Antibodies to Epstein-Barr Virus in Patients with Cryptococcosis

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Antibody levels to the Epstein-Barr virus, the etiological agent for heterophile-positive infectious mononucleosis, have been demonstrated in high titer in a number of lymphomas as well as infectious mononucleosis. Recent reports have suggested that the elevated antibody levels to Epstein-Barr virus may be the nonspecific result of disordered cell-mediated immunity. This study of patients with cryptococcosis was therefore undertaken to examine another disorder of known etiology associated with a defect in cell-mediated immunity. In this study we found that antibody levels in cryptococcosis patients, including a group specifically demonstrated to be anergic to a series of skin test antigens, were no different than those in matched normal controls. At the present time, therefore, it is unlikely that elevated antibody levels can be explained solely on the basis of depressed cellular immunity.

Following its description in long-term cell cultures derived from the lymph node of a patient with Burkitt's lymphoma (4) the Epstein-Barr virus (EBV) has been considered as the possible causative agent or at least a cofactor in the etiology of Burkitt's tumor (6). The serological studies associating high EBV antibody titers with lymphoproliferative diseases such as Burkitt's lymphoma (7, 18), chronic lymphocytic leukemia (17), Hodgkin's disease (12, 14, 15), and other lymphomas (13) have not clarified the role of this virus in these tumors, and the etiological relationship between EBV and human cancer has been questioned because of the reports of elevated antibody titers in nonmalignant diseases of disordered cell-mediated immunity (10, 24). The one report of elevated antibody titers to EBV in patients with a disease of nonviral etiology, lepromatous leprosy (23), could not be confirmed in our own small series (P. H. Levine, A. I. Sutnick, and J. I. Reisher, unpublished data). Therefore, we attempted to look at cryptococcosis, another disease where disseminated infection has been thought to be related to a defect in host cellular immunity (3), and where underlying diseases predisposing to this infection (20) have been associated with elevated EBV titers (10, 12-15, 17, 24).

MATERIALS AND METHODS
Serum samples were retrieved from 63 patients with disseminated cryptococcosis who were referred to the National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health, Bethesda, Md. All patients selected had been evaluated for the presence of underlying disease, particularly lymphoproliferative disorders, sarcoidosis, and conditions requiring sustained treatment with corticosteroids, and no contributing factors could be detected. Cryptococcosis skin tests (1) were performed on 48 of the 63 patients during or after antifungal therapy. A battery of other skin test antigens (candida, trichophyton, mumps, and tuberculin) was used to be certain the patients were not anergic. Anticytotoxiccal antibodies were also measured as previously described (2). Sera from 63 normal individuals matched for age and sex were provided by the Virus Cancer Program. In addition, 21 sera were selected from patients with familial Mediterranean fever who had been on the wards of the NIAID at the time of the disseminated cryptococcosis patients. Sera from the three groups of patients were coded and titered for antibody to the EBV-associated viral capsid antigen (6) using a single batch of antigen prepared from the P3HR-1 cell line. Antibody to the EBV-associated early antigen (8) was determined using methods previously described. All sera were read on the same day that the titrations were performed. Previously standardized test sera were coded and also tested blindly in each day's run as an additional quality control.

RESULTS
Analysis of the antibody titers against the EBV-associated viral capsid antigen and early antigen revealed no differences between the sera of the cryptococcosis patients and either of the two control groups (Table 1). There was no
apparent relationship between the EBV titers, skin tests, or anticytotoxic antibody tests (Table 2). Twelve of the 48 patients with disseminated cryptococcosis who were tested with cryptococcal did not demonstrate skin reactivity, and five of these 12 patients were also unreactive when skin tested with candida, mumps, trichophyton, or intermediate-strength tuberculin. The geometric mean titer of the EBV-associated antigens in the five anergic patients or the seven patients with selective unreactivity against cryptococcal skin test antigen was no different than in the 36 patients with positive cryptococcal skin tests. An equivalent proportion of patients in the reactive and anergic group had elevated antibody levels which did not exceed the percentage of normal controls with elevated antibody titers. Two patients with disseminated cryptococcosis died of their disease while under observation. One of the patients had an elevated antibody titer to the EBV capsid antigen, but titers to the early antigen were normal in both patients.

