component. EAC-rosette-forming cell testing was then performed essentially as described above for EA-RFC, using a ratio of lymphocytes to EAC of 1:75.

(iv) Detection of surface-immunoglobulin-bearing cells. Effector lymphoid cells and target cell line populations were tested for the presence of membrane-bound immunoglobulin that was detectable by immunofluorescence by using a fluoresceinated goat anti-human immunoglobulin reagent (Hyland Laboratories, Inc., Costa Mesa, Calif.). Details of the assay used have been described elsewhere (7, 8). In the anti-immunoglobulin column-passage cell populations, the percentage of B-lymphocytes with immunoglobulin expressed on the cell surface did not exceed 0.5%.

Cytotoxicity assay. (i) 

51Cr labeling of target cells. Samples of the different target cell lines were washed three times in RPMI 1640 medium, and then 5 × 10⁶ cells were placed in 5-ml plastic tubes (type 2054; Falcon Plastics, Oxnard, Calif.) and centrifuged at 140 × g for 5 min. A 100-μl portion of sodium chromate (specific activity, 1 mCi/ml; New England Nuclear Corp., Lachine, Quebec, Canada) in 900 μl of RPMI 1640 medium supplemented with the same amounts of antibiotics and fetal bovine serum FBS that were used for the tissue cultures was added to each tube, and the cells were allowed to react in the pellet form for 60 min at 37°C. Then the cells were washed four times in RPMI 1640 medium, counted, and adjusted to a concentration of 10⁷ cells per ml.

(ii) 51Cr release assay. The actual parameters used to assess the cytotoxic activities of the lymphocytes from the different individuals tested have been described extensively by other workers (36). Essentially, 100 μl of chromium-labeled target cells (concentration, 10⁵ cells per ml) was added to each well of a 96-well microtiter V bottomed sterile plate (type 3040; Falcon). Then 50-μl portions of effector lymphocytes were added to two different concentrations; 2 × 10⁶ and 0.5 × 10⁶ cells per ml were added to give effector-to-target cell ratios of 10:1 and 2.5:1, respectively. All experiments were done in triplicate, with some wells receiving a preparation of tissue culture alone without effector cells to assess the spontaneous release of 51Cr by the different target cells used. In all cases, the total reaction volume was 150 μl, and the plates were incubated for 16 h at 37°C in a humidified CO₂-containing incubator. Then the plates were centrifuged at 200 × g for 5 min, and 100 μl of the supernatant was carefully harvested from each well for counting of the radioactivity. Maximum chromium release was obtained not by conventional hypotonic lysis of a corresponding sample of radiolabeled target cells, but by suspension of the cells in wells parallel to those containing the test effector lymphocytes and counting for the overall radioactive chromium incorporated within each cell line.

Results

Cytotoxicity of blood lymphocytes against EBV genome-carrying lines versus K-562 cells. In the first part of this study, we investigated whether WBL or enriched T-cells from IM patients or both differed from the corresponding lymphocyte populations from normal individuals in their capacity to kill the following three different types of target cells (Table 1): (i) K-562; (ii) Raji, B35M, and P3HR-1; and (iii) B85.

As Fig. 1 shows, WBL from either IM or normal donors very efficiently killed K-562 cells. Although WBL from IM patients tended to be more efficient in their cytotoxic effect on K-562 cells, the difference was not statistically significant. This effect may represent increased nonspecific functional aggression of active effector cells in NK activity. Furthermore, this K-562 cell killing was completely abolished in IM patients to the same extent as in healthy controls after enrichment of T-cells after anti-immunoglobulin column passage.

When the cytotoxic potentialities of WBL and enriched T-lymphocytes from IM patients and normal controls were tested on the three BL-derived EBV genome-carrying cell lines (Raji, B35M, and P3HR-1), the same phenomenon was observed; removal of Fc receptor-bearing cells significantly diminished the cytotoxic activity, and the lymphocytes from IM patients were generally more efficient than those from healthy subjects, although the difference was not statistically significant, except that enriched T-cells from IM patients reacted against B-35M cells, since the cytotoxic activity of EA-RFC-depleted lymphoid cells was not totally abolished compared with normal lymphocytes as effectors.

