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Clinical, virologic, and serologic evidence of Epstein-Barr virus infection in association with childhood pneumonia

To explore the association of Epstein-Barr virus infection with childhood pneumonia we studied two patients whose mononucleosis-like illnesses were accompanied by pneumonia. Both had virologic and serologic evidence of current or recent EBV infection. We then analyzed the sera of 71 children (age range, 14 months to 9 years) with pulmonary infiltrates for the presence of four classes of antibody to EBV. Antibody responses consistent with current or recent EBV infection were found in 15. Two children had IgM antibodies to the EBV viral antigen at titers ≥ 1:160, indicating current infection, and all 15 patients had antibody to components of the early antigen complex, suggesting recent infection. A fourfold rise or drop in one or more EBV-specific antibody classes was noted in eight patients within 30 days following onset of clinical illness. Few patients had clinical features suggesting infectious mononucleosis. Eight of the 15 with serologic evidence of current or recent EBV infection also had clinical or serologic evidence of infection with another pathogen—bacterial, viral, or mycoplasmal. Thus, in childhood pneumonia, EBV may be a primary, co-primary, or secondary pathogen; it may be reactivated in the course of infection with another agent, or possibly, by suppressing immune function, it may precipitate infection with some other organism.

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In the United States, at least 40% of children become infected with Epstein-Barr virus before adolescence. In most cases the infections are subclinical or so mild as to go unrecognized. Occasionally the infection may present as classical infectious mononucleosis, or it may take the form of a neurologic or hematologic disorder. In isolated instances, some infants with serologic evidence of current EBV infection have respiratory illnesses such as bronchopneumonia, but whether EBV is the etiologic agent in such cases is unclear. When the primary infection is accompanied by such an unusual illness, the clinician rarely considers the possibility that EBV may be playing a role. To implicate EBV infection in such children, especially since heterophile antibodies are usually absent, it is necessary to measure antibodies to a variety of EBV-specific antigens. The appearance and disappearance of certain of these antibodies in characteristic time sequence provide evidence for current or recent infection with this agent. Isolation of the virus from the blood and oropharyngeal secretions provides further proof of the presence of infection.
We briefly report here two patients with infectious mononucleosis-like illnesses accompanied by pneumonia who had virologic and serologic evidence of current or recent EBV infection. They served as a stimulus to study systematically the possible association of EBV infection with episodes of childhood pneumonia. Therefore, we analyzed the virus-specific serologic responses of 71 children with radiographic evidence of parenchymal lung infection. Antibody patterns consistent with current or recent EBV infection were found in 15 children.

CASE REPORTS

Patient A, a 9-month-old black boy, presented with fever, irritability, and cough. A chest radiograph revealed right upper and left lower lobe infiltrates. On admission to hospital the temperature was 39.4°C, the patient was tachypneic, and posterior cervical adenopathy was noted. The white blood cell count was only slightly elevated, and there was a marked mononuclear cell predominance with a few atypical lymphocytes. Results of the Monotest (Wampole Laboratories) were negative. A subsequent complete blood count showed leukocytosis with “many” atypical lymphocytes. The right upper lobe lesion cleared within three days, but the left lower lobe infiltrate persisted and shifted location. Fever subsided, and the patient was discharged on the fifth hospital day. EB virus was recovered from peripheral blood on the thirteenth day and from saliva on the fourth, thirteenth, and fifty-ninth days following onset.