**DISCUSSION**

Although prospective studies (5, 21) have clearly shown that EBV causes infectious mononucleosis, EBV titers have been reported to be elevated in a number of other diseases (6, 7, 10, 12-15, 17-19, 23, 24) for unexplained reasons. The detection of significantly increased titers in a group of patients with disease known to be caused by agents other than EBV could be interpreted as demonstrating the nonspecificity of the elevated EBV titers. A preliminary report by Papageorgiou and his colleagues (22), subsequently confirmed on a larger number of pa-

<table>
<thead>
<tr>
<th>Study</th>
<th>No.</th>
<th>Mean age</th>
<th>VCA*</th>
<th>EA*</th>
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<tr>
<td></td>
<td>Total</td>
<td>Males</td>
<td>Females</td>
<td>Total</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>63</td>
<td>44</td>
<td>19</td>
<td>50.3</td>
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<tr>
<td>Familial Mediterranean fever</td>
<td>20</td>
<td>17</td>
<td>3</td>
<td>39.6</td>
</tr>
<tr>
<td>Matched normal controls</td>
<td>63</td>
<td>44</td>
<td>19</td>
<td>48.7</td>
</tr>
</tbody>
</table>

*This table shows the similarity of EBV antibody patterns in the patients with disseminated cryptococcosis and controls. The patients did not show significantly higher geometric mean titers to the EBV-associated antibodies, and the percentage of patients with high antibody titers was also comparable to that of the normal controls.

* VCA, Viral capsid antibody.
* EA, Antibody to the early antigen.
* All titers are reciprocals of the numbers.
* GMT, Geometric mean titer.

<table>
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<tr>
<th>Titer</th>
<th>Skin test results (48 patients)</th>
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<tbody>
<tr>
<td></td>
<td>Crypto-</td>
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<tr>
<td></td>
<td>cocin</td>
</tr>
<tr>
<td>VCA</td>
<td>≤320*</td>
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<tr>
<td></td>
<td>≥640*</td>
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<tr>
<td>EA</td>
<td>≤10</td>
</tr>
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<td></td>
<td>≥20</td>
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* Anergy as a nonspecific cause of elevated EBV titers is unlikely, as indicated by the predominance of low viral capsid antibody (VCA) and antibody to the early antigen (EA) in the patients with negative skin tests.
* CTMP, Candida, trichophyton, mumps, and purified protein derivative.
* All antibody titers are reported as reciprocals.

patients by the same group (23), indicated that 22% of patients with lepromatous leprosy and impaired cellular immunity had elevated EBV titers, which suggested to these authors that disordered cell-mediated immunity per se might be a significant factor in determining the height of the individual's EBV titer. This concept led us to examine another disease of known etiology where the dissemination of an organism normally well controlled by most individuals could be interpreted as a presumptive indication of disordered cell-mediated immunity.

Our study, which did not reveal any difference between EBV titers in patients with cryptococcosis and matched controls, indicates that
depression of cellular immunity by itself is not the usual cause for elevated EBV titers. A comparison of EBV titers and studies of cell-mediated immunity in Hodgkin's disease (9, 19) has shown no correlation between the general immune response and EBV titers, and the studies by Papageorgiou and his colleagues have not been confirmed by others. Furthermore, there is evidence that age affects the level of EBV antibody (11, 16), and it is not certain if the high-titered lepromatous leprosy patients in Papageorgiou's study (23) were the same age as the controls. At the present time, therefore, there has been no conclusive link between EBV and any disease of known etiology, other than infectious mononucleosis.

In evaluating the cause of an elevated EBV titer, several possibilities must be considered. As in infectious mononucleosis, a regular elevation of EBV titers may be indicative of a viral etiology, and EBV remains as a strong candidate in the search for a cause of certain human tumors, particularly lymphomas and nasopharyngeal cancer. A second possibility, one which explains the elevation of EBV antibodies in certain normal individuals, is that the EBV antibody level is controlled by genetically related factors which correlate with susceptibility to cancer (16). A third possibility, the observation that elevated titers are occasionally seen in individuals with depressed cell-mediated immunity, has not yet been answered conclusively, but, as a general explanation for elevated EBV titers, it is an unlikely one in view of the number of individuals with depressed cellular immunity and normal EBV titers.

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LITERATURE CITED