Overall, as determined by chi-square analysis, the cytotoxic behavior of lymphocytes from IM
<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Reference</th>
<th>EBV genome status&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Age of cell lines in culture (months)</th>
<th>% of cells expressing:</th>
<th>Surface</th>
<th>Fc receptors</th>
<th>Complement receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562</td>
<td>Chronic myelocytic leukemia</td>
<td>25</td>
<td>-</td>
<td>&gt;12</td>
<td>0</td>
<td>93.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Raji</td>
<td>BL tissue</td>
<td>37</td>
<td>+</td>
<td>&gt;12</td>
<td>1.8</td>
<td>1.8</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>B35M</td>
<td>BL tissue</td>
<td>31</td>
<td>+</td>
<td>&gt;12</td>
<td>0</td>
<td>1.8</td>
<td>98.6</td>
<td></td>
</tr>
<tr>
<td>P3HR-1</td>
<td>BL tissue</td>
<td>15</td>
<td>+</td>
<td>&gt;12</td>
<td>0</td>
<td>28.1</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>B85</td>
<td>WBL of EBV-seropositive leukemic adult</td>
<td>30</td>
<td>+</td>
<td>&gt;12</td>
<td>1.2</td>
<td>0.6</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>BJA-B</td>
<td>BL tissue</td>
<td>27</td>
<td>-</td>
<td>&gt;12</td>
<td>100.0</td>
<td>6.9</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td>BJA-B95-8</td>
<td>In vitro EBV-converted BJA</td>
<td>10</td>
<td>+</td>
<td>&gt;12</td>
<td>100.0</td>
<td>7.0</td>
<td>48.9</td>
<td></td>
</tr>
<tr>
<td>BJA-P3HR-1</td>
<td>In vitro EBV-converted BJA</td>
<td>10</td>
<td>+</td>
<td>&gt;12</td>
<td>100.0</td>
<td>2.8</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>BJA-B1</td>
<td>In vitro EBV-converted BJA</td>
<td>10</td>
<td>+</td>
<td>&gt;12</td>
<td>100.0</td>
<td>1.0</td>
<td>57.0</td>
<td></td>
</tr>
<tr>
<td>BJA-A5</td>
<td>In vitro EBV-converted BJA</td>
<td>10</td>
<td>+</td>
<td>&gt;12</td>
<td>100.0</td>
<td>0.8</td>
<td>16.11</td>
<td></td>
</tr>
<tr>
<td>C74-PP-1</td>
<td>CBL infected with throat washing of normal seropositive donor&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>&lt;7</td>
<td></td>
<td>66.7</td>
<td>1.7</td>
<td>69.2</td>
<td></td>
</tr>
<tr>
<td>C85-PP-2</td>
<td>CBL infected with throat washing of normal seropositive donor&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>&lt;7</td>
<td></td>
<td>1.5</td>
<td>2.6</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>C134-MA-J</td>
<td>CBL infected with throat washing of IM patient</td>
<td>+</td>
<td>&lt;7</td>
<td></td>
<td>3.4</td>
<td>1.3</td>
<td>71.4</td>
<td></td>
</tr>
<tr>
<td>C134-PR-IM</td>
<td>CBL infected with throat washing of IM patient</td>
<td>+</td>
<td>&lt;7</td>
<td></td>
<td>13.7</td>
<td>0.8</td>
<td>86.7</td>
<td></td>
</tr>
<tr>
<td>C134-M-2-IM</td>
<td>CBL infected with throat washing of IM patient</td>
<td>+</td>
<td>&lt;7</td>
<td></td>
<td>7.53</td>
<td>4.4</td>
<td>78.4</td>
<td></td>
</tr>
<tr>
<td>C191-B95-8</td>
<td>CBL infected with cell-free extract of B95-8 cells&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>&lt;7</td>
<td></td>
<td>73.1</td>
<td>2.0</td>
<td>65.3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> As determined by the presence (+) or absence (−) of EBV-induced nuclear antigen and EBV DNA in the cells.

<sup>b</sup> All of the cord blood-derived lines (CBL) were established in our laboratory as reported previously (26, 28).

<sup>c</sup> See reference 29.
patients against the three BL cell lines and against the EBV genome-negative K-562 cells did not differ from the behavior of lymphocytes from normal donors.

When cells of the spontaneously in vitro-derived cell line B-85 were used as targets for both IM and normal lymphocytes, no statistical difference in the cytotoxic activity of IM lymphocytes was observed, whether they were depleted of EA-RFC or not.

