Molecular Evidence of Ocular Epstein-Barr Virus Infection

Ocular manifestations have been attributed to the Epstein-Barr virus (EBV), largely on the basis of seroepidemiologic data. Two patients who developed conjunctival disease as the presenting feature of EBV infection are reported, each confirmed by in situ hybridization of EBV genome in affected tissue biopsy specimens. Recognition of EBV-induced ocular disease as an initial presentation of clinical EBV infection is important to the practitioner because of the ubiquitous nature of this herpesvirus.

Epstein-Barr virus (EBV) is a herpesvirus that infects >90% of the population [1]. Initial exposure to EBV during infancy or early childhood typically produces subclinical infection, but when the exposure is delayed to adolescence, it often manifests as infectious mononucleosis. Acute mononucleosis syndrome represents a self-limited lymphoproliferation of infected B cells and the expansion of virus-specific T cells that are activated to control the infection. Typical symptoms include fever, pharyngitis, lymphadenopathy, and splenomegaly. Periorbital edema has been reported in up to one-third of cases [2, 3]. After primary exposure, the virus persists lifelong in a latent, nonproductive state. Nucleic acid hybridization studies have implicated the virus in a variety of human cancers, to include posttransplantation polyclonal B cell proliferative disorders [4]. Previous reports have ascribed numerous ocular manifestations to EBV infection, including ocular granulomatous syndrome, conjunctivitis, dry eye, keratitis, uveitis, choroiditis, retinitis, papillitis, and ophthalmoplegia [5]. These associations have been made largely on the basis of acute seroconversion to EBV or concurrent symptoms of infectious mononucleosis, but have lacked definitive molecular evidence of virus etiology. Here we present 2 cases of ocular disease that result directly from latent EBV infection, as confirmed by in situ nucleic acid hybridization.

Patient A was a previously healthy 5-year-old boy who presented to an ophthalmologist with a 2-week history of mild right upper eyelid ptosis and conjunctival mass. The ocular process was painless, and there was no itching, discharge, or visual disturbance. The parents denied that the child had any recent illness or febrile episode. Ocular examination revealed right eyelid ptosis without fullness or erythema. There was a diffusely infiltrating, nontender conjunctival lesion of the superior fornix and bulbar conjunctiva (figure 1A). The lesion had a “salmon patch” appearance with multiple lymphoid follicles on the superior surface, consistent with the appearance of a possible lymphoid malignancy [6]. The remainder of the examination findings were within normal limits, except for an enlarged right preauricular and several small palpable submandibular lymph nodes.

The patient was referred to a pediatric oncologist to exclude a lymphoid malignancy. Evaluation included a complete blood count, blood chemistry, serologic studies, CSF analysis, CT examination of the chest, abdomen, and pelvis, MR scan of the brain, and a bone scan. The only abnormal findings were atypical lymphocytes on the blood smear, elevated lactate dehydrogenase (LDH, 1190 U/L), alanine aminotransferase (ALT, 554 U/L), and aspartate aminotransferase (AST, 308 U/L) levels, as well as serologic evidence of acute EBV infection (virus capsid antigen [VCA], 1:640; Epstein-Barr nuclear antigen [EBNA], negative; early antigen [EA], 1:1280; IgM, 1:160). Serologies for antibodies to toxoplasma, cytomegalovirus (CMV), and HIV were negative.

Excisional biopsy of the lesion was performed without complication, revealing a moderately firm, unencapsulated mass that was adherent to the overlying conjunctiva anteriorly. Since the excision, there has been no disease recurrence through 18 months of follow-up.

