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INFECTIOUS DISEASES

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GRAND ROUNDS

MAY 12, 2005

VIRAL ISSUES IN THE

SOLID ORGAN TRANSPLANT PATIENT

BIOGRAPHICAL INFORMATION

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ACADEMIC INTERESTS ARE CENTRALIZED IN THE AREA OF THE IMMUNOCOMPROMISED HOST, WITH SPECIAL INTEREST IN THE TRANSPLANT RECIPIENT

"This is to acknowledge that Suzanne Wada, M.D. has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Wada may be discussing "off-label" uses in her presentation."

Overview:

Solid organ transplantation is a cost-effective and viable medical modality for a variety of end-stage organ diseases. Despite the success of living donor allografts (Figure 1)

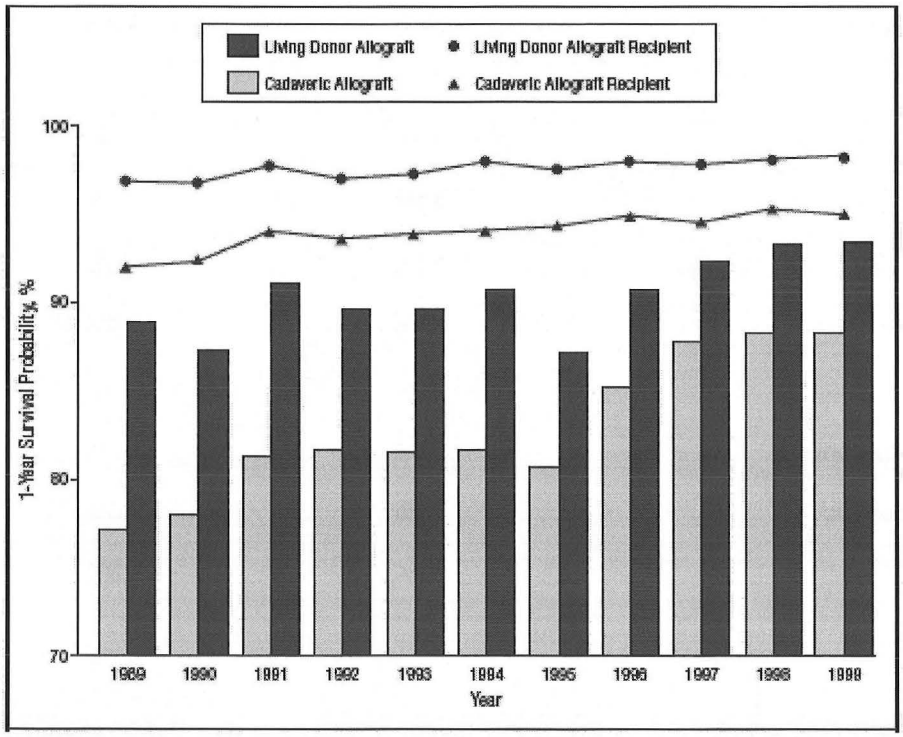

Figure 1. One-year survival probabilities for first cadaveric and living donor allografts and their recipients, adjusted for age, sex, race, and primary diagnosis. Despite impressive increases in cadaveric allograft survival, living donor allograft survival is consistently superior. Source: US Renal Data System 2002 Annual Data Report.

(1), transplantation rates are limited by lack of organ donation and viable organs. Current statistics are listed in Table 1 (2) and Table 2 (2).

Unos Data

Total waiting list	90k
Kidney	61k
Pancreas	2k
Kidney/Pancreas	2500
Liver	17k
Intestine	186
Heart	3k
Lung	4k
Heart-Lung	200

Table 1

Graft Survival

	1-Year	5-Year
Kidney		
Living donor	94%	76%
Cadaveric	88%	63%
Liver		
Living donor	76%	73%
Cadaveric	80%	63%
Pancreas (with kidney)	84%	69%
Heart	84%	68%
Lung		
Living donor	72%	57%
Cadaveric	76%	40%
Intestine	66%	20%

Table 2

Organ transplantation continues to push the limits of modern medicine in transplantation occurring in older, sicker patients; as well as the use of marginal organs. (Transplantation in older, sicker patients and the use of marginal organs mandates its own infectious disease issues as will be discussed.) The marginal organ is described in Table 3 (1).

The Marginal Donor

Non-heart-beating status
Age>60 Years
Hypertension
Diabetes Mellitus
Hepatic C
Renal Disease
Prolonged Perioperative Ischemia
Abnormal renal biopsy finding at time of organ retrieval

Table 3

Transplantation and its infectious diseases issues are a dynamic, continually evolving field. This lecture and protocol, hopefully, illustrate the inherent complexity but also the logic that is involved with infectious disease issues in the solid organ transplant recipient. Long-term graft survival is improving but remains inadequate (1). For example, the principle causes of renal allograft loss beyond the first posttransplantation year are shown in Figure 2 (1) (patient death is the principle cause of loss of a functioning graft) (1). The main cause of death remains cardiovascular followed by infection and malignancy (1).

