Clinical and Laboratory Evaluation of Infants and Children With Epstein-Barr Virus-Induced Infectious Mononucleosis: Report of 32 Patients (Aged 10–48 Months)

By Charles A. Horwitz, Werner Henle, Gertrude Henle, Mace Goldfarb, Paul Kubic, Richard C. Gehrz, Henry B. Balfour, Jr., Gary R. Fleisher, and William K. Krivit

Previous studies in Ghana and the United States have shown that the great majority of primary Epstein-Barr virus (EBV) infections in infants remain silent and fail to evoke hematologic and heterophil antibody responses typical of infectious mononucleosis (IM). In the present study, a search was made for cases of IM among children less than age 49 mo and a diagnosis of this disease was made in 32 patients. The great majority had fever, tonsillar pharyngitis, prominent cervical lymphadenopathy, and respiratory symptoms. There were significant differences in serologic responses between the 11 infants and toddlers aged 10–24 mo and 21 young children aged 26–48 mo. In particular, heterophil antibody responses were detected in only 3 of 11 infants (27.3%) compared with 16 (76.2%) of 21 children. The infants also had less prominent viral capsid antigen (VCA) specific IgM antibody responses (63.6% versus 100%) and a lower frequency of peak IgG anti-VCA titers of ≥1:320 (45.4% versus 76.2%). In addition, only 45% of the infants showed antibody responses to early antigens that were directed against the restricted (R) component in 2 and against the diffuse (D) component in 3. In contrast, anti-D responses of the type seen in 80–95% of young adults with IM were already present in 11 (52.4%) of the 21 children aged 26–48 mo. Peripheral blood smears from all patients showed numerous atypical lymphocytes and easily fulfilled standard hematologic criteria for IM. At the conclusion of this study, EBV antibody tests were performed on serum samples from 53 other sick patients of similar age whose blood smears lacked the minimal morphological criteria for IM. In only 4 cases (4/53, 7.5%) was there evidence for a recent or current primary EBV infection, thus validating the present morphological criteria as guidelines for determining which young children require EBV-specific serodiagnostic studies.

Infectious Mononucleosis (IM) is largely a disease of adolescents and young adults from more affluent population segments of economically advanced nations. The disease is practically unknown in most other parts of the world where, according to seroepidemiologic surveys, infections by the Epstein-Barr virus (EBV), the cause of IM, occur generally under the age of 3 yr.1 These observations have led to the suggestion that primary EBV infections in early childhood are usually asymptomatic. Recent prospective studies in Ghana and the United States have established that the great majority of primary EBV infections in children under the age of 2 yr remain indeed silent.2,3 Nevertheless, classical signs of IM among young children have been recorded occasionally in the literature.4–8 The present study was concerned with a search for cases of IM in young children under 4 yr of age.

A practical approach to the diagnosis of IM includes evaluation of both hematologic and heterophil antibody responses.9,11 When peripheral blood smears meet the minimal morphological criteria for IM and a rapid slide test for heterophil antibodies is positive following serum absorption with guinea pig kidney, the diagnosis of IM is established. The presence of such heterophil antibodies with specific absorption traits, accompanied by both a relative and absolute atypical lymphocytosis, is, with rare exceptions, limited to primary EBV infections, rendering EBV-specific serologic tests unnecessary. When the rapid slide agglutination test is negative but blood smears suggest IM, as observed in less than 5% of young adults with proven IM, EBV-specific serodiagnostic tests are indicated to establish the diagnosis.12–14 According to our previous experience with acutely ill adults, virus-specific serodiagnostic tests rarely show evidence of a current or recent primary EBV infection if the peripheral blood smears fail to reveal significant numbers of atypical lymphocytes. Thus, virus-specific serologic studies of heterophil-antibody-negative patients are initiated in the laboratory of one of us (C.A.H.) only if blood smears meet the minimal morphological criteria for IM.

