Development of Cell-Mediated Immunity to Epstein-Barr Herpesvirus in Infectious Mononucleosis as Shown by Leukocyte Migration Inhibition

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Received for publication 20 September 1976

Eight patients with infectious mononucleosis, aged between 8 and 24, were studied for cell-mediated immunity by the in vitro leukocyte migration inhibition test at acute and convalescent stages. Follow-up studies were also carried out at up to 4 months after clinical illness. Cell-mediated immunity to Epstein-Barr virus (EBV) in the peripheral leukocytes from these patients was absent or incipient in all cases during the acute phase, although it was present in lymphocytes from a biopsied lymph node obtained from one of the patients. In contrast, cell-mediated immunity to EBV was detected readily in peripheral leukocytes obtained during convalescence and in the follow-up studies. A blocking factor that abrogated leukocyte migration inhibition induced by EBV antigen was detected in acute and convalescent sera obtained from six of eight patients, whereas serum antinuclear autoantibodies were detected in the two patients whose sera failed to block leukocyte migration inhibition. When sera were fractionated, this blocking effect was observed only in the serum immunoglobulin G fractions. In follow-up studies, neither the blocking factor nor the antinuclear autoantibodies were found in the sera collected.

It has been established that the Epstein-Barr herpesvirus (EBV) causes infectious mononucleosis, a benign lymphoproliferative illness (13). Epidemiological evidence suggests that the occurrence of clinical infectious mononucleosis at the individual level and its prevalence at the population level are related inversely to the age of EBV infection (6, 7, 14, 18) and that serological status is an index of protection (6, 27), since the disease arises only in individuals who have no serological evidence of previous exposure to the virus.

Cell-mediated immunity (CMI) is considered a primary factor in defense against infection with most viruses (1, 23). However, transient anergy as a manifestation of generalized depression of CMI associated with a variety of viral diseases and administration of viral vaccines has been reported (3, 25, 26, 28). In an earlier study (19) we were able to demonstrate the presence of CMI to EBV in normal subjects who had serum antibody to EBV and showed that CMI to EBV in five patients with acute infectious mononucleosis was not detected, despite the presence of antibody to EBV capsid antigen, but appeared in the convalescent phase of the illness. A blocking factor to EBV was also found in the acute and convalescent sera from these patients but not in normal subjects with serum EBV antibody (19). The presence of a blocking factor and the absence of CMI specific to EBV in acute infectious mononucleosis are of special interest, because the virus has also been incriminated in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma (12, 15, 30).

In this communication, we report on results obtained from studies carried out on another series of eight patients and confirm our findings reported earlier on CMI to EBV in patients with infectious mononucleosis (19).

MATERIALS AND METHODS

Subjects. Eight patients, aged between 8 and 24, with clinically diagnosed and hematologically confirmed infectious mononucleosis (International Classification of Diseases 075) were studied for CMI to EBV by the leukocyte migration inhibition test. None of the subjects was receiving medication known to cause depression of CMI, and all had enjoyed prior good health. Hyperbilirubinemia was detected in three patients, and elevated serum transaminase levels were detected in four of them during the acute phase of illness. Heterophile antibodies were detected in five, and hepatosplenomegaly was detected in six, whereas lymphadenopathy

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and general malaise were found in all patients. Multiple heparinized blood samples were collected at the acute stage and at convalescence, when all clinical symptoms and hematological abnormalities had disappeared. In one case, leukocytes derived from a cervical lymph node biopsy were available at the acute stage of the disease. All patients were tested for CMI to EBV repeatedly in follow-up studies for up to 4 months after the clinical illness. In addition, six normal subjects, aged between 18 and 26, were studied. Four were randomly chosen from a group of medical students volunteering for this study, whereas those remaining were selected because they did not have antibody (seronegative) to EBV. At the time of the study, all were enjoying sound health and none had a history of drug addiction.

