Epstein-Barr Virus in a Malignant Lymphoproliferative Disorder of B-Cells Occurring after Thymic Epithelial Transplantation for Combined Immunodeficiency

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Abstract

A fatal disseminated polyclonal malignant lymphoproliferative disorder of B-cells (immunoblastic sarcoma) developed shortly after a second thymic epithelial peritoneal implant in a 5-yr-old girl with combined immunodeficiency. The immunodeficiency was characterized by low T-cell numbers and function, very low levels of thymic hormone, dysgammaglobulinemia, and an inability to mount a primary antibody or cell-mediated response to new antigens. At necropsy, the thymus fulfilled morphological criteria for thymic dysplasia.

Epstein-Barr virus (EBV) antigen and DNA were identified in neoplastic infiltrates in the lymph nodes and thymus by immunofluorescence for the EBV nuclear antigen and by EBV-specific complementary RNA/DNA hybridization. No antibodies to nuclear antigen, early antigen, or viral capsid antigen of EBV were identified in the serum.

The concurrence of these events suggests that the thymic epithelial implant itself may have been instrumental in the pathogenesis of this neoplasm. It is proposed that the thymus may have provided factors which indirectly potentiated the proliferation of EBV-infected B-cells, possibly by induction of nonspecific T-helper cells and perhaps through other thymic humoral factors.

It is suggested that some forms of immunoblastic sarcoma, even when polyclonal, and especially those which arise in immunocompromised hosts, may, in some instances, represent an opportunistic form of EBV-induced B-cell neoplasia.

Fatal B-cell lymphoproliferative disorders complicating transplantation of cultured thymic epithelium in CID have been described recently (3). The authors suggested that EBV may have been responsible for the induction of the neoplasm in a manner similar to that proposed in XLP (25). Studies to test this postulate were not performed.

This report documents the presence of EBV in neoplastic cells of a malignant B-cell lymphoproliferative disorder (immunoblastic sarcoma) in a patient with CID following implantation of cultured thymic epithelium. Because of the close temporal relationship between thymic epithelial implantation and the clinical emergence of the neoplasm, the possible pathogenetic role of the thymic epithelium as a contributing factor in B-cell neoplasia is discussed.

Materials and Methods

Case Report. Immunological studies were performed on C. C., a white female, at the age of 2 years, because of persistent Hemophilus influenza pneumonia and hyper-IgA dysgammaglobulinemia. She was born at term to a mildly preeclamptic Para 1 Gravida 1 mother. There was no family history of immunodeficiency, and consanguinity was denied. Previous hospital admissions, beginning at 3 months of age, were for failure to thrive, gastroenteritis, anemia, persistent thrush, conjunctivitis, and bronchopneumonia. She had received 4 immunizations with diphtheria-pertussis-tetanus (DPT) and one of rubella-rubella. At the time of these studies, no abnormalities were noted on physical examination. Immunoglobulins (mg/dl) were: IgG (1950); IgM (55); IgA (1620); and IgD (20). IgE was 20 IU/ml. The IgA was polyclonal on immuneelectrophoresis. Antibody titers were: rubella, 1: 256; diphtheria, 1:320; tetanus, nil; anti B, nil (Blood Group A, Rh positive). (The diphtheria titer subsequently became undetectable.)

Classical and alternative pathway hemolytic complement were normal. Delayed skin tests to Monilia and dermatophytin were positive, and the Schick skin test indicated immunity. ERFC were 20%, and cells bearing surface membrane immunoglobulin were 14%. There was virtually no mitogen response to phytohemagglutinin, concanavalin A, pokeweed mitogen, and allogeneic cells. Thymic hormone (measured by M. Dardenne, Paris, France) was 1:8 (very low for age). Incubation of lymphocytes with thymosin fraction V (provided by A. Goldstein, Washington, D. C.) effected a 7% augmentation of ERFC. Lymphocyte adenosine deaminase and nucleoside phosphorylase were present in normal to elevated levels. Typhoid immunization elicited no antibody response; sensitization to dinitrochlorobenzene was negative. HLA typing of blood lymphocytes revealed A2, A26, B18, and BW54. Subsequent admissions were for 3 episodes of pneumococcal septicaemia with different serotypes, recurrent otilitis, pneumonia, staphylococcal infections, intractable diarrhea, and severe, prolonged varicella.

