Infections caused by herpes simplex virus, varicella-zoster virus and Epstein-Barr virus in organ transplant recipients, and their diagnosis

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Les infections provoquées par l'herpès simplex, l'herpèsvaricellaet le virus Epstein-Barr chez les receveurs de transplantations d'organes et leur diagnostic

RÉSUMÉ Le patient immuno supprimé court un risque d'infection grave à herpesirus. Les infections à herpès simplex (HSV), à herpesvaricella (VZV) et au virus Epstein-Barr (EBV) sont associées à des syndromes caractéristiques dans cette population. De façon typique, les infections à HSV et VZV provoquent des lésions mucocutanées; le diagnostic est généralement confirmé à l'aide de cultures de tissus ou de microscopies à fluorescence. La disponibilité d'agents antiviraux efficaces et de techniques précises pour...
MORE THAN 80 HERPESVIRUSES HAVE BEEN ISOLATED from a wide variety of animal species (1). Only seven herpesviruses have been isolated from humans, including herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2), cytomegalovirus (CMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), and the newly identified human herpesvirus 7 (HHV-7).

All human herpesviruses share some biological properties, such as their ability to cause latent infections and to infect epithelial cells and cells of the immune system. In contrast, major differences exist in their genome structure, specific features of their replicative cycle, their effects on host cells, and the clinical syndromes associated with their infection.

Herpesviruses are an important cause of morbidity and mortality in humans. Immunosuppressed patients are especially at risk of severe infections due to decreased immunosurveillance and loss of control of herpesvirus latency. Infections with herpesviruses are usually followed by latency, which is the capacity of the virus to remain in the cell with only partial expression of its genome.

This review will focus on infections due to HSV, VZV and EBV in organ transplant recipients.

HERPES SIMPLEX VIRUS

During primary infection with HSV-1 or HSV-2, the viral genome replicates in the skin or epithelium and virions then ascend by retrograde axonal transport in sensory nerve cells. HSV-1 causes the common cold sore and is the most common viral cause of blindness and sporadic encephalitis. It also causes aggressive infection in immunosuppressed patients.

In kidney allograft recipients, HSV infection occurs at rates as high as 70% (2), but visceral dissemination is relatively rare. In the most recent review of herpes simplex infections in heart-lung transplant recipients (3), 45% of recipients were HSV antibody-positive at transplantation. Four of the 23 seropositive patients developed culture-proven superficial HSV lesions. In comparison, HSV pneumonia was diagnosed on six occasions in five patients. All occurred in patients who were HSV seropositive. Only one patient with HSV mucositis developed HSV pneumonia. One of the five patients died as a result of the HSV pneumonia despite therapy with intravenous acyclovir. A definite diagnosis of HSV pneumonia can be done only with a tissue biopsy; however, if biopsies are performed very early in infection it may not be possible to find characteristic inclusions by light microscopy. The results of tissue culture may give useful information and if they are positive for CMV and herpes simplex, immunohistochemical and DNA probe procedures may enhance specificity of diagnosis.

HSV infection may also involve other visceral organs such as the colon and liver, and these conditions usually have a fulminant course (4,5). Acyclovir should be effective for treatment, but anecdotal reports suggest that invasive visceral disease occurs with strains that are acyclovir-resistant (6).

**Herpes simplex diagnosis**: Because infections with herpes simplex in immunosuppressed patients can have a fulminant course, it is important to collect specimens as early as possible in the course of the illness. Ideally cultures should be taken from cutaneous lesions that have reached the vesicle stage. The rate of viral isolation decreases as the lesions develop from vesicles to crusts. Swabs should be moistened with transport media, and for maximum isolation rate, specimens should be transported to the laboratory on the day of sampling. To maintain infectious virions, it is useful to inoculate cell culture at the bedside before and during transportation. There are several rapid techniques to demonstrate HSV infections, such as immunofluorescence of infected cells. Cells are collected with a large bore needle from the base of the vesicles, smeared onto glass slides and fixed in methanol. Virus-specific antigens are identified using monoclonal antibodies tagged with fluoroscein. The vesicular fluid can also be analyzed by electron microscopy which will allow the detection of virions. Although serology can be useful in identifying patients who are at risk of acquiring reactivated infection, it is of very limited value during illness. Other techniques have been used for diagnosis confirmation, such as DNA amplification and in situ hybridization.
zation to demonstrate visceral disease. Strains of HSV less sensitive to antiviral drugs have been observed in immunocompromised patients treated for long periods. Although this is not a widespread problem in the treatment or prophylaxis of HSV infections, it is increasingly seen in patients who have received long term therapy with antiviral agents.