Patient B was an 8-year-old boy who developed painful, bilateral conjunctivitis and eyelid swelling ~70 days after a T cell-
Figure 1. Gross and microscopic appearance of conjunctival lesions from patient A (A, C, E, and G) and patient B (B, D, F, and H). (A) Salmon-colored lesion on the bulbar conjunctiva; (B) lid edema and membranous conjunctivitis. Hematoxylin-eosin staining of conjunctival lesions demonstrates (C) leukocytic infiltrate with occasional large, multinucleated Reed-Sternberg-like cells; and (D) collections of cells with large pleomorphic nuclei consistent with a large cell lymphoproliferative process. Immunoperoxidase staining for CD3 (E, F) demonstrates infiltrating T cells identified by brown cytoplasmic staining, particularly in the section from patient A (E). In situ hybridization of conjunctival biopsy with digoxigenin-labeled antisense EBV RNA probes (G, H) reveals cells with darkly stained nuclei positive for EBV; same sections hybridized to control, sense probes show no staining (not shown).
depleted matched-unrelated bone marrow transplantation for Wiskott-Aldrich syndrome. Before the development of ocular findings, the patient had had a 2-week course of fever, cough, and nasal congestion. Ophthalmic examination revealed bilateral lid edema, as well as erythema and bilateral membranous conjunctivitis with 3° conjunctival edema (figure 1B). The corneas were clear. Swelling of the eyelids and conjunctival tissues progressed over the next week to compromise eye opening. The patient also complained of right ear pain. Otolaryngologic examination revealed a bulging mass in the right external auditory canal and nasopharyngeal swelling. At the onset of symptoms, the patient was receiving therapeutic cyclosporin A and prednisone for graft-versus-host disease. Abnormal laboratory findings at the time of the ophthalmic disease included a WBC count of 1700/mm³ with 28% lymphocytes, as well as elevated LDH (2334 U/L), ALT (95 U/L), and AST (212 U/L) levels. EBV serology obtained before the transplantation showed evidence of previous infection (VCA, 1:80; EBNA, 1:80; EA, negative; IgM, negative). CT examination of the head, neck, and chest revealed near complete opacification of sinuses without bony destruction or nasopharyngeal fullness, but no cervical adenopathy or pulmonary disease. To establish the etiology of the ocular disease, a surgical biopsy of the right supratemporal conjunctiva was obtained.

The patient received 2 doses of EBV-specific cytotoxic T lymphocytes (CTL) 4 weeks apart. These cells are prepared according to institutional protocol and offered to all recipients of T cell–depleted, allogeneic bone marrow transplants for the prevention of EBV-related lymphoproliferative disease [7]. Immunosuppression (cyclosporin A and prednisone) was discontinued 3 weeks after the first dose of CTL was given. Two to 3 weeks after the second dose of CTL, abatement of ocular and nasopharyngeal symptoms was noted. Eyelid and conjunctival edema decreased to permit eye opening, and conjunctival injection improved.

In situ hybridization. Small noncoding EBV RNAs (EBERs) were detected using virus-specific riboprobes [8]. De-waxed paraffin sections of biopsy material were hybridized to digoxigenin-labeled sense (control) and antisense EBER riboprobes. Hybridization was detected with an alkaline-phosphatase–conjugated digoxigenin-specific antibody, according to the manufacturer’s protocol (Boehringer Mannheim, Indianapolis, IN).

PCR for EBV DNA. Mononuclear cells were collected from peripheral blood by density centrifugation (Ficoll; Pharmacia Biotech, Piscataway, NJ), and DNA was extracted from isolated cells (QIAamp Blood Kit; Qiagen, Valencia, CA). Total cellular DNA was tested for EBV using PCR primers specific to the BamH1C fragment of EBV DNA (primer 1, GCCTACACACCAACTATAGC; primer 2, GGAAGACAACCCACAGACACC) that amplify a 201-bp sequence of the EBER region. After a 25-cycle semiquantitative PCR reaction [9], amplified products were transferred to a nylon membrane, hybridized to a digoxigenin-labeled probe, and detected via chemiluminescence (Genius Kit; Boehringer Mannheim, Indianapolis, IN). The lymphoblastoid cell line IB4 (with 2 EBV genome equivalents per cell) was used as quantitative control.

Histology. The conjunctival lesion from patient A was an epithelium-lined soft tissue fragment showing diffuse infiltration by polymorphous leukocytes. The infiltrate was comprised predominantly of lymphocytes, as well as plasma cells and neutrophils (figure 1C). Rare large, multinucleated Reed-Sternberg–like cells were also noted. The lesion was designated an atypical polymorphous reactive process.

The conjunctival biopsy from patient B revealed nodules of lymphocytic cells with large pleomorphic nuclei interspersed with fewer small cells that had hyperchromatic nuclei (figure 1D). The remaining conjunctiva was edematous and congested. Findings were consistent with a large cell lymphoproliferative process. The same process was also identified in biopsies of swollen nasopharyngeal tissues.

Immunohistochemical staining of conjunctival tissue from patient A showed strong positive staining for CD20 (a pan B cell marker) in a follicular and diffuse scattered pattern, consistent with an EBV-driven lymphoproliferative process (data not shown). In this immunocompetent patient, abundant CD3° T cells were identified infiltrating this B cell lesion (figure 1E). This contrasts with the conjunctival biopsy from the immunocompromised patient (patient B), also positive for CD20 expression, but showing far fewer infiltrating CD3° T cells (figure 1F).

The conjunctival mass of CD20° cells excised from patient A was EBV positive, as demonstrated by EBER in situ hybridization (figure 1G). The conjunctival biopsy from patient B also showed substantial numbers of EBV-positive cells (figure 1H) among the small infiltrating cell population, as did biopsies from the nasopharynx and a mass identified in the external auditory canal of this patient (data not shown). EBER hybridization signals were localized to the nuclei in all tissue samples.