Cultured thymic epithelium was given i.p. at 3 years, 10 months and at 4 years, 9 months of age. One day following the first thymic implantation, the percentage of B-cells increased to 48% with all heavy chain classes represented. This subsequently declined, while the percentage of ERFC doubled. Prior to the second thymic epithelial graft, the percentage of B-cells was again elevated to 35%, and the serum IgA was 6000 mg/dl. Immediately following the graft, the percentage of ERFC again increased, and there was a small increase in the response to phytohemagglutinin and concanavalin A (Table 1). Three weeks following the second graft, the patient became febrile, developed generalized progressive lymphadenopathy, hepatosplenomegaly, etc.
jaundice, neutropenia, pulmonary infiltrates, otitis, and sepsis with occurred 10 weeks following the second graft.

Thymic Epithelial Culture. Culture of thymic epithelium was performed according to the technique of Hong et al. (8) on thymic tissue obtained from patients undergoing cardiac surgery.

Serum Antibodies. Serum samples were analyzed for antibodies to EBNA, EA, and VCA by indirect immunofluorescence using methods described previously (9).

Tissue Antigens. Frozen tissue specimens were tested for the presence of EBNA with an antibody-positive human serum and a negative serum as a control (26).

Detection of EBV DNA. EBV DNA was detected in aliquots of extracted DNA by the technique of complementary RNA/DNA hybridization (23, 24). All determinations were performed in duplicate.

Results

Pathology. The lymph node was effaced by a cell population representing the spectrum of B-cell transformation from small lymphocyte to plasma cell (Fig. 1). The majority were large to medium-sized lymphoblasts, plasmacytoid lymphoblasts, and mature plasma cells, some multinucleated. Many demonstrated intense cytoplasmic pyroninophilia. Lesser numbers of large binucleated cells having some features of Reed-Sternberg cells were identified. Mitoses were numerous. Capsular and vascular invasion by malignant cells was prominent. Erythro- and lymphohagocytosis by histiocytes were occasionally observed. Electron microscopy substantiated these findings but failed to demonstrate viral particles within the neoplastic cells. Immunofluorescent studies of frozen tissue revealed moderate numbers of plasma cells with intracytoplasmic IgM and IgA. Only a few cells contained IgG. Both κ and λ light chains were present in roughly equal numbers. Tumor cell surface marker studies revealed 20% ERFC, 20% immunoglobulin-bearing cells, 2% Fc rosette-forming cells, and 14% complement receptor-bearing cells. In the population of cells showing surface immunoglobulin, all heavy and light chain classes except IgE were represented.

At necropsy the thymic peritoneal implants could not be recovered.

Virtually all lymph nodes were involved in a manner histologically identical to the biopsy material with the most extensive involvement in the abdomen. Neoplastic infiltrates were also found in the liver, spleen, thymus, lungs, lymphoid tissue of gastrointestinal tract, pancreas, bone marrow, and central nervous system. (Sections of surgical and autopsy tissue were reviewed by Dr. R. J. Lukes who confirmed the diagnosis of immunoblastic sarcoma of B-cells.)

The thymus was minute and composed of small lobules surrounded by edematous connective tissue. The gland was devoid of Hassall’s corpuscles and lacked definitive cortex and medulla. The lobules were infiltrated by plasma cells and immunoblasts; only occasional small lymphocytes were observed.

In lymph node, a high percentage of EBNA-containing cells were present (Fig. 2a). In the thymus, scattered aggregates of EBNA-positive cells were also seen (Fig. 2b). No EBNA-containing cells were identified in the sample from the spleen.

An average of 15 and 17 EBV genomes/cell was found as indicated in Table 2. Since the tissue specimens contained normal as well as neoplastic cells, the actual number of EBV genomes in the neoplastic cells is probably higher.

Two serum samples from the patient, one at age 2 years, the other just prior to her demise, were analyzed for antibodies to EBNA, EA, and VCA. Both samples were negative.

Seven months after the second thymus had been obtained for culture, serological studies were performed on thymus donor as well as blood donors whose blood had been given for culture, serological studies were performed on thymus donor as well as blood donors whose blood had been given prior to and at the time of cardiac surgery. Antibodies to VCA but not to EBNA were present in the thymus donor. Both antibodies were detected in the blood donors.