**VARICELLA-ZOSTER VIRUS**

VZV is the cause of chickenpox and herpes zoster (shingles). In the immunocompromised host it may cause disseminated and occasionally fatal infections. The pathogenesis of VZV and chickenpox involves a primary asymptomatic infection of the oropharyngeal or conjunctival epithelium followed by viremia, viral replication in reticuloendothelial cells, and secondary viremia followed by infection of the skin and mucous membranes. Shingles is the counterpart of the common cold sore in varicella-zoster virus infections. The reactivation of a latent infection in sensory ganglia results in active replication of the virus, anterograde axonal transport, liberation of virions from the axons and, finally, dermalomatous skin lesions.

VZV continues to be, with CMV, the most important cause of herpesvirus infections in immunocompromised patients. The probability of VZV infection is approximately 30% by one year after bone marrow transplantation. Most cases occur within the first nine months after transplantation. Peak occurrence of infection occurs during the fourth and fifth month after transplantation. Fifteen per cent of patients presenting with VZV infection develop varicella and 84% develop zoster. Although the frequency of visceral dissemination and mortality is higher in patients with varicella than in patients with localized zoster, the incidences of subsequent visceral dissemination (36.6%) and death (18.3%) in patients with cutaneous dissemination of zoster are comparable to those in patients with varicella. The most significant risk factor for the development of disseminated VZV infection is acute and chronic graft-versus-host disease. Visceral disease involving the liver or lungs is manifest by elevated results of liver function tests and transient pulmonary infiltrates. Severe infections are more common in patients who develop an infection within nine months after transplantation than in those who have a later onset of the disease (32% versus 19%, P<0.05). The mortality rate of VZV infection in bone marrow transplant recipients is approximately 10%. The mortality rate in patients with herpes zoster is around 6.6% and in patients with varicella it is approximately 28%. Diffuse interstitial pneumonia develops in the majority of patients with fatal disease (87%). Some patients with fatal infection do not develop skin lesions during the course of the illness. Among those patients with visceral dissemination, the mortality rate is 55% (7). The incidence of varicella-zoster in renal transplant recipients is around 7% (8). Dissemination rates in solid organ transplants range from 25 to 50% (9). Postherpetic neuralgia is also an important sequela of zoster infection, cranial nerve herpes zoster may also cause corneal scarring, loss of vision and Bell's palsy with loss of hearing (Ramsay-Hunt syndrome). A recent report suggests that VZV infections may present with clinical deterioration, fever and abnormal liver function tests and evidence of pancreatic involvement, two to five days before a skin eruption becomes apparent (10).

**VZV diagnosis:** VZV rapidly loses infectivity if not transported in suitable media. Swabs of lesions or vesicular fluid aspirates should be put immediately into viral transport medium and inoculated into tissue culture as soon as possible. It is recommended that sucrose phosphate (0.2 M) should be used for transport to preserve the stability of VZV. As with herpes simplex infection, cells scraped from the base of skin lesions may be used for direct immunofluorescence staining for detection of VZV viral antigens. This technique can easily be used for confirmation of diagnosis. Electron microscopy examination of vesicle fluid is used for confirmation of the herpetic nature of skin lesions but cannot separate VZV from HSV infections. Methods for direct viral nucleic acid detection by dot hybridization or DNA amplification have also been developed and are quite sensitive and specific for confirmation of diagnosis. Antibody assays to demonstrate active VZV infection are of limited value in immunosuppressed patients. Most of these patients have reactivated infection. The demonstration of antibody to VZV by a wide variety of tests including complement fixation, fluorescent antibody membrane antigen and enzyme immunoassay is usually helpful in defining populations at risk among immunocompetent patients who are found to be seronegative. In immunocompromised patients, the presence of antibodies to VZV does not indicate a decreased risk for zoster or varicella.