We have now applied these diagnostic principles to studies in infants and young children in whom diagnostic problems occur because physicians lack famil-
iarity with IM in this age group. In general, these problems relate to atypical clinical presentations (respiratory or neurologic) and to the myriad of reactive lymphocytoses that accompany childhood exanthems and other viral infections. In addition, heterophil-antibody-negative cases are much more common in childhood, making virus diagnostic studies mandatory to confirm the diagnosis in many cases. Using morphological guidelines to determine when heterophil antibody and virus diagnostic studies are indicated, we have confirmed primary EBV infections accompanied by signs of IM in 32 children 10–48 mo of age. We report here data from these cases, including significant age-related differences between infants and toddlers aged 10–24 mo and young children aged 25–48 mo. As a corolla to this study, we attempted to confirm the validity of our selection process by searching for evidence of primary EBV infections in sera of children whose blood smears lacked minimal morphological criteria for IM.

MATERIALS AND METHODS

Routine Laboratory Tests

The presence of heterophil antibodies was determined in a 2-hr tube test with horse red cells following differential absorption of the serum with guinea pig kidney (GPK) and beef (BF) red blood cell suspensions. The data were interpreted as positive if the GPK-absorbed titer was greater than the simultaneously performed BF-absorbed titer. Heterophil antibodies were also determined in selected cases by the immune adherence hemagglutination assay (IAHA) with positive titers considered to be ≥1:40. Cold agglutinins (CAs) were measured at 4°C with 2% human group O red blood cells. Specificity of CAs was determined by parallel titrations against both adult and cord red blood cells. Cold agglutinins with maximal reactivity against cord cells were considered anti-"i," whereas those with preferential reactivity against adult cells, anti-"I." Serum bilirubin, alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT), and glutamic oxaloacetic transaminase (SGOT) levels were determined mainly by standard micromethods. Normal values for children less than the age of 5 yr are: bilirubin <1.0 mg/100 ml, AP <350 μU/ml, GGT <26 μU/ml, and SGOT <26 μU/ml.

Epstein-Barr Virus (EBV) Related Serology

Antibodies to viral capsid antigens (VCA) and the D (diffuse) and R (restricted) components of the EBV-induced early antigen (EA) complex were titrated by indirect immunofluorescence. Antibodies to the EBV-associated nuclear antigen (EBNA) were determined by anticomplement immunofluorescence using techniques that have been described previously. As reviewed earlier, serologic evidence of current or recent primary EBV infection in initial serum specimens was provided by one or more of the following criteria: presence of VCA-specific IgM antibodies, high titers of VCA-specific IgG antibodies (≥1:320), detection of anti-D (≥1:10), and absence of anti-EBNA (<1:2). The results were confirmed when subsequent serum samples showed a decline or disappearance of VCA-specific IgM antibodies and of anti-D and the emergence of anti-EBNA. A past EBV infection was identified by constant VCA-specific IgG and EBNA antibody titers in serum specimens obtained during the acute and convalescent phase. Most of these titers ranged between 1:10 and 1:160 and were associated with the absence of IgM antibodies and usually also of anti-D. Low titers of anti-R are occasionally observed late in the course of IM in teenagers and also among healthy blood donors who maintain constantly high levels of VCA-specific IgG. The absence of all EBV-related antibodies excludes past as well as recent EBV infections.

Antibodies to Cytomegalovirus (CMV)

These were determined by immunofluorescence using test kits obtained from Electro-Nucleonics Corp., Bethesda, Md. Positive results were recorded (≥1:16) when typical inclusion bodies were identified in CMV-infected cell slides. Positive results were recorded only after the presence of rheumatoid factors was excluded by appropriate tests at 25°C.

Case Selection

Between 1972 and 1980, heterophil antibody studies with horse red blood cells were performed at the request of physicians on serum samples from about 200 acutely ill children less than 5 yr of age. Accompanying blood smears were routinely evaluated for atypical lymphocytes by one of us (C.A.H.), and EBV-specific diagnostic studies were initially pursued only on the 34 cases that met minimal morphological criteria for IM; i.e., at least 50% mononuclear cells, ≥10 atypical lymphocytes/100 WBC, and significant lymphocytic heterogeneity.