Cytotoxicity of blood lymphocytes against in vitro EBV-converted lymphoma-derived cell lines. When cell line BJA-B and its in vitro EBV-converted sublines were used as target cells in parallel with K-562 cells for assessing NK activity, the same cytotoxicity pattern described above (Fig. 1) was observed with K-562 cells, confirming that EA-RFC depletion and, consequently, NK annihilation were achieved very effectively (Fig. 2). When these lines were tested as target cells for lymphocytes from IM patients and controls, two different cytotoxicity patterns were observed. In the first pattern the cytotoxic activity of IM WBL was slightly greater than the cytotoxic activities of control lymphocytes for BJA-B, BJA-B-95-8, and BJA-A5 cells; the removal of EA-RFC did not affect to any significant extent the capacity of enriched T-cells from IM patients to kill any of these targets (Fig. 2). Depletion of Fc receptor-bearing cells from the lymphocytes of normal individuals also did not decrease the cytotoxic activity of the lymphocytes; in fact, it enhanced their cytolytic capacity. In the second pattern, P3HR-1-converted BJA-B sublines BJA-P3HR-1 and BJA-B1 were killed to a greater extent by lymphocytes from IM patients than by lymphocytes from a healthy control, although the difference was not statistically significant; EA-RFC depletion significantly diminished the cytotoxic activities of both types

FIG. 1. Cytotoxicity of IM and normal effectors against EBV genome-positive cell lines. The K-562 cell line was used as an EBV genome-negative control for NK activity. The results are expressed as percent specific lysis ±1 standard deviation. In each experiment the following measurements were made: IM effectors at an effector-to-target cell ratio of 10:1 (column A), normal effectors at an effector-to-target cell ratio of 10:1 (column B), IM effectors at an effector-to-target cell ratio of 2.5:1 (column C), and normal effectors at an effector-to-target cell ratio of 2.5:1 (column D). T, Enriched T-cell population. When IM and normal effectors were compared, the P values at effector-to-target cell ratios of 10:1 and 2.5:1, respectively, were as follows: for enriched T-cells and K-562 cells, not significant (NS) and NS; for enriched T-cells and Raji cells, NS and NS; for enriched T-cells and B35M cells, 0.01 and NS; for enriched T-cells and P3HR-1 cells, NS and NS; for enriched T-cells and B85 cells, NS and NS; for WBL and K-562 cells, NS and NS; for WBL and Raji cells, NS and 0.05; for WBL and B35M cells, NS and NS; for WBL and P3HR-1 cells, NS and NS; and for WBL and B85 cells, NS and NS. When WBL and enriched T-cells were compared, the P values at effector-to-target cell ratios of 10:1 and 2.5:1, respectively, were as follows: for IM cells and K-562 cells, 0.001 and 0.005; for IM cells and Raji cells, 0.0125 and 0.005; for IM cells and B35M cells, 0.01 and not determined; for IM cells and P3HR-1 cells, 0.0025 and not determined; and for IM cells and B85 cells, 0.05 and NS.
of lymphocytes but did not totally abolish the activities compared with K-562 cells. This finding was essentially identical to what we observed with the three BL-derived cell lines described above (Fig. 1). Thus, especially when EBV genome-negative cell line BJA-B was compared with its EBV-converted sublines as sources of target cells, no EBV-specific recognition characteristics by T-cells could be attributed to the lymphocytes from IM patients.

**Cytotoxicity of peripheral blood lymphocytes against in vitro EBV-transformed cord blood lymphoid cell lines.** In the experiments described above, only BL cell lines and one lymphoid cell line derived in vitro from an EBV-seropositive leukemic individual were used as target cells. For the vast majority of these cell lines, IM-derived lymphocytes were more efficient killers than cells obtained from normal controls. The specificity for EBV-related membrane components could not be established despite the use of highly purified T-cell subpopulations deprived of NK cells. To investigate further the reactivity of IM WBL toward EBV-infected cells, we used a battery of in vitro-derived lymphoid cell lines obtained after in vitro EBV infection of cord blood cells from four different newborn donors. These donors were not infected in utero with EBV (8); despite this, the resulting cell lines behaved in vitro and expressed EBV-induced antigens like the adult-derived cell lines (28).

As Fig. 3 shows, the cytotoxic potentials of lymphocytes from both IM and normal individuals toward cord blood lymphoid cell lines were relatively small compared with the cytotoxic potential observed with K-562 cells. Overall, whole blood effector cells behaved similarly whether they were obtained from IM patients or from normal subjects. However, as a group the enriched T-cells from all normal individuals were more efficient in killing lymphoid cell lines than the purified T-cells from IM patients were.

