NOTES

Antibody Responses to Two Epstein-Barr Virus (EBV) Nuclear Antigens (EBNA-1 and EBNA-2) During EBV Primary Infection in Children Born to Mothers Infected with Human Immunodeficiency Virus

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A variety of antibody response patterns to the latent Epstein-Barr nuclear antigen (EBNA) family have been described in different groups of subjects infected with Epstein-Barr virus (EBV). The purpose of this study was to characterize the immune response to two EBNA proteins, EBNA-1 and EBNA-2, in a population of children who were born to mothers infected with HIV and who underwent EBV seroconversion. Serial serum specimens from 33 children (nine were infected with HIV, and 24 were not infected) were evaluated for the presence of antibodies to EBNA-1 and EBNA-2 by anticomplement immunofluorescence. All the EBNA serology profiles observed for children in our study who were not infected with HIV were consistent with those described for immunocompetent hosts with acute EBV infection, i.e., development of antibodies to EBNA-1, often preceded by the appearance of a humoral immune response to EBNA-2. In contrast, following EBV primary infection in HIV-infected children, antibodies to EBNA-2 arose after antibodies to EBNA-1 and tended to persist. Further studies are needed to investigate the role of EBNA-2 serology as a prognostic marker in HIV-infected children.

Serological testing is the method of choice for the diagnosis of primary infection with Epstein-Barr virus (EBV). Classically, antibodies to the viral capsid antigen and—in 80% of the cases—antibodies to the early antigen component of EBV appear first, followed by a delayed response to Epstein-Barr nuclear antigens (EBNA) [1]. Titers of antibody to viral capsid antigen and to EBNA should persist for many decades in immunocompetent individuals, and, in the context of reactivated EBV infection, the production of antibodies to early antigen is enhanced. However, in many immunosuppressed individuals, the antibody response to EBNA is markedly reduced or fails to appear altogether [1–3].

The EBNA family of EBV nuclear antigens consists of six known proteins, among which are EBNA-1 and EBNA-2. EBNA-1 is essential for maintenance of the EBV episome; EBNA-2 plays a central role in the immortalization process and was shown to transactivate another EBV gene, namely that encoding the latent membrane protein 1, which is also involved in immortalization of B cells [4]. The serological response to EBNA-1/EBNA-2 has been well characterized in EBV-seropositive individuals and in patients in the acute phase of infectious mononucleosis. The purpose of this study was to characterize the immune response to EBNA-1 and EBNA-2 in a population of children who were born to mothers infected with HIV and who underwent EBV seroconversion.

Materials and Methods

As part of another study, we documented EBV primary infection by means of serial serology testing in 37 of 65 children (18 were infected with HIV and 47 were not infected) born to HIV-infected mothers and observed prospectively since birth at the Centre Maternel et Infantile sur le SIDA of Sainte-Justine Hospital (Pedneault et al., unpublished data). From the time of EBV seroconversion, 33 of the 37 children who developed
Therefore, all the EBNA serology profiles observed in children are summarized in table 1 according to their HIV infection status. It is of interest that the profiles observed in HIV-infected children were quite distinct from those seen in the children who were not infected. Antibodies to EBNA-1 arose first and persisted thereafter, whereas antibodies to EBNA-2 appeared at a later time in four of the five children with HIV infection who had an interpretable EBNA serology and more than one EBNA-positive serum specimen. In three of these four subjects, antibodies to EBNA-1 were first detected within 6 months of EBV seroconversion, followed by the emergence of antibodies to EBNA-2 less than 3 months thereafter. On the basis of the availability of sera, we were able to document that antibodies to both EBNA-1 and EBNA-2 persisted in each of the three patients for at least another 10 months, 23 months, or 27 months. In the fourth case, antibodies to EBNA-1 were already present at the time when EBV seroconversion was documented. Antibodies to EBNA-2 arose 29 months later, and both sets of antibodies remained detectable for at least 29 months thereafter. Total EBNA and EBNA-1/EBNA-2 serologies were not interpretable for three HIV-infected subjects because of the presence of antinuclear antibodies.

In contrast, when more than one EBNA-positive serum specimen was available per subject, all children who were not infected with HIV either developed antibodies to EBNA-2 before building an immune response to EBNA-1 or mounted an antibody response strictly against EBNA-1. Most of the children who were not infected with HIV had an EBNA-1-positive and EBNA-2-negative serological profile sometime between 3 and 11 months after documentation of seroconversion (data not shown). The four children who were not infected with HIV and who had persistently positive EBNA-2 antibodies had ≤ 13 months of serological follow-up. EBNA serology was interpretable for all children in this population.

When the first five (i.e., expected or normal) profiles described in table 1 were pooled and compared with the sixth unexpected profile (EBNA-1 alone followed by EBNA-1 and EBNA-2) among the children who were infected with HIV vs. those who were not infected, the difference was found to be statistically significant ($P = .0006$).

