Short Review

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IS ASSOCIATED WITH EPSTEIN-BARR VIRUS

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SUMMARY : The post-transplant lymphoproliferative disorder (PTLD) is defined in large part by their occurrence following organ transplantation and by their histopathological appearance. The vast majority is associated with Epstein -Barr virus (EBV) infection. Their recognition is important because underdiagnosis as a not otherwise specified reactive process may lead to continued immunosuppression and progression of disease and overdiagnosis as a conventional lymphoma may lead to inappropriate and potentially fatal chemotherapy. In this article pathogenesis, clinical cause, and therapy of PTLD have been presented and the role of EBV described.

Key Words: Epstein-Barr virus, post-transplant lymphoproliferative disease.

INTRODUCTION

Epstein-Barr virus is a ubiquitous lymphocryptovirus belonging to the subfamily of gamma herpes virinae. This agent probably evolved and spread among the old world primates since the divergence of apes from monkey about 30 million years ago (1). EBV is 180 μm in diameter and consists of an outer membrane, an icosahedra nucleocapsid with 162 capsomers, and a cord of protein and DNA. The viral membrane has spike-like projections on its outer surface which are composed of one or two species of high- molecular- weight glycoprotein's (1). Other protein components of the membrane include a nonglycosylated membrane matrix protein (140 kilo Daltons), a minor glycoprotein (120 glycoprotein), and a major inner membrane glycoprotein (85 kilo Daltons). The nucleocapsid and core consist of at least 15 polypeptides ranging from 20 to 150 kilo Daltons in size (1). The EBV genome is a double stranded DNA molecular of approximately 172000 base pairs; as in other herpes viruses, the molecule is divided into unique, internal repeat, and terminal repeat domains. The genome encodes approximately 80 proteins. The function of many of the genes involved in viral replication has been inferred from their homology to herpes simplex virus genes; however, genes expressed during latent infection of B cells do not have recognized counterparts in other human herpes viruses (2).

Genomic analyses of Epstein-Barr virus isolated from around the world have identified two broad families of EBV, designated type A and type B, respectively. Type A EBV is ubiquitous, while type B EBV is commonly isolated only from parts of Africa endemic for malaria and Burkitt's lymphoma.

Type A EBV can effect oropharyngeal epithelial cells and peripheral blood lymphocytes, while type B EBV, when found in healthy Western populations, is seen in the oropharynx and only rarely in the peripheral blood (3).

ANTIGENS OF EBV

The EBV genome is one of the largest pieces of eukaryotic DNA encoded 80 to 100 genes; only 11 are expressed during latent infection and in tumor cells. The proteins encoded by these 11 genes are found either in the

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nucleus or at the cell surface. These include the EBNA 1-6; three latent membrane proteins (LMP1, 2A and 2B); and two abundant EBV encoded RNA, so called latency III.

The state of latent infection is maintained by the EBNA-1 protein; it binds to a nucleotide sequence, termed ORIP, which is part of the viral origin of DNA replication. The binding of EBNA-1 to ORIP allows the viral genome to be maintained in the nucleus of the B cell.

EBNA-2 is essential for cell growth transformation since an EBV isolate which is deleted for a region including EBNA-2 is unable to transform B lymphocytes.

EBNA-3B and EBNA-3C are encoded by three tandem arrayed genes which are distantly homologous. Two latent gene products, the EBV - encoded RNAs (EBERs), are the most abundant RNAs expressed *in vitro*; they do not code for proteins and are dispensable for transformation *in vitro*. LMP-1, acts as a direct oncogene in transformation assays.

The virus can be reactivated from latently infected B lymphocytes by certain chemicals or by antibody. These stimuli lead to expression of the EBV BZLFI gene product, or ZEBRA (Z EBV replication activator) protein, an immediate early gene product. The ZEBRA protein acts as the switch that triggers viral replication in latently infected B cells. This protein also transactivates expression of ZEBRA itself (4, 5).