PCR for EBV DNA. Elevated levels of peripheral blood EBV DNA were not identified from patient A. In contrast, peripheral blood EBV DNA from patient B was highly elevated (>40 genome equivalents/0.1-ng DNA) at the onset of ocular symptoms, compatible with systemic lymphoproliferative disease. Middle ear fluid obtained from patient A at the time of conjunctival biopsy was also strongly positive for EBV DNA by PCR (>400 genome equivalents/2-µL fluid). Concomitant with clinical improvement in patient B, peripheral blood EBV DNA returned to normal levels (<40 genome equivalents/100-ng DNA).

EBV is an infection of mucosal surfaces and lymphoid tissues. Signs and symptoms such as pharyngitis and lymphadenopathy are a consequence of B cell infection and corresponding virus-specific cytotoxic T cells at these sites. On the basis of seroepidemiologic data, EBV infection has been reported to affect all segments of the eye, but ocular manifes-
tations most commonly associated with acute mononucleosis include periorbital edema (10%–20% of children and adolescents [2, 3]) and follicular conjunctivitis (up to 38% of cases [10]). Infectious processes in the conjunctiva often provoke true follicle formation, as in patient A, which tend to elevate the epithelium and be clinically evident [11]. EBV conjunctival disease might be predicted on the basis of ocular anatomy: in contrast to the avascular cornea, the conjunctival epithelium rests on a stromal layer replete with blood vessels and lymphoid aggregations corresponding to the mucosa-associated lymphoid tissue (MALT) of the gut and bronchi [12]. EBV-infected lymphocytes probably disseminate along a MALT pattern [13], which possibly explains the 2 cases of conjunctival disease described in this report, as well as the conjunctival findings described during acute mononucleosis.

Seroepidemiologic studies have previously implicated EBV in anterior segment eye diseases, including conjunctivitis, dacryoadenitis, episcleritis, keratitis, and iritis [5], as well as posterior segment processes such as retinitis and uveitis [14]. These observational associations suggest that EBV causes ocular disease, a proposal strengthened by identification of EBV genome by PCR in all ocular tissues except the optic nerve in normal cadaveric globes [15]. Even more conclusive direct evidence for a pathogenic role was obtained by in situ hybridization studies of B cell lymphoproliferations in lacrimal gland biopsies from patients with Sjögren’s syndrome [16]. Immunohistochemistry was used to suggest EBV as the etiology of a conjunctival mass in an adult with acute, symptomatic EBV infection [17]. However, the present report is the first to localize EBV EBERs (markers of latent infection [18]) to the nuclei of infiltrating cells within conjunctival lesions and firmly establishes EBV as a cause of these ocular manifestations.

In the first case in this report, unsuspected acute EBV infection (evident by serology, atypical lymphocytes, and elevated aminotransferases) manifested as an isolated conjunctival mass in a previously healthy and otherwise asymptomatic child. In the second case, systemic lymphoproliferative disease after bone marrow transplantation manifested predominantly as ocular and otolaryngologic abnormalities, including bilateral lid edema, conjunctivitis, and conjunctival edema. These 2 cases are unified by the predominance of the ocular findings and their appearance as the presenting feature of EBV infection. Although these cases are probably exaggerations of more typical ocular findings in acute infectious mononucleosis, they indicate that EBV infection in healthy and immunocompromised patients may present first with ocular signs and symptoms.

Like other symptoms of EBV, ocular manifestations are likely to be self-limited in healthy individuals. The conjunctival mass identified in the acutely infected healthy child was heavily infiltrated with CD3+ T cells (figure 1E). Although the lesion was surgically excised, the T cell infiltrate that was identified predicts that the ocular lesion would have been controlled by the intact cellular immune response of this healthy host. This contrasts with the progressive disease observed in the child without adequate T cell immunity. The extensive ocular and otolaryngologic EBV disease observed in the bone marrow transplant recipient did not improve until immunosuppressive therapy was withdrawn and EBV-specific cytotoxic T cells were infused as immunotherapy. It is important to note that ocular disease in this patient served as a practical indicator of systemic EBV infection: reduction in eyelid edema, conjunctival injection, and conjunctival edema tightly coincided with the decrease in blood EBV DNA measured by semiquantitative PCR. These cases illustrate the protean manifestations of EBV ocular disease, which may range from self-limited disease in healthy individuals to harbinger of more serious systemic EBV disease among vulnerable patient populations.