Detection of VZV-specific antigens in biopsy material by immunofluorescence is rapid and reliable. For this purpose, monoclonal antibodies or antiserum specific for VZV is normally used.

**EPSTEIN-BARR VIRUS**

EBV causes heterophil antibody-positive acute infectious mononucleosis in young adults. The pathogenesis of acute infectious mononucleosis involves the replication of the virus in the oropharyngeal epithelium, followed by infection of B lymphocytes and an intense humoral and cellular immune response that results in the classical manifestations of infectious mononucleosis. These include lymphadenopathy, fever and sore throat. Active EBV replication, characterized by virus shedding in saliva, may be prolonged for weeks or months after infectious mononucleosis and during episodes of immunosuppression. EBV is also associated with lymphoproliferative disorders in organ transplant recipients and HIV-seropositive individuals. EBV may
also have an etiological role in nasopharyngeal carcinoma and African Burkitt's lymphoma.

**EBV in the oropharynx:** Oropharyngeal epithelial cells provide the target for the replicative cycle of EBV in acute infectious mononucleosis and in the chronic carrier state (11,12). EBV DNA and RNA can be demonstrated in oropharyngeal epithelial cells by in situ hybridization (13). Infectious virions can be detected by lymphocyte transformation in the saliva of individuals who acquire primary infections with EBV. Several studies have shown that 15 to 27% of EBV-seropositive individuals shed EBV in oropharyngeal secretions (12-14). Other studies suggest that almost all EBV-seropositive individuals shed some virus, with the levels of excretion remaining constant in specific individuals over long periods of time (15). Whether the oropharynx is the source of infectious virions that cause infection and immortalization of B cells remains controversial, but after the administration of acyclovir, the oropharyngeal EBV shedding is inhibited, while the level of B cells infected with EBV is unchanged (16).

**EBV and B lymphocytes:** EBV binding to B lymphocytes occurs by a specific interaction between the major viral envelope glycoprotein gp340 and the cellular receptor for the C3d component of complement (C3dR, also known as CD21 or DR2) (17). EBV-associated diseases, such as Burkitt's lymphoma, infectious mononucleosis and diffuse lymphoproliferative disorders, arise from infection of the B cell lineage. EBV induces normal B lymphocytes to proliferate in vitro and to become activated in long term cell lines that can be propagated in vitro indefinitely (18). The induction of indefinite B cell proliferation in vitro is termed 'transformation' or 'immortalization'. Although infection of lymphocytes by EBV is a necessary step in achieving B cell activation and immortalization (19), it has been shown that the transfer of EBV receptors to EBV receptor-negative cells, which allows the virus to infect cells and express some viral antigens, does not result in immortalization (20). After primary EBV infection of B cells in vivo, up-regulation of human interleukin-10, a potent B cell growth factor, is seen in vitro (21), which may explain the hyperproliferative response of B lymphocytes after infection.

Lymphoproliferative disorders occurring in organ transplant recipients have immunological features that in most cases identify them as polyclonal, and more rarely, as monoclonal in origin. The morphological characteristics exhibited by B cell lymphoproliferation in immunosuppressed patients include diffuse polymorphic B cell hyperplasia and diffuse polymorphic lymphoma (22). Most lymphoproliferative tumours contain EBV (23), and often occur in patients developing a primary EBV infection (24,25). Spontaneous regression often occurs in oligoclonal or polyclonal tumours after the dose of immunosuppressive medications is decreased (25), instead of the usual aggressive and fatal course seen in monoclonal lymphomas. Lymphoproliferative disorders in organ transplant recipients are the result of immortalization of B cells caused by EBV after primary infection, or the proliferation of latently infected cells unchecked by deficient T cell responses.

The analysis of the pattern of EBV gene expression with monoclonal antibodies in B cell tumours from eight organ transplant recipients demonstrated a latent/nonproductive type of infection (27).