Viral replication occurs in a fraction of the initially infected B lymphocytes, with subsequent infection of a large population of B cells, which disseminate the infection to other tissues. The primary immune response to EBV infection involves natural killer (NK) cells and CD4+ suppressor T cells. In usual circumstances, this is followed by a secondary EBV - specific immune response mediated by class I HLA restricted CD8+ cytotoxic T cells, which control the proliferation of EBV latently infected B cells (6).

However, transplantation with concomitant immunosuppressant interferes with EBV control mechanisms in the patient and may lead to uncontrolled EBV - driven B cell lymph proliferation in susceptible patients with development of PTLD (7).

MECHANISMS FOR EVADING THE IMMUNE SYSTEM

One mechanism of evading host responses is to limit the number of viral gene products that are expressed. When EBV replicates, about 90 of its genes are expressed. However when it latently infects B cells *in vitro*, only 10 genes are expressed.

A second mechanism that EBV uses for evading the immune system is interference with cytokine activity. Epstein-Barr virus encoded a gene product, BCRF1 that has more than 80% sequence homology with human interleukin IL-10. A third mechanism that the virus can use for immune evasion is to interfere with CTLs that would otherwise limit its spread. Killing of virus - infected cells requires that CTLs recognize viral peptides on the surface of infected cells in the contex of major histocompatibility complex (MHC) class I molecules.

The laboratory diagnosis of an EBV infection relies on techniques to demonstrate the virus, viral antigen, or viral DNA in clinical material and on serologic responses. The virus can be isolated from saliva, and peripheral blood or lymphoid tissue by immortalization of normal human lymphocytes. EBV is present in the saliva of many immunosuppressed patients. Up to 20% of healthy adults will also yield virus positive throat washings.

Techniques used for detection of EBV include serology, immunohistochemistry, southern blotting, polymerase chain reaction (PCR), in situ hybridization (ISH), complement- fixing (CF) and gel - precipitating (GP). The molecular biologic techniques are the most sensitive (8).

EPIDEMIOLOGY

Infectious mononucleosis (IM) is a lymphoproliferative disorder associated with fever, pharyngeal inflammation, and cervical lymphadenopathy. Primary infection is serologically characterized by the appearance of anti-virus capsid antigen (VCA) IgM and anti- early antigen (EA) IgM and transient development of anti-EA IgG in approximately 80% of infected individuals. Anti-VCA IgG, which persists throughout the life of normal individuals, occurs almost concurrently and continues to increase during the course of IM (9).

Burkitt's lymphoma (BL) is monoclonal proliferations of malignant B lymphocytes. Irrespective of whether they carry the Epstein - Barr genome, these tumor cells have been shown consistently to have one of the specific reciprocal chromosome translocation, t (8;14), t (2;8) or t (8;22), involving the long arm of chromosome 8 and chromosome 14, 2 or 22. The later chromosomes have been shown recently to carry genes for immunoglobulin heavy and light chains (10). Epidemiologic studies have linked Hodgkin's disease, which occurs for 30% to 50% of malignant lymphomas in Western countries, to previous IM. IM patients

have a twofold to fourfold increased risk of HD; the risk increases with age at exposure to EBV. Patients with HD often have higher titers of EA/VCA antibodies against EBV.

The expression of latent membrane protein (LMP), EB early region (EBER) transcripts and BZLF1 protein (in a small fraction of LMP- positive cases) in RS cells suggests that EBV is not merely a silent passenger in HD (11).

Nasopharyngeal carcinoma (NPS) is unique among epithelial malignancies. NPS is constantly associated with the Epstein-Barr virus regardless of the patient's origin. The EBV DNA is contained in malignant epithelial cells, usually in a latent form, without production of viral particles (12).

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD)

Post-transplant lymphoproliferative disease (PTLD) results from Epstein-Barr virus (EBV) in induced proliferation of B cells in the immunosuppression transplant population. Hence, it is not surprising that immunosuppression and primary EBV infection represent the two major risk factors for disease. The reported frequency of PTLD ranges from approximately 1% in renal transplant recipients to 9% in heart-lung transplant patients.