RESULTS

The data will be discussed in terms of infants aged 10–24 mo (11 cases) and aged 26–48 mo (21 cases). Pertinent clinical, hematologic, biochemical, and serologic data from 8 of the 11 patients aged 10–24 mo with current or recent EBV infections are recorded in Table 1. Significant cervical lymphadenopathy was present in 10 of 11 children (90.9%), pharyngitis and fever were noted in at least 8 of the children, and respiratory symptoms were observed in 7. Six patients initially presented with respiratory illnesses, one with acute Bell’s palsy (II), and another with generalized petechiae (I). Leukemia was the admitting diagnosis for 4 patients. Liver function studies were abnormal in 3 of 5 children tested. IM-type heterophil antibodies were detected by the horse cell differential absorption test in 3/11 patients (27.3%), including one child whose heterophil antibodies were only detected in a follow-up serum sample at the very low titer of 1:28 after GPK absorption (VIII). The Monospot test (Ortho Diagnostics) was positive in 2 of the 3 heterophil-positive children. The immune adherence hemagglutination test (IAHA) for heterophil antibodies was performed on 7 of the 8 heterophil-negative cases and gave titers of ≥1:20 in all instances. Cold agglutinins of anti-"i" type were detected in 1 of 8 children tested.

EBV-specific serodiagnostic tests revealed current or recent primary EBV infections in all 11 patients. The primary EBV infections were documented by the
CHILDHOOD IM

Table 1. Clinical and Laboratory Data From 8 Infants and Toddlers With Infectious Mononucleosis (Aged 10-24 mo)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, Sex</th>
<th>Clinical Presentation</th>
<th>Days After Onset</th>
<th>Lymphocytes (Atypicals) per 100 WBCs</th>
<th>Heterophil*</th>
<th>Anti-VCA (IgM)</th>
<th>Anti-VCA (IgG)</th>
<th>Anti-EA</th>
<th>Anti-EBNA</th>
<th>Additional Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10, M</td>
<td>Initial respiratory; later ecchymoses, petechiae; lymphadenopathy (4 +)</td>
<td>2</td>
<td>80 (Many)</td>
<td>GP B</td>
<td>80 10 &lt;10 2</td>
<td>80 10 &lt;10 2</td>
<td>80 10 &lt;10 2</td>
<td>80 10 &lt;10 2</td>
<td>Platt count 15,000/cu</td>
</tr>
<tr>
<td>II</td>
<td>11, M</td>
<td>Initial respiratory; later Bell’s palsy; lymphadenopathy (4 +)</td>
<td>7</td>
<td>76 (30)</td>
<td></td>
<td>&lt;7 &lt;7 &lt;7 70</td>
<td>&lt;7 &lt;7 &lt;7 70</td>
<td>&lt;7 &lt;7 &lt;7 70</td>
<td>&lt;7 &lt;7 &lt;7 70</td>
<td>83 mlU/ml</td>
</tr>
<tr>
<td>III</td>
<td>17, M</td>
<td>Fever, tender abdomen; lymphadenopathy (4 +)</td>
<td>2</td>
<td>90 (70)</td>
<td></td>
<td>&lt;7 &lt;7 80 80</td>
<td>&lt;7 &lt;7 80 80</td>
<td>&lt;7 &lt;7 80 80</td>
<td>&lt;7 &lt;7 80 80</td>
<td>Initial Dx: leukemia</td>
</tr>
<tr>
<td>IV</td>
<td>18, M</td>
<td>Ear aches; not very sick</td>
<td>5</td>
<td>84 (&gt;50)</td>
<td></td>
<td>&lt;7 &lt;7 160 160</td>
<td>&lt;7 &lt;7 160 160</td>
<td>&lt;7 &lt;7 160 160</td>
<td>&lt;7 &lt;7 160 160</td>
<td>Amoxicillin rx.</td>
</tr>
<tr>
<td>V</td>
<td>18, M</td>
<td>Fever, tonsillitis</td>
<td>23</td>
<td>80 (&gt;60)</td>
<td></td>
<td>&lt;7 &lt;7 80 80</td>
<td>&lt;7 &lt;7 80 80</td>
<td>&lt;7 &lt;7 80 80</td>
<td>&lt;7 &lt;7 80 80</td>
<td>Amoxicillin rx.; many</td>
</tr>
<tr>
<td>VI</td>
<td>21, M</td>
<td>&quot;Croup;&quot; no lymphadenopathy</td>
<td>3</td>
<td>71 (&gt;50)</td>
<td></td>
<td>448 28 160 160</td>
<td>&lt;7 &lt;7 320 320</td>
<td>&lt;7 &lt;7 320 320</td>
<td>&lt;7 &lt;7 320 320</td>
<td>X-ray: interstitial pneumonia; SGOT 21; anti-I 1:448</td>
</tr>
<tr>
<td>VII</td>
<td>24, M</td>
<td>Fever; lymphadenopathy</td>
<td>2</td>
<td>72 (60)</td>
<td></td>
<td>&lt;7 &lt;7 160 160</td>
<td>&lt;7 &lt;7 160 160</td>
<td>&lt;7 &lt;7 160 160</td>
<td>&lt;7 &lt;7 160 160</td>
<td>SGOT 50; Pos FANA; CMV-igm 1:32-</td>
</tr>
<tr>
<td>VIII</td>
<td>24, F</td>
<td>Respiratory illness; lymphadenopathy</td>
<td>16</td>
<td>63 (17)</td>
<td></td>
<td>&lt;7 &lt;7 10 10</td>
<td>&lt;7 &lt;7 640 640</td>
<td>&lt;7 &lt;7 640 640</td>
<td>&lt;7 &lt;7 640 640</td>
<td>1:84; Anti-&quot;I&quot; CA's</td>
</tr>
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</table>