**EBV diagnosis:** Diagnosis of primary EBV infections in immunocompetent individuals is based on a typical clinical picture which usually includes a triad of symptoms including sore throat, fever and lymphadenopathy; there may also be a characteristic hematological picture with atypical lymphocytosis and a serological response. Confirmation of the diagnosis of infectious mononucleosis largely depends on the demonstration of heterophil antibodies in a patient's serum. These antibodies are detected just preceding or during the acute phase of infectious mononucleosis, and decrease to undetectable levels six months post infection (28). The production of heterophil immunoglobulin (lg) M antibodies after EBV infection may be explained by the greater susceptibility to EBV infection of cell precursors committed to IgM production than of those committed to IgA or IgG production (29).

**Antibodies to EBV-associated antigen:** The detection of antibodies to specific EBV antigens is useful for identifying the small percentage of individuals who develop heterophil-negative infectious mononucleosis-like disorders caused by EBV, and may also distinguish EBV-induced infectious mononucleosis from malignant diseases having features in common with primary EBV infection. The most useful test for the serological diagnosis of primary EBV infection is the IgM antibody to the viral capsid antigen (VCA). These antibodies appear early during the acute phase of infection, but disappear soon after the acute illness. VCA-IgG is used to determine whether a person has been infected with EBV. VCA-IgG antibodies appear soon after the onset of primary EBV infection, and it is difficult to demonstrate rising antibody titres unless the first serum is obtained very early during the acute phase of the illness. Because these antibodies persist at a high titre for a long period, they have only limited diagnostic value.

IgG antibodies specific for EBV early antigen are detected during acute or active EBV infection (30). Two types of early antigens have been described. The first is the 'diffuse' or 'D type' and is detected in both the nucleus and cytoplasm of infected cells. The second early antigen is termed 'restricted' or 'R type' and is detected only in the cytoplasm. Antibodies to the D type are detected in 70 to 80% of patients with acute EBV infection. These antibodies appear early during acute illness and decrease to undetectable levels during the convalescent phase of the illness. There are obvious limitations to the use of early antigen IgG (EA-IgG)
antibodies in the diagnosis of active or reactivated EBV infections. Immunosuppressed patients and some nonimmunosuppressed individuals do not demonstrate EA-IgG antibodies during active EBV infection (24). Some asymptomatic individuals who do not have detectable EBV in throat washings demonstrate persistently high titres of EA-IgG antibodies (12).

EBV-induced nuclear antigens (EBNA) were initially demonstrated using the anticomplement immunofluorescence procedure. Antibodies against EBNA are detected weeks after the primary infection. There are six distinct EBV nuclear antigens (EBNA-1 to EBNA-6). Following infectious mononucleosis, antibodies to EBNA-2 are the first to appear, reaching peak titres after several weeks, and then declining to low or undetectable levels. Antibodies to EBNA-1 emerge several weeks or months after anti-EBNA-2 and gradually rise and persist indefinitely.

Detection of EBV infection by tissue culture: The 'gold standard' for identification of viruses in clinical specimens is tissue culture, whereby infectious EBV virions are detected by their ability to cause transformation of B lymphocytes. Infection of lymphocytes may then be confirmed by detection of EBNA using immunofluorescence. EBV is detected in oropharyngeal secretions of patients with primary infections, in EBV-seropositive immunosuppressed patients and in some asymptomatic EBV-seropositive individuals. Ten to 60% of asymptomatic EBV-seropositive individuals and 90 to 100% of heterophil-positive infectious mononucleosis patients shed EBV in the oropharynx, as detected by the lymphocyte transformation assay. There are several factors which influence the detection of EBV by the lymphocyte transformation assay, including: EBV serostatus of the B cell donor; transforming activity of the EBV strain; history of the previous EBV infections; time since a primary EBV infection; integrity of the immune system; and in vitro conditions used in the assay. Several important factors should be considered when lymphocyte transformation is used to detect EBV. First, cord blood lymphocytes or lymphocytes from EBV-seronegative donors should be used for the assay because lymphocytes from EBV-seropositive individuals may contain EBV-infected cells or memory T cytotoxic cells specific for EBV-infected cells, which may inhibit the outgrowth of transformed lymphocytes. Second, transformation defective EBV strains may not be detected by this system. Sixbey et al (13) found that approximately half the individuals in whom EBV DNA was demonstrated in oropharyngeal secretions were negative for EBV by the lymphocyte transformation assay, and that most of the EBV DNA-positive, lymphocyte transformation-negative individuals were infected with an EBV variant that was transformation defective. There is some evidence that this transformation defective variant, or type 2, virus is widespread in some parts of North America (13). Oropharyngeal shedding of EBV may persist at low levels in asymptomatic individuals for weeks or months after an initial episode of infectious mononucleosis. The present author has followed patients who excrete EBV for up to three years after infectious mononucleosis. Asymptomatic EBV-seropositive patients who are immunodeficient have a high rate of EBV excretion. The levels of oropharyngeal excretion of EBV may have a predictive value at the onset of illness in immunosuppressed individuals. Theoretically, lymphocyte transformation could detect as few as 30 virions, since one in 30 virions is infectious (31). Nevertheless, individuals excreting low or high levels of the virus will not be differentiated by lymphocyte transformation unless a limiting dilution assay of the transformed cells is used to quantify the level of virions excreted. This assay would not take into account the degree of transforming activity of different EBV strains.