Leukocyte migration inhibition test. Freshly collected, heparinized peripheral blood samples were allowed to sediment by gravity for 1 h at 37°C. Dextran was used to assist sedimentation when separation of erythrocytes from plasma was insignificant after 30 min. The leukocyte-rich plasma fractions were collected and centrifuged at 800 × g for 5 min. After washing three times in Hanks balanced salt solution containing heparin (100 IU/ml), the leukocytes were suspended in growth medium (RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, standard amounts of antibiotics [penicillin, 100 IU/ml; streptomycin, 100 µg/ml], and 2% of a 1.4 M N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid solution) at a concentration of 10⁶ cells per ml. Leukocyte migration inhibition was performed according to Lai et al. (16), using a crude EBV antigen prepared from PHR-1 cells (16), and the area of leukocyte migration for each tube was measured (19) without knowing its origin. The percentage of inhibition was calculated according to Lai et al. (19), and its significance was determined statistically.

Test for serum blocking factor. Sera collected at the acute stage, at convalescence, and in the follow-up studies were tested for the presence of blocking factor. Twofold dilutions of each serum sample were made, beginning with 1:5, and each serum dilution was mixed with EBV antigen or control antigen. The mixtures were used to challenge heterologous and autologous leukocytes after a brief incubation at 37°C, as described by Lai et al. (19).

Fractionation of serum. Three of the eight sera collected from patients with acute infectious mononucleosis were fractionated into immunoglobulin G (IgG) and IgM fractions in a column (2.5 by 100 cm; Pharmacia, Uppsala, Sweden), using Sphadex G-200 (Pharmacia), at a flow rate of 20 ml/h. The fractions were then examined at 280 nm in a spectrophotometer (Beckman Instruments, Purley, United Kingdom) before being used in immunodiffusion and immunofluorescent and immunoelectroosmophoretic studies. Presence of blocking factor in each fraction was tested as described.

Immunodiffusion. Immunodiffusion of serum fractions was carried out by using commercially available immunoplates (Hyland Laboratories, Inc., Costa Mesa, Calif.). In addition, each fraction was tested against rabbit antihuman IgG and rabbit anti-human IgM (Behringwerke, A.G., Marburg/Lahn, W. Germany) according to the method of Ouchterlony (24) in 0.8% agarose (Seakem agarose, Marine Colloids, Inc., Springfield, N.J.) buffered at pH 7.6.

Detection of serum EBV antibody by immunofluorescence. The presence of IgM and IgG antibodies to EBV in serum samples and in serum fractions was determined by indirect immunofluorescence (11, 18), using SH-RP cells (20) previously ultraviolet irradiated for the enhancement of virus capsid antigen (17).

Immuinolectroosmophoresis. Immunoelectroosmophoresis of serum IgG fractions was carried out according to Ouchterlony (24) with rabbit antihuman serum and antihuman IgG (Behringwerke) in 0.8% agarose buffered at pH 7.3, using a Vokam power pack (Shendon Scientific, United Kingdom) and a Kohn tank (Shendon Scientific) with constant current at 8 A.

Serum ANF. The presence of antinuclear antibodies (ANF) in serum samples was tested for by indirect immunofluorescence, using acetone-fixed smears of EBV-nonproducing Raji cells and human embryonic lung fibroblasts grown in tissue culture.

RESULTS

Migration inhibition response to antigens. Peripheral leukocytes from normal subjects seropositive to EBV responded to EBV antigen by marked inhibition (+20%), as determined statistically in leukocyte migration inhibition tests, whereas peripheral leukocytes from EBV-seronegative adults failed to respond (Table 1). In contrast, peripheral leukocytes from patients with infectious mononucleosis collected during the acute phase of the disease did not respond to EBV antigen (Tables 2 and 3).