*pHeterophil studies with horse red blood cells; GP, guinea pig kidney-absorbed; Bf, beef cell-absorbed agglutinins. Antibody titers to Epstein-Barr viral capsid antigens (VCA), the "D" and "R" components of the early antigen (EA) complex and to EBV-associated nuclear antigen (EBNA). CA, cold agglutinins. Titers are expressed as reciprocals of serum dilutions.

The presence of IgG antibodies to VCA in association with the absence of antibodies to EBNA (<1:2). IgM-VCA antibodies were at significant levels (≥1:20) in 7 children (63.6%, current infections), at a doubtful level of 1:10 in 2 (18.2%), and found absent (<1:10) in patients II and VIII (recent infections). Peak anti-VCA-IgG levels were ≥1:320 in 5 patients (45.4%) and between 1:40 and 1:160 in the remaining 6. Anti-EA responses were encountered in 5 of the 11 patients, 3 times as anti-D (27.2%) and twice as anti-R (18.2%). Anti-EBNA responses were <1:2 in 9 children (81.8%), 1:2 in 1 case (9.1%), and inconclusive in the last patient due to the presence of antinuclear antibodies. In patients VII, a positive result was recorded in the CMV-IgM test and a dual CMV infection could not be excluded; however, cross-reactivity by sera from patients with primary EBV infections is a more likely explanation for the positive CMV-IgM reaction.

Data from the patients aged 26-48 mo with primary EBV infections revealed significant cervical lymphadenopathy in all 21 cases (100%) and many had generalized lymphadenopathy. Pharyngitis was present in 16 patients and absent or not noted in the other 5, while fever was noted in at least 13, usually of more than 5 day duration. At least 5 patients had prominent respiratory symptomatology, and in 2 patients prominent airway obstruction was noted due to cervical and tonsillar lymphoid hyperplasia. One of the latter patients responded to therapy with prednisone. In 1 case, inguinal lymphadenopathy was interpreted as an incarcerated hernia and an unnecessary exploratory procedure performed. In the postoperative period, generalized lymphadenopathy developed and blood smears then led to the correct diagnosis of heterophil-antibody-negative IM. All 21 patients had blood smears that easily met IM criteria and most had over 60% lymphocytes and ≥20 atypical forms per 100 WBCs. IM-specific heterophil antibodies were detected in the horse cell differential absorption tube test in 16 of 21 cases (76.2%). The IAHA test was performed on all 5 of the heterophil-negative samples and gave titers of 1:40 (barely positive) in 3 cases and <1:20 in the other 2. Cold agglutinins of anti-"I" type were detected in 3 of 17 patients tested. Either SGOT or GGT, or both, was mildly to moderately elevated (highest SGOT 236 mIU/ml) in 9 of the 11 children tested.