### Discussion

The pattern of antibody responses to EBNA-1 and EBNA-2 during the course of acute EBV infection in immunocompetent subjects has previously been described. According to Henle et al., following EBV primary infection, antibodies to EBNA-2 appear first and should decline thereafter to lower but persistent levels or to nondetectable levels. Several weeks to months later, antibodies to EBNA-1 arise and should persist indefinitely [8].

On the other hand, Niederman and Miller described two main patterns of antibody responses to total EBNA and to EBNA-1 in uncomplicated infectious mononucleosis: the emergence of antibodies to EBNA could precede by several months or coincide with the appearance of antibodies to EBNA-1 [9]. Therefore, all the EBNA serology profiles observed in children

### Table 1. Profiles of antibody responses to two Epstein-Barr virus (EBV) nuclear antigens (EBNA-1 and EBNA-2) among 33 children who were EBV seroconverters.

<table>
<thead>
<tr>
<th>EBNA-1 and EBNA-2 serology profiles*</th>
<th>HIV-positive ($n = 9$)</th>
<th>HIV-negative ($n = 24$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBNA-2 only</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>EBNA-2 alone followed by EBNA-1 and EBNA-2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>EBNA-2 alone followed by EBNA-1 alone</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>EBNA-1 and EBNA-2 followed by EBNA-1 alone</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>EBNA-1 only</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>EBNA-1 alone followed by EBNA-1 and EBNA-2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Not interpretable†</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Only one EBNA-seropositive serum sample</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Only one not interpretable serum sample</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Unless stated otherwise, EBNA-1 and EBNA-2 profiles were established on at least two EBNA-positive serum specimens per subject.
† Because of the presence of antinuclear antibodies.

EBV primary infection (nine were infected with HIV, and 24 were not infected) had at least one serum specimen for which EBNA serology was positive or not interpretable because of the presence of antinuclear antibodies.

A total of 116 frozen serial serum samples from the 33 children who underwent EBV seroconversion were collected at birth and, whenever possible, every 3 months thereafter and were evaluated. Serum specimens were kept frozen at −70°C until testing. Anticomplement immunofluorescence, as described by Reedman and Klein [5], was used to determine titers of antibody to EBNA on Raji cells fixed in acetone/methanol (1/1). Titers of antibody to EBNA-1 and EBNA-2 were determined by anticomplement immunofluorescence with use of L cells transfected with EBNA-1 (kindly donated by Dr. George Miller, Yale University, New Haven, CT [6]) and BJAB cells transfected with EBNA-2 (kindly donated by Dr. Clare Sample, St. Jude Children’s Research Hospital, Memphis, TN [7]) when total EBNA serology was positive or not interpretable.

A $\chi^2$ test with Yates’ correction was performed to determine whether observed differences in the EBNA-1 and EBNA-2 serology profiles between the children infected with HIV and the children who were not infected with HIV were statistically significant.
in our study who were not infected with HIV are consistent with those described above for acute EBV infection in the immunocompetent host.

However, the kinetics of antibody responses to EBNA-1 followed by EBNA-2 in a HIV-infected population of children, as shown in this study, had not been previously described. Data from several investigators suggest that antibody responses to the EBNAs can vary in the context of immunosuppression. Production of antibodies to EBNA is often reduced, if not abolished, in transplant recipients, and most of the studies on the production of these antibodies have been done on this group of patients [1–3]. Similarly, Miller et al. found that antibodies to EBNA-1 were not detected in 12 (80%) of the 15 serum specimens collected from children with AIDS and tested in their study [10].

On the other hand, Seigneurin et al. showed a 68% (70/103) EBNA-2-seropositivity rate among HIV-infected individuals, as compared with only 8% (10/125) in a control population [11]. Furthermore, and in accordance with our own results, the same investigators demonstrated seroconversion to EBNA-2 in one renal transplant adult recipient and in one HIV-infected adult after seropositivity to EBNA and EBNA-1 was documented [11]. Finally, Henle et al. also reported ratios of EBNA-1/EBNA-2 antibody titers well below 1.0 in patients with AIDS [8].

In summary, following EBV primary infection, children infected with HIV seem to develop an immune response to EBNA-1 and EBNA-2 that differs from that observed in those who are not infected with HIV. In HIV-infected children, antibodies to EBNA-2 arise after antibodies to EBNA-1 and seem to persist. It is conceivable that because of their immunosuppressed state, the HIV-infected infants may have persistent circulating B cells expressing EBNA-2 that results in production of antibodies to this antigen. Since EBNA-2 is essential for the immortalization process induced by EBV, further studies are needed to investigate whether the emergence or persistence of antibodies to EBNA-2 represents a useful serological marker for HIV-infected children presenting with lymphocytic interstitial pneumonitis or whether the emergence or persistence of these antibodies can be used to identify individuals at greater risk of EBV-associated lymphoproliferative diseases.

Acknowledgments

The authors thank Marc Dumont for statistical analysis of the data and Mary Cassidy for secretarial assistance.

References