<table>
<thead>
<tr>
<th>Normal subjects</th>
<th>Serum IgG titers* to EBV</th>
<th>Leukocyte migration inhibition (mean % ± SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>10</td>
<td>72.3 ± 2.8 (+)</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>37.4 ± 1.7 (+)</td>
</tr>
<tr>
<td>13</td>
<td>160</td>
<td>46.5 ± 1.4 (+)</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>31.2 ± 2.1 (+)</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>-3.7 ± 2.1 (-)</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>9.8 ± 2.3 (-)</td>
</tr>
</tbody>
</table>

* Assayed by indirect immunofluorescence (11, 18), using SH-RP cells (17, 20).

* Freshly collected leukocytes were used in the leukocyte migration inhibition test. Mean percent inhibition ± standard error (SE) was determined from a minimum of four replicate cultures. Twenty percent was considered positive, and <15% was considered negative. Peripheral leukocytes from all subjects failed to respond (<15%) to control antigen from Raji cells.
although IgG and IgM antibodies to EBV could be detected in their sera. Detailed results of two cases are given in Table 2. Since the pattern of results obtained in testing serial blood samples for the remaining cases did not differ significantly (with none showing significant mean percentages of migration inhibition) during the acute stage of disease, when clinical symptoms and hematological abnormalities as well as IgG and IgM antibodies to EBV were present, results obtained from the first blood samples collected at initial consultations are representative; these results are given in Table 3. However, despite the fact that peripheral leukocytes from patient no. 4 in the acute stage of disease repeatedly gave insignificant migration-inhibitory response to EBV antigen, lymphoid cells from a biopsied lymph node taken at this phase gave a marked migration-inhibitory response (Table 3).

At convalescence, when all clinical symptoms and hematological abnormalities had abated and when heterophile antibodies as well as IgM antibody to EBV had fallen to undetectable levels, significant inhibition (≥20%) of migration could be detected when peripheral leukocytes from these patients were challenged by EBV antigen (Tables 2 and 3). This reactivity did not appear to wane and could be detected in peripheral leukocytes of these patients repeatedly, up to 4 months after the illness. Detailed results of two cases are given in Table 2, whereas representative results for all cases, obtained from testing the first blood samples collected at convalescence and the last samples collected in follow-up, are given in Table 3.

When challenged by control antigen prepared from Raji cells, peripheral leukocytes from normal adults and from patients at different stages of infectious mononucleosis did not respond.

Serum antibodies to EBV. All sera collected at the acute and convalescent phases of infectious mononucleosis as well as in follow-up studies had serum IgG antibody to capsid anti-
The tested antigen in this study was EBV, and the collected blood samples were from individuals with infectious mononucleosis.

Leukocytes were assayed by immunofluorescence (18), using SH-RP cells (17, 21).

Mean percent inhibition ± SE was determined from a minimum of four replicate cultures. Twenty percent was considered positive, 15% was considered negative, and in-between was considered equivocal. Peripheral leukocytes from all subjects failed to respond to control antigen from Raji cells.

Acute infectious mononucleosis is defined clinically and hematologically in the presence of specific IgM to EBV and/or Paul-Bunnell heterophile antibodies. Results are those obtained from the first blood samples collected at initial consultations.

Convalescence is defined by the absence of clinical, hematological, and serological evidence of infectious mononucleosis.

Follow-up studies were carried out up to 4 months after clinical illness. Results are those obtained from the last blood samples.

Leukocytes from a lymph node biopsy of this patient at the acute phase of infectious mononucleosis tested against EBV antigen and control antigen gave mean percentages of inhibition of 24.3 ± 1.8 and 7.2 ± 1.3, respectively.