The presence of antibodies to VCA in association with the absence or low titers of antibodies to EBNA, as well as the detection of IgM antibodies to VCA at levels of 1:40 to 1:320, established the diagnosis of...
current primary EBV infections in all 21 patients. Levels of IgG antibodies to VCA at titers of ≥1:320 were noted in 16 children (76.2%) and at levels of 1:40 to 1:160 in the other 5. Anti-EA responses were present in 17 of the 21 cases (81%), which were directed against D in 11 instances (52.4%) and against R in 5 (23.8%) and initially against D (1:10), later against R (1:10) in 1 patient (4.8%). Anti-EBNA responses were initially absent (<1:2) in 16 patients (76.2%), present at serum dilution of 1:2 in 3 patients (14.3%), 1:5 in 1 (4.8%), and not measurable because of antinuclear antibodies in the last patient.

During the course of this study, blood smears from 2 other infants (aged 10 and 13 mo) also met minimal morphological criteria for IM, but their sera failed to reveal antibodies to either EBV- or CMV-related antigens. The cause of their IM-like illnesses remained undetermined. Three additional children aged 16–46 mo had clinical illnesses resembling IM and were heterophil-positive; however, their blood smears failed to meet minimal morphological criteria for IM. EBV-specific serodiagnostic tests revealed that 2 of these 3 children had a recent or current primary EBV infection, respectively, while the third patient had evidence of an old EBV infection. The last patient may have had a recent silent EBV infection that preceded his current illness (juvenile rheumatoid arthritis).

At the conclusion of our study, serum samples from 50 other infants less than 49 mo of age whose blood smears did not meet morphological criteria for IM were evaluated for EBV-specific antibodies. EBV-specific serodiagnostic tests revealed that 35 were still susceptible to EBV; that is, antibodies to VCA were not detected (<1:10). Thirteen had evidence for past EBV infections; i.e., IgG antibodies against VCA and EBNA at titers ranging from 1:5 to 1:160 and no (<1:10) anti-EA and IgM antibodies to VCA. The 2 remaining patients had evidence for a recent primary EBV infection based on the detection of high titers of only IgG antibodies to VCA and absence of antibodies to EBNA (<1:2). One of these patients, aged 13 mo, had primarily a respiratory illness with 2 separate bouts of bilateral pneumonia. She had a viral-type blood smear that did not fulfill criteria for IM, splenomegaly, elevated cold agglutinins, and responded poorly to several antibiotics. There was evidence for dual infection with EBV and CMV, in that CMV was isolated from the urine and high antibody titers (≥1:64) were noted in the CMV-specific macroglobulin (CMV-IgM) and complement fixation tests. The other patient, aged 24 mo, had necrotizing tonsillitis that failed to respond to several antibiotics. Although he had both an absolute and relative lymphocytosis, atypical lymphocytes were not identified in significant numbers, and the EBV-specific serodiagnostic studies were not initially pursued.

**DISCUSSION**

This study of IM-like illnesses in children aged 10–48 mo has shown that the established morphological hematologic criteria are quite useful, especially in heterophil-antibody-negative patients, for selection of those cases requiring EBV-specific serodiagnostic studies. Thus, of 34 acutely ill young children whose blood smears strongly suggested IM, primary EBV infections were confirmed in 32 of 34 cases: 28 as current and 4 as recent infections. Neither antibodies to EBV nor CMV were detected in the sera from the other 2 patients. For controls, EBV-specific serodiagnostic tests were performed on sera from 53 young children whose blood smears did not meet morphological criteria for IM. Of these, 35 had no antibodies to EBV, 14 gave evidence of past EBV infections, and in only 4 was there evidence of primary EBV infections: 1 current and 3 recent. Two of the last 4 patients were originally submitted for EBV studies because tests for heterophil antibodies were positive. Although the number of subjects is small, it appears that even in very young children a careful evaluation of blood smears allows one to determine with a relatively high degree of accuracy when a diagnosis of IM is likely and which heterophil-negative cases need EBV-specific serodiagnostic studies. A recent study of IM in college students has suggested that the initial evaluation of a peripheral blood smear is a more cost-effective method for approaching the diagnosis of IM than heterophil antibody reactions; e.g., Monospot tests.