Detection of EBV infection by hybridization and by DNA amplification: Nucleic acid hybridization is a sensitive and specific method for demonstrating virus-specific DNA in clinical samples (12,24). Unlike lymphocyte transformation assay, the nucleic acid hybridization system may detect noninfectious viral DNA and nontransforming variants with EBV. In addition, clinical specimens under long term storage for longitudinal studies may be analyzed for the presence of viral DNA (32). DNA amplification also allows for a detailed analysis of the genomic structure of EBV.

Early studies established that there is at least a 90% homology between EBV DNA of different origins (33). The areas of heterogeneity include the sequence of the EBNA-2 gene which has been useful in the typing of EBV isolates. Striking differences in the EBNA-2 encoded region have been found in isolates from Central Africa and strains isolated from North America. EBV isolates may be of two types according to the structure of the EBNA-2 gene or protein: type 1 or A, and type 2 or B. EBNA-2A strains have been found in healthy EBV-seropositive individuals in the United Kingdom and Australia (34). In contrast, EBNA type 2B has been found in up to 40% of Burkitt's cell lines in the peripheral blood lymphocytes of 20% of adults in Africa (34,35), and in 40% of patients with HIV infection (36). Amplification by polymerase chain reaction of the EBNA-2 gene can be used to characterize EBV isolates based on the high divergence of both the protein and DNA levels between the type 1 and type 2 variants. Type 2 transforms B cells less efficiently than type 1. In contrast to previous reports (25), Sixbey et al (13) found that infection by the type 2 variant is widely distributed.
in healthy EBV-seropositive individuals in North America. In their study of 34 individuals who excreted EBV DNA in throat washings, 41% were shedding type 2 virus, 50% were shedding type 1 and 9% were infected by both strains of the virus. In comparison, we found that of 34 organ transplant recipients who shed EBV DNA in the oropharynx, 79% shed EBV-1, 11.8% shed EBV-2, and 8.8% shed both EBV-1 and 2 (25), in contrast to our findings in lymphocytes of HIV-seropositive individuals in whom only type 2 strains were demonstrated, despite the prevalence of both types 1 and 2, in oropharyngeal epithelial cells (37).

**EBV infection in organ transplant recipients:** Organ transplantation is a frequent form of treatment that requires immunosuppressive therapy and puts the patient at risk of developing opportunistic infections and de novo cancers. The risk of neoplastic disease is increased approximately 100-fold in organ transplant recipients, but it varies with the degree of immunosuppression, the immunosuppressive regimen employed, and the nature of the allograft. Except for skin cancers, the most common malignancies in this population are the lymphoreticular cancers associated with EBV. High titres of EBV-specific antibodies are frequently demonstrated in patients with immune deficiency (38). The high titres of EBV-specific antibodies probably reflect an increased activation of the virus in immunodeficient states. In contrast, many patients with severe EBV infections are immunodeficient and do not exhibit the typical clinical or immune responses that are present in normal individuals. For instance, some immunodeficient patients do not develop heterophil antibodies or EBV-specific IgM antibodies after primary infection (27,38), and tests necessary for diagnosis, such as nucleic acid hybridization or lymphocyte transformation, are not generally available. In one study, 50% of organ transplant recipients developing primary EBV infection did not have a specific IgM response to EBV VCA (25).