### TABLE 3. Antibody and leukocyte migration-inhibitory response to EBV antigen in patients with infectious mononucleosis

<table>
<thead>
<tr>
<th>Case of infectious mononucleosis, no.</th>
<th>Stage</th>
<th>Days after onset</th>
<th>Serum antibody to EBV</th>
<th>Paul-Bunnell heterophile antibody titer after absorption with guinea pig kidney</th>
<th>Migration inhibition (mean % ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute</td>
<td>24</td>
<td>80 64</td>
<td>0</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>64</td>
<td>80 0</td>
<td>0</td>
<td>32.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>179</td>
<td>80 0</td>
<td>0</td>
<td>31.4 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>Acute</td>
<td>6</td>
<td>20 128</td>
<td>0</td>
<td>7.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>41</td>
<td>80 0</td>
<td>0</td>
<td>28.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>190</td>
<td>80 0</td>
<td>0</td>
<td>42.1 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>Acute</td>
<td>15</td>
<td>40 32</td>
<td>84</td>
<td>16.2 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>84</td>
<td>40 0</td>
<td>0</td>
<td>37.2 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>110</td>
<td>40 0</td>
<td>0</td>
<td>39.8 ± 1.7</td>
</tr>
<tr>
<td>4'</td>
<td>Acute</td>
<td>11</td>
<td>320 32</td>
<td>84</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>92</td>
<td>160 0</td>
<td>0</td>
<td>24.1 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>222</td>
<td>160 0</td>
<td>0</td>
<td>32.7 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>Acute</td>
<td>16</td>
<td>320 16</td>
<td>280</td>
<td>6.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>65</td>
<td>320 0</td>
<td>0</td>
<td>47.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>145</td>
<td>320 0</td>
<td>0</td>
<td>46.4 ± 2.0</td>
</tr>
<tr>
<td>6</td>
<td>Acute</td>
<td>10</td>
<td>10 128</td>
<td>0</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>69</td>
<td>640 0</td>
<td>0</td>
<td>33.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>140</td>
<td>320 0</td>
<td>0</td>
<td>30.6 ± 1.9</td>
</tr>
<tr>
<td>7</td>
<td>Acute</td>
<td>13</td>
<td>160 120</td>
<td>56</td>
<td>−4.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>49</td>
<td>160 0</td>
<td>0</td>
<td>28.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>150</td>
<td>160 0</td>
<td>0</td>
<td>29.8 ± 1.6</td>
</tr>
<tr>
<td>8</td>
<td>Acute</td>
<td>18</td>
<td>40 64</td>
<td>56</td>
<td>6.7 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>60</td>
<td>40 0</td>
<td>0</td>
<td>52.7 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>285</td>
<td>40 0</td>
<td>0</td>
<td>38.7 ± 2.8</td>
</tr>
</tbody>
</table>

* Assayed by immunofluorescence (18), using SH-RP cells (17, 21).
* Freshly collected leukocytes and EBV antigen were used in the leukocyte migration inhibition test.
* Mean percent inhibition ± SE was determined from a minimum of four replicate cultures. Twenty percent was considered positive, 15% was considered negative, and in-between was considered equivocal. Peripheral leukocytes from all subject failed to respond to control antigen from Raji cells.
* Acute infectious mononucleosis is defined clinically and hematologically in the presence of specific IgM to EBV and/or Paul-Bunnell heterophile antibodies. Results are those obtained from the first blood samples collected at initial consultations.
* Convalescence is defined by the absence of clinical, hematological, and serological evidence of infectious mononucleosis.
* Follow-up studies were carried out up to 4 months after clinical illness. Results are those obtained from the last blood samples.
* Leukocytes from a lymph node biopsy of this patient at the acute phase of infectious mononucleosis tested against EBV antigen and control antigen gave mean percentages of inhibition of 24.3 ± 1.8 and 7.2 ± 1.3, respectively.

gen of EBV. In two of the patients, a significant rise in IgG titers could also be detected. Serum IgM antibody to EBV was detected in all acute sera; this varied in individuals and ranged from 1:16 to 1:128 (Table 3). However, there is no correlation between the levels of specific antibodies (IgG and IgM) to EBV and the level of leukocyte migration-inhibitory response (Tables 2 and 3).