In the present study most patients had clinical evidence compatible with IM; i.e., significant cervical lymphadenopathy and often tonsillar pharyngitis and respiratory symptoms. The laboratory data disclosed several important differences between infants under the age of 2 yr compared with older children and young adults with IM. These data are summarized in Table 2. Most noteworthy, heterophil antibody responses were encountered in only 27.3% (3/11) of infants less than 2 yr of age, in 76.2% (16/21) of children between the ages of 25 and 48 mo, and, as observed earlier, in over 96% of young adults with IM. Antibody responses to the EA complex were noted in only 5 (45.5%) of 11 infants, 2 were directed against R as commonly seen in silent infections of infants, but 3 were against D. In the older children, 17 (81%) of 21 responded with antibodies to EA, 5 to R, 12 to D, 1 of whom subsequently developed antibodies to R. In
adolescents and adults, the incidence of anti-EA responses is 80%-95%, which nearly always are directed against D.24 The present data thus confirm earlier reports that anti-R responses are more commonly encountered in infants and toddlers than in older children and young adults.15,24 It is not clear, however, when the switchover from anti-R to anti-D occurs, but it probably happens in a gradual manner between the ages of 18 and 48 mo. On occasion, young adults with IM also develop sole or late antibody responses to the R component but usually in association with protracted clinical manifestations or late recurrent symptoms of IM.25 In the latter setting, anti-R may be a reflection of an intermittent activation of the persistent EBV carrier state. The age-related differences in EBV-specific serologic responses have been attributed to a more limited spread of the virus and absence of significant lymphadenopathy in very young patients.2 Such an explanation does not hold, however, for the clinically overt EBV infections with striking lymphoproliferation and very prominent lymphadenopathy seen in the majority of our patients.

REFERENCES


<table>
<thead>
<tr>
<th>Age Group (No.)</th>
<th>Significant Lymphadenopathy</th>
<th>Sore Throat or Pharyngitis</th>
<th>Positive Heterophil Test*</th>
<th>Anti-'V' Cold Agglutinins</th>
<th>Abnormal Liver Function Studies</th>
<th>Anti-VCA (lgM) ≥ 1:10†</th>
<th>Anti-VCA (lgG) Peak ≥ 1:320</th>
<th>Anti-EA (D vs RI)†</th>
<th>Initial Anti-EBNA (&lt; 1:2)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–24 mo (11)</td>
<td>90.9%</td>
<td>72.8%</td>
<td>27.3%</td>
<td>12.5%</td>
<td>60.0%</td>
<td>63.6%</td>
<td>45.5%</td>
<td>45.5%</td>
<td>81.8%</td>
</tr>
<tr>
<td>26–48 mo (21)</td>
<td>100%</td>
<td>76.2%</td>
<td>76.2%</td>
<td>27.7%</td>
<td>81.8%</td>
<td>100%</td>
<td>76.2%</td>
<td>81.0%</td>
<td>76.2%</td>
</tr>
<tr>
<td>Young adults</td>
<td>80%–90%</td>
<td>90.0%</td>
<td>&gt;96.0%</td>
<td>35%–40%</td>
<td>&gt;96.0%</td>
<td>90%–100%</td>
<td>50%–60%</td>
<td>85.0%</td>
<td>80%–85%</td>
</tr>
<tr>
<td>15–25 yr (&lt;300)</td>
<td>(Mainly) D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Figures (%) represent maximum sensitivity for heterophil antibody detection using horse red blood cell suspensions.
†Antibody titers to Epstein-Barr viral capsid antigen (VCA), the "D" and "R" components of the early antigen (EA) complex, and to EBV-associated nuclear antigen (EBNA).
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