Patient B, a 17-year-old white girl, developed nausea, dizziness, headache, puffy eyes, sore throat, cervical adenopathy, and conjunctivitis three weeks prior to admission. One week later a heterophile antibody test was positive and the patient began complaining of difficulty breathing and dysphagia. These symptoms persisted and chest radiographs taken two days before hospitalization revealed diffuse, patchy pneumonia. The patient did not improve on a regimen of oral dicloxacillin and was admitted to hospital dehydrated, coughing, and in mild respiratory distress. Positive findings included a temperature of 38.8°C, bilateral otitis media, injected tonsils with exudate, coarse rhonchi and signs of consolidation over both lower lung fields, and a slightly enlarged liver. The white blood cell count was 13,800 with a left shift. Blood gas values revealed arterial oxygen desaturation which improved on 40% O2 by mask. Chest radiographs showed multiple infiltrates (Fig. 1). The Monotest was positive and the ox hemolysin titer was 1:2,800. The hospital course was characterized by five days of spiking fevers, chest radiographs showing further consolidation in the lower lung fields, and severe lower airway obstruction with a moderate restrictive defect. Cultures of blood, urine, sputum, and throat were negative for bacterial pathogens, including Mycobacterium tuberculosis. The titer of cold agglutinins was 1:64 and Mycoplasma pneumoniae complement fixation titers rose from 1:8 to 1:128. The antibiotic regimen...
Table I. The serologic survey: Clinical groups to which the 71 patients with pneumonia were assigned

| Group*         | No. patients | Laboratory criteria                                                                 |
|---------------|--------------|-------------------------------------------------------------------------------------|
| I. Bacterial pneumonia | 5            | Positive blood culture and/or lung aspirate for *S. pneumoniae*, CRP positive at 1:50; † leukocyte count \( \geq 18,000/\text{mm}^3 \); ESR \( \geq 30 \text{ mm/hr} \) |
| II. Possible bacterial pneumonia | 7            | Same as Group I, except for negative cultures                                          |
| III. Viral or *M. pneumoniae* pneumonia | 14           | Fourfold or greater rise in CF antibodies to a viral respiratory pathogen or *M. pneumoniae* (influenza, 3; parainfluenza, 5; *M. pneumoniae*, 4; adenovirus, 2; RSV, 1)†‡ |
| IV. Etiology unknown     | 45           |                                                                                      |

The total number of patients is 71.

*For further explanation of the criteria used in placing the patients in these groups, see reference 7.
†Hyland CR-test.
‡Complement fixation tests were performed by the Virus Laboratory Division of the Connecticut State Department of Health.

The serologic survey. Between July 1, 1975, and May 1, 1976, 156 children (mean age, 40 months; range, 1 month to 16 years) with radiographic evidence of pneumonia were seen in the emergency room of the Yale-New Haven Hospital. They were originally studied prospectively to determine the value of C-reactive protein in differentiating bacterial from viral pneumonia. On the basis of clinical and laboratory evaluation, the patients were assigned at that time to one of four clinical categories (Table I). For the present investigation, serum was available from 71 members of the original group (mean age, 46 months; range, 14 months to 9 years). Paired sera were available from 58 patients and single sera from 13 (acute, 5; convalescent, 8). Convalescent sera were collected 10 to 30 days after the acute specimen.

Serologic tests. All acute and convalescent sera were screened at a dilution of 1:10 for the presence of IgG antibodies to EB viral capsid antigen. Serial twofold dilutions of those containing antibodies to EBV-VCA were then prepared to measure the precise titer of IgG and IgM antibodies to VCA by indirect immunofluorescence. Sera were not absorbed with IgG-coated latex particles. All positive sera were also tested for antibodies to EBV nuclear antigen by the method of anticomplement indirect immunofluorescence and for antibodies to both the diffuse and restricted components of the EBV early antigen complex. To distinguish between the two EA patterns of intracellular fluorescence, pairs of slides bearing antigen smears were fixed in acetone and methanol. Most or all of the restricted component disappears following methanol fixation.

The paired sera were also examined by CF tests for evidence of recent or current infection with other respiratory virus pathogens, including adenoviruses, respiratory syncytial virus, influenza A and B, the parainfluenza viruses, and *M. pneumoniae*. These tests were performed by the Laboratory Division of the Connecticut State Department of Health.