ANF. ANF was detected in two of the eight patients with acute infectious mononucleosis (cases 4 and 5, Table 4). Nuclear staining was patchy or speckled, and, occasionally, peripheral or rim nuclear staining was observed.
Table 4. Effect of sera from normal adults and from patients with infectious mononucleosis on leukocyte migration induced by EBV

<table>
<thead>
<tr>
<th>Sera from case no.</th>
<th>Stage</th>
<th>Serum titer of blocking factor</th>
<th>Migration inhibition (mean % ± SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autologous leukocytes†</td>
</tr>
<tr>
<td>IM 1</td>
<td>Acute</td>
<td>20</td>
<td>3.4 ± 1.2*</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>10</td>
<td>4.1 ± 1.7*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>28.7 ± 1.6</td>
</tr>
<tr>
<td>IM 2</td>
<td>Acute</td>
<td>160</td>
<td>1.7 ± 2.1*</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>40</td>
<td>4.3 ± 1.8*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>43.2 ± 1.1</td>
</tr>
<tr>
<td>IM 3</td>
<td>Acute</td>
<td>160</td>
<td>-2.6 ± 1.1*</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>20</td>
<td>5.4 ± 2.2*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>42.1 ± 2.1</td>
</tr>
<tr>
<td>IM 4†</td>
<td>Acute</td>
<td>0</td>
<td>32.7 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>&lt;5</td>
<td>18.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>34.8 ± 1.4</td>
</tr>
<tr>
<td>IM 5†</td>
<td>Acute</td>
<td>0</td>
<td>17.2 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>80</td>
<td>2.1 ± 1.6*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>41.7 ± 1.8</td>
</tr>
<tr>
<td>IM 6</td>
<td>Acute</td>
<td>320</td>
<td>9.1 ± 1.7*</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>40</td>
<td>6.4 ± 2.4*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>47.2 ± 1.5</td>
</tr>
<tr>
<td>IM 7</td>
<td>Acute</td>
<td>40</td>
<td>3.2 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>320</td>
<td>5.1 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>30.6 ± 1.5</td>
</tr>
<tr>
<td>IM 8</td>
<td>Acute</td>
<td>160</td>
<td>8.7 ± 1.2*</td>
</tr>
<tr>
<td></td>
<td>Convalescent</td>
<td>160</td>
<td>9.8 ± 1.8*</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>0</td>
<td>37.3 ± 1.7</td>
</tr>
<tr>
<td>NA 11</td>
<td></td>
<td>0</td>
<td>57.2 ± 2.1</td>
</tr>
<tr>
<td>NA 13</td>
<td></td>
<td>0</td>
<td>37.8 ± 2.7</td>
</tr>
<tr>
<td>NA 15</td>
<td></td>
<td>0</td>
<td>8.2 ± 1.8</td>
</tr>
</tbody>
</table>

* IM, Infectious mononucleosis patient; NA, normal adult.
† Titer measured as the reciprocal of the greatest serum dilution at which the blocking effect on EBV-induced migration inhibition of autologous leukocytes is still detectable.
‡ Freshly collected autologous leukocytes or heterologous leukocytes whose migration was known to be inhibited by EBV were challenged by exposure to EBV antigen and test serum in the leukocyte migration inhibition test. Percent inhibition is expressed as mean ± SE, derived from a minimum of four replicate cultures; 20% or greater is positive, less than 15% is negative, and in-between is equivocal. In all cases insignificant migration inhibition responses were obtained when autologous or heterologous leukocytes were challenged by serum alone, in the absence of EBV antigen. * Indicates abrogation of expected leukocyte migration inhibition.
§ In IM patients, migration inhibition was determined by using autologous leukocytes collected at follow-up studies. Migration inhibition values of these leukocytes to EBV in the presence of heat-inactivated fetal bovine serum are given in Table 3.
§§ Migration inhibition was determined by using leukocytes from a normal subject whose migration was known to be inhibited by the EBV antigen preparation in the presence of heat-inactivated fetal bovine serum; percent inhibition was 23.4 ± 1.2.
* Sera collected at the acute phase of infectious mononucleosis and freshly frozen at -20°C.
*† Sera collected at convalescence and freshly frozen at -20°C.
* Follow-up studies at up to 4 months after clinical infectious mononucleosis. Sera were freshly collected.
* ANF detected in acute sera.
rum titers of ANF (IgG and IgM) in the two patients were low, at 1:8 (case 5) and 1:16 (case 4); treatment of the sera with mercaptoethanol resulted in decreases in serum titers but failed to abolish nuclear staining.