Individuals who undergo iatrogenic immunosuppression, such as organ transplant recipients, patients with congenitally acquired immunodeficiency and HIV-seropositive individuals, are prone to developing active EBV infections and EBV-associated diseases such as hepatitis, encephalitis, bone marrow suppression, hemorrhage, pneumonitis, further immunosuppression resulting in opportunistic infections, and lymphoproliferative disorders (23). After renal transplantation, 1 to 13% of patients develop lymphoproliferative disorders (23).

We recently analyzed the quantitative oropharyngeal EBV shedding measured by a dot blot assay. The genotypes of isolates determined by DNA amplification were studied in 23 renal and 23 cardiac recipients over the first post-transplant year. In this population, five patients developed lymphoproliferative disorders and two additional patients with B cell tumour and renal transplantation were studied from the time of diagnosis. A significantly high level of EBV was found in primary versus reactivated infections. Higher levels of EBV were also found in patients who developed lymphoproliferative disorders than in patients who did not. Patients with the highest EBV shedding had the poorest serological responses, suggesting that patients with high viral antigen load had defective humoral responses to EBV. All patients who developed lymphoproliferative disorders and those without lymphoproliferation shed EBV type 1. In the same study, during the first post-transplant year, EBV excretion was detected at one or more sampling times in 82% of 23 renal and 95% of 23 cardiac and heart-lung transplant recipients by the dot blot assay. When both dot blot and DNA amplification were used, EBV was detected in 91% of renal and 96% of cardiac and heart-lung transplant patients. Analysis of the quantitative oropharyngeal EBV shedding in this population demonstrated two subgroups of individuals: EBV-seropositive patients who excreted less than 100 pg of EBV DNA per microgram of human DNA and were more likely to be intermittent shedders, and patients who demonstrated more than 1000 pg of EBV DNA per microgram of human DNA who were more likely to be persistent shedders.

The peak value of EBV shedding observed in individual patients in the first six months after transplantation was used to compare patients' groups. There was no significant statistical difference between renal and cardiac patients. Patients experiencing primary EBV infection had higher peak levels of EBV than patients seropositive before transplantation. Patients who received antithymocyte globulin or OKT3 antibody for induction or rejection therapy shed significantly higher peak levels of EBV than those who received neither therapy.

A fall in anti-EBNA IgG titre was observed in 15% of seropositive patients. Two of the seven patients who developed lymphoproliferative disorder were EBV-seropositive before transplantation; these two patients did not have significant serological responses to EBV at diagnosis.

The incidence of lymphoproliferative disorders after solid organ transplantation varies from centre to centre, and depends largely on the serological EBV status before transplantation and on the immunosuppressive regimen used. At the University of Alberta Hospital, lymphoproliferative disorder has been diagnosed in 11 transplant recipients: four of 62 (2.5%) receiving cadaveric kidneys, five of 69 heart recipients (7.2%), and two of 10 heart-lung or lung transplant recipients (20%). Patients who developed lymphoproliferation also developed significantly higher peak levels of EBV than those who did not. However, this reflects the high incidence of primary EBV infections in this population. Of seven patients who developed primary EBV infection after transplantation, five developed lymphoproliferative disorders. The two renal and cardiac transplant recipients who experienced primary EBV infection, but
did not develop lymphoproliferation, had symptomatic primary and reactivated CMV infection at eight and seven weeks after transplantation, respectively. Both were treated with a reduction immunosuppression and the cardiac transplant recipient also received a two-week course of ganciclovir therapy, which may explain the absence of lymphoproliferative disorders.

The role of the oropharyngeal epithelium in the outgrowth of transformed B cells resulting in B cell tumours in immunosuppressed patients remains unknown. Antiviral therapy at diagnosis has achieved only limited success. It is possible that prophylactic antiviral therapy initiated immediately after transplantation could prevent high peak EBV levels and perhaps decrease the level of EBV transformed B cells and the risk of developing lymphoproliferation. Patients who are EBV-seronegative before transplantation and receive an organ from an EBV-seropositive donor, or blood products from an EBV-seropositive donor, are at high risk of developing invasive B cell tumours, and, therefore, should be followed more closely for signs of lymphoproliferation.

REFERENCES