Virus isolation. To test for EB virus in Patients A and B, sputum and throat swab material were mixed with RPMI 1640 medium supplemented with 5% fetal calf serum and antibiotics. After freeze-thawing and filtering the samples, they were mixed with Ficoll-Hypaque separated human umbilical cord lymphocytes in a standard microtitre plate transformation assay. Peripheral blood samples were examined for the presence of leukocyte-associated virus in similar fashion. All transformation events were scored at the end of six weeks. Specimens of oropharyngeal materials were also tested for the presence of respiratory viruses by inoculation of tissue cultures (Rhesus monkey kidney, human placental fibroblasts, Hep 2, Vero) using standard techniques. The cultures were examined every few days for cytopathic effect and were tested by hemadsorption at seven and nine days. Our culture techniques were inadequate for isolation of coronaviruses, *Legionella*, and *M. pneumoniae*.

RESULTS

Primary EBV infection and pneumonia in two patients studied virologically and serologically. EB virus was isolated from both children with mononucleosis-like illnesses...
EBV antibody patterns in children with pneumonia: Serologic study. The time relationships of the appearance, rise and decline of the various antibodies in primary EBV infection are shown in Fig. 2. Of the 71 patients whose sera were examined for antibodies to EBV, 28 (40%) had IgG antibodies to EBV-VCA, reflecting either current or previous infection. Among this group of 28 seropositive individuals, evidence of current or recent infection was present in 15 (Table II). Two had high levels of IgM antibodies to VCA, indicating acute infection with EBV at the time of their clinical presentation. Both of the IgM antibody-positive individuals were in the group with pneumonia of unknown etiology. Two patients (Nos. 4 and 5) had VCA-IgM titers of 1:5. Since these low levels of IgM may reflect residual antibody following acute infection or the confounding influence of rheumatoid factor, these patients are placed in the “recent infection, probable” group.

All 15 patients with suggestive evidence of current or recent infection had antibodies to the EBV-induced early antigens. In most instances these “early” antibodies were directed exclusively or primarily against the restricted component of the EA complex, a finding which agrees with previous studies of primary infection in children. Ten of the 15 already had antibodies to EBNA, indicating that they were probably first seen with pneumonia at least six to eight weeks following the onset of their EBV infection, and perhaps even later. With only one exception, no serum containing anti-EBNA antibody contained IgM antibodies to EBV. Of the 15 patients with current or recent infection, paired sera were available from 13; in eight, a fourfold rise and/or fall in one or more antibody classes were demonstrated.

Review of the hospital records of the 15 patients with serologic evidence of EBV infection did not reveal any unusual or unexpected clinical or laboratory features. All the children were febrile and all but one had elevated white blood cell counts. Six patients had atypical lymphocytes seen on blood smear, but they were very few in number. Only one patient had clinically significant cervical lymphadenitis; one other had exudative tonsillitis. There was radiographic evidence of hepatic enlargement in five patients; one of these also had marked splenomegaly. There was nothing specific about the pulmonary infiltrates, and there was no predilection for any lobe or for either lung. Hilar lymphadenopathy was not observed. In one child, as in Patient A, the infiltrates shifted location and involved successively the perihilar regions of the right upper, the right lower, and the left lower lobes.

DISCUSSION

The results of this study provide evidence for an apparent association between some episodes of childhood
Table II. The serologic survey: Antibody responses in 15 pneumonia patients with evidence of current or recent EBV infection