Discussion

The EBV is a herpesvirus with a striking tropism for human B-lymphocytes. In vitro, the virus transforms B-lymphocytes which have a normally limited life span into permanently replicating lymphoblastoid cell lines. Some of these EBV DNA-carrying cell lines can grow as malignant neoplasms after heterotransplantation into immunologically deficient rodents (1,
2, 29). The virus appears to have direct oncogenic potential in marmosets (28) and owl monkeys (6). Both the cell lines and the experimental neoplasms express EBNA. This virally determined nuclear protein antigen is a consistent indicator of the EBV genome within the nucleus of the virally infected cell (14).

In man, infection with the virus most often results in clinically quiescent seroconversion. The virus is the etiological agent of infectious mononucleosis and has also been implicated in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma (10, 22, 39). The virus has also been observed in one case of immunoblastic lymphadenopathy (38). This plurality of pathological expression is believed to depend largely on the immunocompetence of the host. In the self-limited forms of EBV infection, host T-cell responses arrest the potentially explosive and generalized proliferation of virally infected B-lymphocytes. Elsewhere in this issue, Klein et al. (12), Rickinson et al. (27), and Henle and Henle (7) discuss immune responses to EBV in detail.

In contrast, males with XLP possess a postulated genetically determined weakened ability to form antibodies to EBV and to mount vigorous cytotoxic T-cell response to EBV-infected lymphocytes. Infection with the virus results in 4 types of phenotypic expressions of XLP: agammaglobulinemia; aplastic anemia; fatal infectious mononucleosis; or B-cell lymphoma. Immunodeficiency to the virus is thought to permit the lethal proliferation of B-cells frequently observed in this syndrome (25).

Immunoblastic sarcoma has been observed among the B-cell lymphomas complicating XLP. This neoplasm is a malignant proliferation of B-lymphoblasts with varying degrees of plasmacytic differentiation and may be unique among lymphomas in that it is often polyclonal. Although the neoplasm may arise in an ostensibly normal host, a significant percentage develops in patients with antecedent immune disease or lymphoproliferative cancer (16). It is also an infrequent but significant complication of prolonged immunosuppressive therapy (19) and has been described in at least one renal transplant patient in association with presumptive serological evidence of EBV infection (4). Immunoblastic sarcoma and a related entity, immunoblastic lymphadenopathy (18), were recently observed to emerge following thymic epithelial grafting for CID (3). Although an EBV-induced oncogenic mechanism analogous to that of XLP was raised by these authors, studies to demonstrate EBV were not performed.

The immediate significance of the case reported here resides in its documentation of EBV in a polyclonal lymphoproliferative disorder of B-cells which is indistinguishable both by its histopathology and biological behavior from immunoblastic sarcoma. The case indeed raises the possibility that there exists at least one type of immunoblastic sarcoma which is an opportunistic form of EBV infection in which a host response is either deficient or completely lacking.

The absence of EBV-specific antibodies in this patient both prior to and following thymic transplantation precludes determination of the time when viral infection occurred. It is plausible that the thymic graft may have contained EBV-infected B-lymphocytes. This possibility must be considered since the thymic donor's serum contained antibodies to VCA and because the donor was twice the recipient of blood from donors whose serum was later found to contain antibodies to both VCA and EBNA. It must be emphasized that these studies were performed 7 months after transplantation and not shortly after transplantation when the data could have been more conclusive. However, it is also possible the infection was acquired by other means given the ubiquity of the virus in the environment. It is noteworthy that EBV-associated lymphoproliferation has not been described in CID after bone marrow transplantation. Yet, EBV infection has been documented following bone marrow transplantation for acute leukemia (30), indicating that bone marrow, in addition to supplying hematological and immunological presursor cells, is a potential source of EBV-infected B-lymphocytes.