When tested at convalescence and again in follow-up studies up to 4 months after clinical illness, ANF was not detected by indirect immunofluorescence in sera from any patient.

Serum blocking factor. Of the eight patients tested, a blocking effect on EBV-induced migration inhibition of autologous and heterologous leukocytes was found in six sera collected at the acute phase, in seven collected at convalescence, and in none of the sera collected in the follow-up studies. Detailed results are given in Table 4. The titer of blocking factor in each serum collected from the eight patients with infectious mononucleosis is given in Table 4. The titers of blocking factor do not correlate with serum antibody titers to EBV (Tables 3 and 4). Sera from normal adults did not show any blocking effect on migration inhibition of leukocytes whose migration was known to be inhibited by EBV antigen in the presence of fetal bovine serum (Table 4).

When IgG and IgM fractions of acute sera collected from three of the eight patients were tested, a blocking effect on autologous leukocyte migration inhibition induced by the EBV antigen was observed in the IgG fractions, but not in the IgM fractions.

Immunodiffusion and immunoelectroosmophoresis. Immunodiffusion of IgG and IgM fractions in immunoplates or against antihuman IgG and antihuman IgM standard sera showed that the IgG fractions reacted only with antihuman IgG and not with antihuman IgM. Similarly, the IgM fractions reacted with antihuman IgM only. However, when the IgG fractions were tested with antihuman IgG and antihuman serum by immunoelectroosmophoresis, multiple components were observed (Fig. 1).

**DISCUSSION**

The evidence that infectious mononucleosis is caused by EBV is now extensive, but the basis for the self-limiting course of the illness is still unknown.

In an earlier study, we found that CMI to EBV in the peripheral leukocytes of five patients with acute infectious mononucleosis was not detected by the leukocyte migration inhibition test, but became detectable in convalescence (19). In this study, we confirm and extend this finding in another series of eight patients. The results suggest that CMI to EBV persists for a long period, at least up to 4 months after clinical illness, and probably for life, since it can be detected consistently in EBV-seropositive adults, as shown in Table 1 and in previous reports (8, 16; P. K. Lai, E. M. MacKay-Scollay, and M. P. Alpers, Proc. Aust. Soc. Med. Res., p. 151, 1973).

In the present study, the absence of migration-inhibitory response to EBV in peripheral leukocytes, together with the temporary anergy reported by others (2, 10, 21) during acute infectious mononucleosis, suggests the possibility of generalized immunosuppression. This, however, is difficult to reconcile with the histopathology of lymphoid tissues (4) during the acute phase of infectious mononucleosis. The temporary anergy in these patients may, of course, be explained by antigenic competition in the presence of excessive EBV antigen, and the absence of CMI to EBV in peripheral leukocytes does not preclude its presence in other host tissues. In fact, we have demonstrated a migration-inhibitory response to EBV in lymph node leukocytes obtained from one of the patients (case 4, Table 3) at a time when it was.

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**Fig. 1. Immunoelectroosmophoresis of serum IgG fraction against antihuman IgG and antihuman serum in 0.8% agarose at pH 7.3. The slide was photographed after washing in saline and staining with a 2% amido black solution.**
undetected in circulating leukocytes. This finding is consistent with the morphological changes in lymph nodes in infectious mononucleosis (4), in which the paracortex, a thymus-dependent zone not primarily involved in antibody formation, is found to be hyperactive.