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K. S. Slobod,1,6 J. T. Sandlund,2,6 P. H. Spiegel,7 B. Haik,7 J. L. Hurwitz,3,9 M. E. Conley,3,6 L. C. Bowman,2,3,6 E. Benaim,2,6 J. J. Jenkins,4,6 R. M. S. Stocks,4 Y.-J. Gan,10 and J. W. Sixbey,10

Departments of Infectious Diseases,2 Hematology/Oncology,3 Immunology, and 4 Pathology, and Division of Bone Marrow Transplantation, St. Jude Children’s Research Hospital, and Departments of Pediatrics,6 Ophthalmology,8 Otolaryngology, and 9 Pathology, University of Tennessee, Memphis, Tennessee;10Louisiana State University Medical Center, Shreveport, Louisiana

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Significance of Aspergillemia in Patients with Cancer: A 10-Year Study

The significance of blood cultures positive for Aspergillus species for patients with cancer remains unclear. The significance of aspergillemia in 36 cancer patients over a 10-year period was evaluated. True aspergillemia was rare, occurred late in the course of aspergillosis, and was seen exclusively in patients with hematologic malignancies.

It can be a challenge to determine the importance of Aspergillus fungemia in patients at risk for invasive aspergillosis, because contamination of culture media can occur and yield false-positive results [1]. We examined the significance of blood cultures positive for Aspergillus species at a cancer center by applying previously proposed criteria for differentiating true aspergillemia from contamination [2].

Blood cultures positive for Aspergillus species were identified by reviewing the microbiology culture reports at the University of Texas M. D. Anderson Cancer Center for a 10-year period (1989–1998). Blood cultures for the detection of fungi were performed by use of BACTEC (BACTEC9240 system with BACTEC Plus Aerobic F media; Becton Dickinson Microbiology Systems, Sparks, MD) and an Isolator 10 system (Wampole, Cranbury, NJ), according to a method described elsewhere [3]. Inoculation and plate reading were performed in a laminar air flow type II hood [3]. Standard morphologic methods for the microbiological diagnosis of Aspergillus species were used [4]. We reviewed the medical records of the patients with blood cultures positive for Aspergillus species to determine the underlying malignancy, risk factors, and signs and symptoms of invasive aspergillosis. We determined 3 categories of blood cultures positive for Aspergillus species according to the criteria proposed by Duthie and Denning [2]: definite aspergillemia, probable aspergillemia, and pseudofungemia. Definite aspergillemia was defined as the presence of Aspergillus species in blood associated with histological evidence of hyphae in tissue or the same species of Aspergillus cultured from infected tissue before and after death. Probable aspergillemia was defined as the presence of Aspergillus species in blood and a clinical condition compatible with invasive aspergillosis, but no histopathologic evidence of invasive aspergillosis. Finally, pseudofungemia was defined as the presence of Aspergillus species in blood and a clinical setting not associated with invasive aspergillosis.

We identified 36 cancer patients (7 with definite aspergillemia, 5 with probable aspergillemia, and 24 with pseudofungemia) with blood cultures positive for Aspergillus species during the study period (<.001% of all positive blood cultures and <.01% of all blood cultures positive for fungi; table 1). Definite or probable aspergillemia was seen only in patients with hematologic malignancies (12 of 24 vs. 0 of 12 patients with solid tumors; P = .03). On the other hand, pseudofungemia occurred only in patients with solid tumors (12 of 12 vs. 12 of 24 patients with hematologic malignancies; P = .03). Positive blood cultures in cases of definite or probable aspergillemia occurred late (1 day before death or at autopsy in 7 of 12 patients). In 6 of 7 cases of definite aspergillemia, blood cultures were positive only at autopsy. Therefore, of the 18 patients with hematologic malignancies who had positive cultures of blood obtained antemortem, only 1 had definite aspergillemia. On the other hand, in all cases of pseudofungemia, positive cultures were of blood obtained antemortem. The rate of blood cultures positive for Aspergillus species in patients with hematologic malignancies

Table 1. Significance of blood cultures positive for Aspergillus species in 36 patients with cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Definite</th>
<th>Probable</th>
<th>Pseudofungemia</th>
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<tr>
<td><strong>Aspergillus species</strong></td>
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<td>Aspergillus fumigatus</td>
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<td>5</td>
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<tr>
<td>Other Aspergillus species</td>
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<td>3b</td>
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<tr>
<td><strong>Total with positive blood cultures (n = 36)</strong></td>
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<td>5</td>
<td>24</td>
</tr>
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</table>

NOTE. Data are no. of patients.

a One case of Aspergillus flavus.
b Two cases of Aspergillus flavus, 1 case of Aspergillus flavus.