The fact that both this patient and one of the 3 described by Borzy et al. (3) developed lymphoma shortly after thymic transplantation suggests that thymic epithelium itself may have been instrumental in the pathogenesis of this neoplasm. In the spontaneous leukemia observed in AKR mice and that which follows irradiation in C57BL mice, the thymus is an essential requisite for leukemogenesis since thymectomy dramatically decreases the incidence of leukemia (20). In vitro studies have demonstrated that cultured thymic epithelium can transform thymocytes into neoplastic cells in the AKR system, thus establishing the role of thymic epithelium in at least one form of experimental T-cell neoplasia (33). Although no role has, as yet, been postulated for the thymus in B-cell neoplasia, thymic-dependent humoral factors are known to indirectly influence B-cell expansions by T-helper-B-cell interactions. Hence, it is plausible that the thymic epithelial graft may have facilitated EBV-initiated neoplastic proliferation of B-cells by inducing nonspecific host T-helper cells and perhaps providing other humoral factors.

In the H-2 system of the mouse, T-cell cytotoxicity against cells infected with certain viruses requires recognition of both virus-induced membrane antigens and major histocompatibility antigens of infected cells. These antigens must be shared with the H-2 type of the thymic epithelium present in the host at the time of T-cell maturation (35, 37). Zinkernagel et al. (36) suggested that transplantation of thymic epithelium which does not share certain major histocompatibility antigens with the host may thus leave the recipient deficient with respect to cytotoxicity against its own virally infected cells. Other thymic functions such as nonspecific T-helper induction do not appear to be similarly restricted (11). Thus, it is possible that non-HLA-matched allogeneic thymic epithelial implants in patients with CID may, in addition to inducing T-helper cell function, further facilitate an uncontrolled proliferation of EBV-infected cells because a host cytotoxic T-lymphocyte response is deficient. While this is an intriguing postulate, in vitro evidence to date suggests that, while some components of the cytotoxic response to EBV-infected cells appear to be HLA restricted (21), others are not (17). Further evidence for these notions are

### Table 2

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>No. of determinations</th>
<th>Av. cpm/50 μg DNA hybridized</th>
<th>Av. no. of EBV genome equivalents/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>5</td>
<td>650</td>
<td>15</td>
</tr>
<tr>
<td>Lymph node</td>
<td>5</td>
<td>506</td>
<td>12</td>
</tr>
<tr>
<td>Raji cells EBV-positive</td>
<td>6</td>
<td>8300</td>
<td>191</td>
</tr>
<tr>
<td>Calf thymus EBV-negative</td>
<td>3</td>
<td>35</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*The cpm hybridized to 50 μg Hep 2 DNA (144 cpm) were subtracted to yield hybridization data. Input of EBV complementary RNA was 105 cpm/filter.*
E. R. Reece et al. discussed elsewhere in this issue by Klein et al. (12) and Rickinson et al. (27).

Alternatively, the thymic epithelial cells may have served as the source of the EBV inoculum. The epithelial cells of nasopharyngeal carcinomas have been shown to contain EBV DNA (5, 13, 34) and permit replication of the virus (31, 32). Additionally, oropharyngeal epithelial cells of patients with infectious mononucleosis contain the viral genome (15). Thus, thymic epithelial cells may also be among the primary targets of EBV infection.

In this patient, prior to the first thymic epithelial transplantation, one peripheral blood T- and B-cell analysis revealed a polyclonal B-cell expansion. This was again noted immediately following the first thymic epithelial graft and just prior to the second. The children reported by Borzy et al. have been similar to ours since all 3 had significant numbers of circulating B-cells and 2 had elevated immunoglobulins prior to thymic implantation. These children may represent a group especially vulnerable to an EBV-associated uncontrolled proliferation of B-cells following thymic epithelial grafting. Indeed, the pre-thymic epithelial B-cell expansions and elevated IgA may have indicated the presence of a lymphomatous process given the known association of immunodeficiency and lymphoproliferative disorders.

In summary, transplantation of allogeneic thymic epithelium for combined immunodeficiency may in some patients have a deleterious effect by providing thymic factors which indirectly potentiate the growth of EBV-induced B-cell neoplasms. Because it is possible that cultured thymic epithelium may serve as a source of EBV-infected cells, screening for EBV infection in potential donors seems advisable.

Acknowledgments

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References


Fig. 1. Lymph node biopsy. A, H & E, × 200; B, toluidine blue, epon embedded, × 790.
Fig. 2. A, lymph node demonstrating EBNA immunofluorescence in neoplastic cells, × 790; B, thymus demonstrating EBNA immunofluorescence in neoplastic cells, × 790.
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