| Antibody titer to EBV* | VCA-IgG | VCA-IgM | Acetone fixation | Methanol fixation | Distribution of EA in cells | EBNA |
|------------------------|---------|---------|------------------|------------------|-----------------------------|------|
| Current EBV infection† |         |         |                  |                  |                             |      |
| Patient                | Age     | Classification of pneumonia | Titer | Titer |  |  |  |  |  |
| S1                    | 17 yr   | Mycoplasma vs. primary EBV | 320   | 160   | <5 | <5 | — | <5 |
| S2                    |         |                     | 320   | 160   | <5 | <5 | — | — |
| S1                    | 29 mo   | Etiology unknown | 80    | 160   | 10 | <5 | R  | <5 |
| S2                    |         |                     | 80    | 10    | 20 | <5 | R  | <5 |
| S1                    | 14 mo   | Etiology unknown | 640   | 320   | 20 | 5  | R(D)| <5 |
| S2                    |         |                     | 320   | <10   | 80 | 5  | R(D)| <5 |
| Recent EBV infection, probable‡ | | | | | | | | |
| Patient                | Age     | Etiology unknown | Titer | Titer |  |  |  |  |  |
| S1                    | 9 mo    |                     | 20    | <5    | 10 | 10 | D  | <5 |
| S2                    |         |                     | 40    | <5    | 20 | 20 | D  | <5 |
| S3                    |         |                     | 40    | <5    | 10 | 10 | D  | <5 |
| S2                    |         |                     | 160   | <5    | 5  | <5 | R  | <5 |
| S2                    |         |                     | 640   | <5    | 40 | 20 | D  | 10 |
| S2                    |         |                     | 160   | 5     | 40 | 5  | R(D)| <5 |
| S2                    |         |                     | 20    | <5    | 40 | 5  | R  | 5  |
| S2                    |         |                     | 80    | <5    | 40 | <5 | R  | 5  |
| Recent EBV infection, possible§ | | | | | | | | |
| Patient                | Age     | Etiology unknown | Titer | Titer |  |  |  |  |  |
| S1                    | 9 yr    | Mycoplasma          | 220   | <5    | 5  | <5 | R  | 5  |
| S2                    |         |                     | 20    | <5    | 20 | <5 | R  | 10 |
| S2                    |         |                     | 80    | <5    | 5  | <5 | R  | 10 |
| S2                    |         |                     | 320   | <5    | 10 | 5  | R(D)| 20 |
| S2                    |         |                     | 320   | <5    | 40 | 5  | R(D)| 20 |
| S1                    | 14 mo   | Etiology unknown | 20    | <5    | 20 | 10 | D  | 40 |
| S2                    |         |                     | 20    | <5    | 40 | 10 | R(D)| 40 |
| S1                    | 5-½ yr  | Etiology unknown | 160   | <5    | 20 | <5 | R  | 40 |
| S2                    |         |                     | 320   | <5    | 10 | <5 | R  | 40 |
| S2                    |         |                     | 160   | <5    | 40 | <5 | R  | 40 |

*All antibody titers are expressed as the reciprocal of the highest serum dilution which produced a positive reaction.
†Recent infection: High titer of IgM antibody to VCA, with or without antibody to EA; absence of antibody to EBNA.
‡Recent infection, probable: Low or absent IgM antibody to VCA; IgG antibody to VCA and EA present; antibody to EBNA absent, present in low titer, or appearing in the course of infection.
§Recent infection, possible: No IgM antibody to VCA; presence of antibody to EA, EBNA, and VCA.
|R| = Restricted, D = Diffuse.

When there is at least a fourfold difference in titer between acetone and methanol fixed smears, the principal response is considered to be against the restricted component, but the presence of some diffuse reacting antibody cannot be excluded. If only a twofold difference in titer between acetone and methanol fixed smears is observed, the principal response is considered to be directed against the diffuse component of the EA complex.

pneumonia and current or recent EBV infection. This association has been observed previously in a few babies with bronchopneumonia who had IgM antibodies to EBV, and in the occasional instance of classical IM complicated by pneumonitis. Recently, a renal allograft recipient whose fever and pneumonia were found to be associated with a primary EBV infection was described. In the present group of subjects, the association of EBV with the pneumonic process was suspected clinically only in the two patients whose histories are provided. In the others, the illnesses were milder; based on the results of routine laboratory tests, bacterial, mycoplasmal, or well-recognized viral pathogens were believed to be the etiologic agents.
In the series of 71 patients with pneumonia whose sera were tested, criteria for current infection were met by the two individuals who had high levels of IgM antibody to EBV-VCA. Since these antibodies are transient, peaking about three to four weeks after the acute infection and falling to undetectable levels after eight weeks, it can be safely assumed that the pneumonia episodes in these children were temporally coincident with a primary EBV infection.