The presentation of PTLD is quite heterogeneous because it represents a continuum of diseases that initiates with a mild infectious mononucleosis (IM) -like syndrome, progresses to a more severe polymorphic polyclonal but still benign lymphoproliferation, and terminates at a monomorphic monoclonal malignancy. As a result, patients can exhibit merely localized lymphadenopathy or have disseminated disease involving multiple sites including the lymph nodes, gastrointestinal tract, and transplanted organs. Similarly, the PTLD lesions themselves also exhibit a variety of B-cell phenotypes that range from polymorphous monoclonal populations to those that are monomorphous monoclonal and often lymphoblastoid in appearance (13, 14).

CLASSIFICATION

These lymphoproliferative diseases have unique histology features that can be classified as polymorphic diffuse B cell hyperplasia or polymorphic B cell lymphoma. PT-LPDs are divisible to three distinct categories as follows:

i) plasmacytic hyperplasia: most commonly arise in the oropharynx or lymph nodes, are nearly always polyclonal, usually contain multiple infection events or only a minor cell population infected by a single form of EBV,

ii) polymorphic B-cell hyperplasia and polymorphic B -cell lymphoma; may arise in lymph nodes or various extranodal sites, are nearly always monoclonal, usually contain a single form of EBV, and lack oncogene and tumor suppressor gene alterations; and

iii) immunoblastic lymphoma or multiple myeloma: present with widely disseminated disease, are monoclonal, contain a single form of EBV, and contain alterations of one or more ontogeny or tumor suppressor genes (15,16).

EPSTEIN-BARR VIRUS - DETERMINED CLONALITY IN POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE

The molecular data lend credence to two important aspects of viral pathogenesis;

i) the finding of a homogeneous episomal population in the monoclonal tumors suggests that EBV infection is an early event in tumorigensis that occurs before clonal expansion; and

ii) therapeutic efficacy of acyclovir has been shown only in presence of polyclonal disease but may impact on intermediate stages where liner replicative virus can be found (17).

Transplant recipients are at increased risk for the development of post-transplant lymphoproliferative disorders (PTLDs). PTLD harbor genomes of the Epstein-Barr virus, a herpes virus that immortalizes B cell *in vitro*. At least five viral proteins are required for immortalization. Two of them are particularly important. Latent membrane protein (LMP) has transforming activity in fibroblasts, and Epstein-Barr antigen (EBNA) 2 transactivates the expression of numerous cellular and viral genes. The expression of EBNA2 and LMP are related to the histological and clinical presentation of PTLD. LMP and EBNA2 were found particularly in immunoblasts. The expression of EBNA2 and LMP is related to the differentiation stages of the infected cells and that other viral cellular proteins may contribute to tumor growth (18, 19).

Proteins that confer resistance to apoptosis are recognized as important in the development and progression of lymphomas. BCL-2 in particular, is expressed in a broad range of indolent lymphomas with and without the t (14:18) translocation. The EBV - encoded latent membrane pro-

tein -1 (LMP1) that is commonly expressed in PTLD has been shown to up regulate BCL-2 expression *in vitro*. Another EBV protein, BHRF1, has significant sequence and functional similarity to BCL-2. Both proteins have been shown to protect cells from apoptosis induced by serum withdrawal and a variety of DNA damaging agents.

PTLD treatments by monoclonal antibodies are based on the expression of these molecules, and some recent studies reported successes using anti-CD21 and anti CD24 anti B cell monoclonal antibodies therapy.

CLINICAL FEATURES

Post-transplant lymphoproliferative disease (PTLD) is a complication of immunosuppression that affects 2-5% of organ transplant recipients and is usually related to Epstein-Barr virus (EBV). PTLD may develop after primary infection in an immunosuppressed host or as reactivation of latent virus. EBV seronegative patients who receive transplants from EBV seropositive donors are at increased risk of developing PTLD, making this a particular concern in pediatric transplantation. Other factors that may affect its incidence include the frequency, title dose, and interval of T cell suppressive agents (particularly OKT3) used as treatment for allograft rejection. When it presents as fever and graft dysfunction, the principle suspicion is allograft rejection (19).

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