Although the migration inhibition test has been reported to correlate well with delayed-type hypersensitivity (9) and involves the production of a migration inhibition factor (16, 29), it is still an open question whether cellular immune reaction detected by this assay is exclusively a T cell function. We are aware of the shortcomings in using a single in vitro correlate to detect CMI and are, therefore, developing an antigen-stimulated blastogenic test as well as an interferon release assay to complement our study on cellular immunity to EBV in patients with infectious mononucleosis.

Intense antibody formation in infectious mononucleosis has long been recognized. Humoral antibodies include heterophile antibodies, EBV antibodies, and a battery of hetero-, iso-, and autoantibodies. This intense antibody response is unique among infectious diseases and is equalled only occasionally in Sjögren's disease and systemic lupus erythematosus (5). However, the role of these antibodies, especially EBV-associated antibodies, in the control of infectious mononucleosis is obscure.

Recently, a blocking factor that abrogates CMI to EBV detected by the leukocyte migration inhibition test has been described in sera collected from patients with infectious mononucleosis (19). This serum blocking effect is probably specific to EBV, since it fails to negate CMI response to an unrelated antigen such as one prepared from *Escherichia coli* (19). In the present study, we have demonstrated that acute and convalescent sera collected from patients with infectious mononucleosis were able to abrogate in vitro leukocyte migration-inhibitory response to EBV, whereas sera collected from the same patients in follow-up studies up to 4 months after the illness failed to block EBV-induced leukocyte migration inhibition. It is impossible to say whether this blocking factor is simply an IgG antibody or, more generally, whether it is a product of infected or normal B cells, since although it is found in the IgG fraction, immunoelectrophoretic study of the fraction gave multiple precipitation lines.

The precise function of this blocking factor in vivo is unknown, although it interferes with leukocyte migration inhibition in vitro, as shown in the present study. It is thus not clear whether this blocking action demonstrated in vitro contributes to the intensive but self-limiting lymphoproliferation seen in infectious mononucleosis in vivo.

In acute viral hepatitis, it has been postulated that a hyperactive T cell response is responsible for the development of fulminant hepatitis in 1% of patients and results in severe liver cell necrosis, whereas impaired T cell function is responsible for the development of a hepatitis B antigen carrier state in 10% of patients and gives rise to slow, progressive, chronic liver disease (22). It is possible that the blocking factor detected in this study is part of the normal negative feedback mechanism involved in immunity to virus infection. Destruction of EBV-carrying cells in vivo may result in the release of autoantigen, and this process may have been enhanced by the absence of blocking-factor production as well as by a reduced efficiency of normal control mechanism against autoantibody formation (cases 4 and 5, Table 4).

The interrelationship between the humoral and cell-mediated components of immunity is obviously complex, and further information on this delicate immunological balance in EBV infection may help elucidate the pathogenesis of EBV-related diseases and other persistent viral infections.

ACKNOWLEDGMENTS

We thank F. Deinhardt and L. Wolfe for their discussions and advice and P. Lowder for assistance in preparation of the manuscript.

This work was supported by a collaborative agreement between the Biological Carcinogenesis Unit, International Agency for Research on Cancer, World Health Organization, and the Department of Microbiology, University of Western Australia, and by Public Health Service Contract no. N01 CP 45296 from the Division of Cancer Cause and Prevention within the Virus-Cancer Program of the National Cancer Institute. At the time of writing, P. K. L. was in receipt of a Research Fellowship from the International Agency for Research on Cancer, World Health Organization, Lyons, France. M. P. A. was a Senior Research Fellow of the National Health and Medical Research Council of Australia. E. M. M.-S. would like to thank the Commissioner of Health of Western Australia for permission to publish.

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