Fifteen of the 71 patients had antibody to components of the early antigen complex; ten of the 15 also had antibody to EBNA. Since these two classes of antibody generally appear in the convalescent phase of the infection, the data indicate that when these patients presented with pneumonia, they may have been recovering from recent EB virus infections. This finding is similar to observations made in a group of EBV-infected children who presented with minor complaints compatible with an infectious process. In that study most of the patients were experiencing recent rather than current infection with EBV at the time of clinical presentation. As in all clinical classifications of patients based on serologic data alone, the criteria for placement in one group versus another are somewhat artificial. For example, in the present study, evidence for recent infection is least firm for Patients 10 through 15. The only acute phase antibodies found in their sera were those directed against EA, and such antibodies are sometimes found in disease states apparently unrelated to EBV or in healthy donors.

A surprising number in the present series of patients with pneumonia and serologic evidence of current or recent EBV infection also had antibody to some other known respiratory pathogen (Table II). The significance of such dual infections is not clear; perhaps EBV may occasionally act as a co-pathogen. Alternatively, EBV, by inducing a state of temporary immunosuppression, by precipitating agranulocytosis, or by activating lymphoid cells containing other latent agents, may actually predispose a patient to another infection. We have recently studied two children who presented compelling virologic and serologic evidence for dual infection with EBV and an adenovirus (unpublished data). In addition, a patient has recently been described who developed severe \textit{Mycoplasma pneumoniae} infection as a complication of the immunosuppression induced by infectious mononucleosis: Patient B appears to have responded in similar fashion. Finally, infection with another respiratory pathogen may reactivate latent EBV and induce a transient serologic response to the early antigens, as may have occurred in some of our patients with EBV infection of possible recent onset. Antibody responses to the early antigens induced in other human herpes-virus systems, such as cytomegalovirus or varicella-zoster, occur not only in patients with primary infection, but also in those with recurrent infection. It seems likely that the situation may be similar with EBV.

Although no radiographic findings specifically characterize the pneumonia of infectious mononucleosis, previous reports emphasize hilar lymphadenopathy, strand-like densities in the parenchyma and, rarely, nodular densities and pleural effusion. Roentgenographic findings indistinguishable from those of primary atypical pneumonia, including shifting infiltrates, have also been described. None of the patients whose sera were examined had hilar lymph node enlargement, and the lobar or interstitial infiltrates which were seen did not suggest an unusual etiology. Therefore, based on both the radiographic and serologic data, it is entirely possible that in many of our patients pneumonia was caused by a classical respiratory pathogen. In these instances, EBV may have set the stage for the definitive infection, may have been reactivated, or a nonspecific EBV antibody response may have occurred.

Since we were limited by small volumes of sera, we were not able to screen samples for the presence of heterophile antibody. However, a number of other investigators have consistently shown that heterophile responses are unusual in most primary EBV infections in childhood, except when the infection presents in the form of infectious mononucleosis.

This study re-emphasizes the difficulty in accurately determining the cause of most episodes of pneumonia. In order to ascribe to EB virus an etiologic role in some of the pneumonias of childhood, extensive, prospective epidemiologic and virologic studies are required. Evidence of immunosuppression in such patients would be of great interest, since it would help to explain some of the secondary infections which seem to occur. To further clarify this point, EBV should be included among the pathogens that are sought in bronchial washings and pulmonary tissue from patients with pneumonia. It would be particularly relevant to study immunosuppressed individuals with pneumonia of unknown etiology.

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