**DISCUSSION**

Our results clearly indicate that the T-cell reactivity of lymphocytes from IM patients is
variable, depending upon the EBV-infected cell line used. In some cases, this reactivity is even surpassed by that observed with lymphocytes from healthy individuals (Fig. 3). Furthermore, cytotoxicity of lymphocytes from IM patients is also present at a very significant level with an EBV genome-negative BL cell line (BJA-B) (Fig. 2) and with an EBV-negative cell line (K-562). With regard to the BJA-B target cells, as well as to the EBV-infected cord blood cell lines, enrichment of T-cells leads to a relative increase in the killing activity of lymphocytes from normal donors (Fig. 2 and 3). This contrasts with what was observed when K-562 cells were used as target cells. Taken together, our results do not support the existence of a specific killer T-cell activity against EBV genome-carrying cells in IM patients. This is in contrast to the results reported by other investigators (1, 45). Rather, our data indicate that the CTLs in the acute phase of IM are not specific against an EBV-related antigen (i.e., LYDMA) per se. This in turn raises doubts concerning the validity of the original definition of LYDMA as a lymphocyte (IM CTL)-determined membrane antigen (45). In addition, it is noteworthy that some investigators (21, 22, 41) have recently suggested that the original concept and definition of LYDMA have to be reevaluated. Therefore, considering our data presented above and the results of other investigators (21, 22, 41), we suggest that the cytotoxicity mediated by CTLs from IM patients is not specifically directed against an EBV-related membrane antigen. The recent results of Hutt-Fletcher and Gilbert (17) support this proposal and the data reported above. On the other hand, the role of some lymphocytes (most likely NK cells) in IM patients in the control of EBV infection cannot be excluded. In any event, our data suggest that both T-cells (capable of NK activity) and other lymphoid cells in acute IM are rendered general-
ly more aggressive against lymphoid cells derived from malignant tissues. The reasons for this increased killing activity are presently unknown, but they may be related to an increased stimulation of the immune system which is detected only against lines derived from in vivo malignancies regardless of their EBV genome status and not against in vitro-transformed cell lines after EBV infection. It is tempting to speculate that NK cells may be one of the components of the first line of defense against virus-infected or -transformed cells. Recent studies (2, 17, 21, 22, 33a, 41, 44) appear to suggest these possibilities indirectly. Interestingly, EBV-superinfected cells have been shown to be more sensitive to NK lysis than uninfected cells (2, 33a). It is also noteworthy that in the murine system it has been shown that NK and antibody-dependent cell-mediated cytotoxicity activities against cytomegalovirus-infected targets are detected significantly earlier than specific CTL-mediated cytotoxicity (38). Thus, at present we cannot rule out the possibility that a similar pattern may occur in human subjects undergoing viral infections.

The relatively poor cytotoxicities exhibited by lymphocytes from both IM patients and normal donors toward in vitro EBV-transformed cell lines are in accordance with the results obtained recently by Jondal et al. (20). The difference in susceptibility to lysis between cord blood-derived lines and BL-derived target cells may be due to the many differences (mainly in the cell surface characteristics) between these B-cell lines (32). On the other hand, it is also probable that cord blood-derived lines may be killed by a mechanism(s) other than CTL-mediated cytotoxicity. One reason for the insensitivity of cord blood-derived lines to CTL-mediated lysis could relate to the age of these cell lines, as it has recently been reported by Seeley et al. (43) that younger cultures (<12 months old) may be more resistant to CTL lysis than older lines. Interestingly, all of the cord blood-derived lines used in the present study (Table 1) were only 2 to 6 months old. It is also noteworthy that, in contrast to our results, Seeley et al. (43) found that young target cells were sensitive to CTL from IM patients; the reasons for these discrepancies are not known at present.

In conclusion, the evidence presented above clearly indicates that the T-lymphocytes from patients in the acute phase of IM are not specifically directed against EBV-infected cells per se. Thus, our data are in agreement with the very recent suggestions by other investigators that it is at present difficult to detect an EBV-specific component in the action of T-cells in the acute phase of IM exerted on B-cells (21) and that most, if not all, of the cytotoxicity from IM effectors might be mediated by a type of activated NK cell which is not truly EBV specific (41).

ACKNOWLEDGMENTS

We are extremely grateful to S. A. Smith and his colleagues for their continuous help in providing the blood used in this study. The expert technical assistance of N. Majia is acknowledged.

This investigation was supported by grants from the Canadian Medical Research Council and the Canadian Arthritis and Rheumatism Society. P. C. P. was the recipient of a studentship from the Cancer Research Society, Inc., Montreal, Canada, and J. M. was a senior scholar of the Conseil de la Recherche en Santé du Québec.

LITERATURE CITED