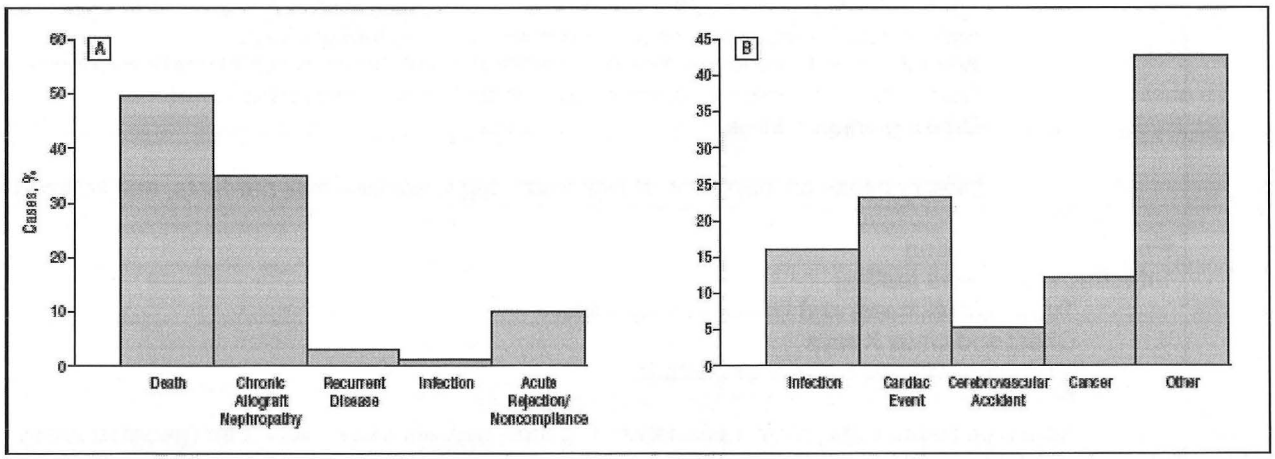

Figure 2. A, Causes of allograft loss. B, Causes of death from postransplantation years 1 through 5. Adapted with permission from Cecka.[74]

Transplant infectious disease issues begin well before any particular solid organ transplant physically occurs. Both the donor and recipient's infectious disease history potentially contribute to the future health of the donor and the success of a functioning allograft. Both the donor and recipient are tested for a variety of infectious disease markers. At the Mayo Clinic, transplant candidates are evaluated by an Infectious Diseases subspecialist. The history focuses on past infections and any unusual exposures. Table 4 (3) represents the pretransplantation infectious disease evaluation

History

Immunosuppressive therapy: type and duration (current or past)
Antibiotic allergies probable or documented
Past medical history: infectious diseases
- Oral: dental caries, sinusitis, pharyngitis, HSV infection
- Respiratory: pneumonia, tuberculosis
- Cardiovascular: valvular heart disease, heart murmur (need for endocarditis prophylaxis)
- Gastrointestinal: diverticulitis, diarrheal disease, hepatitis A, B, or C, intestinal parasitic infection
- Genitourinary: urinary tract infectious, prostatitis, vaginitis, genital herpes, genital warts, syphilis, gonorrhea, pelvic inflammatory disease, chlamydial infection
- Cutaneous skin and nail infectious, varicella, and zoster
- Osteoarticular: osteomyelitis, prosthetic joint(s)
- Childhood illnesses: chicken pox, measles, rubella
- Other: mononucleosis, other infectious diseases not included above

Exposure history
- Travel history: prior residence in or travel to areas associated with the geographically restricted endermic mycoses and/or parasitic disease, especially *S. stercoralis*, malaria, etc.
- Tuberculosis exposure, prior tuberculous skin testing, chest X-ray abnormality
- Risk factors for blood-borne pathogen infection (including HIV)
- Animal and pet exposure (including vaccination status of pets); *Brucella* exposure
- Occupational exposure: farming, animal husbandry, gardening
- Drinking-water source
- Exposure to young children
- Dietary habits consumption of raw meat, unpasteurized milk products, and seafood

Physical examination

Infectious-diseases testing

Tuberculin skin test and limited anergy panel
Chest and sinus X rays
Urine analysis and culture for bacteria
Stool culture and examination for ova and parasites
Serologic tests: CMV, VZV, EBV, HSV, *T. gondii*, syphilis, HBV, HCV, HIV (geographically restricted endemic mycosis if history of exposure present

Vaccinations

Tetanus-diphtheria (update)
Influenza
Pneumococcus
Hepatitis B
H. influenzae type b (pediatric patients)
Inactivated polio vaccine

Table 4. Pretransplantation infections diseases evaluation

performed at the Mayo Clinic. Especially noteworthy are the serological testing and the various vaccines administered well before the actual transplantation. The serological testing reveals the possibility of past exposure/infection or disease to the listed pathogens.

Despite all the advances in transplantation medicine (including advances in surgical technique, transplantation biology and the use of immunosuppressive agents), infection causes significant morbidity and mortality. The optimal approach to infection is prevention; however, failing this, prompt and aggressive diagnosis and therapy is paramount. The sources of infectious agents posttransplantation include: endogenous organisms, the allograft itself and the environment (3). A guiding principle to consider in evaluation of a transplant patient is that the signs and symptoms of infection may be blunted in part by the immunosuppressive therapy, and that the usual diagnostic techniques may be inadequate. Therefore, aggressive and often invasive investigations of seemingly minor symptoms may be warranted.

In general, I think we are fairly successful in the diagnosis and subsequent management/ treatment of various bacterial and fungal pathogens that infect and cause disease in these chronically immunosuppressed solid organ transplant recipients. Robert Rubin's classic timetable (Figure 3) (4) for various infectious diseases in the (renal) organ transplant recipient is quite helpful. However, innumerable factors can affect this timetable such as: basic donor and recipient health, prior donor and recipient infectious diseases, the technical success of the operation, the chosen immuno-suppressive regimen, rejection and subsequent treatment, the environment, etc.

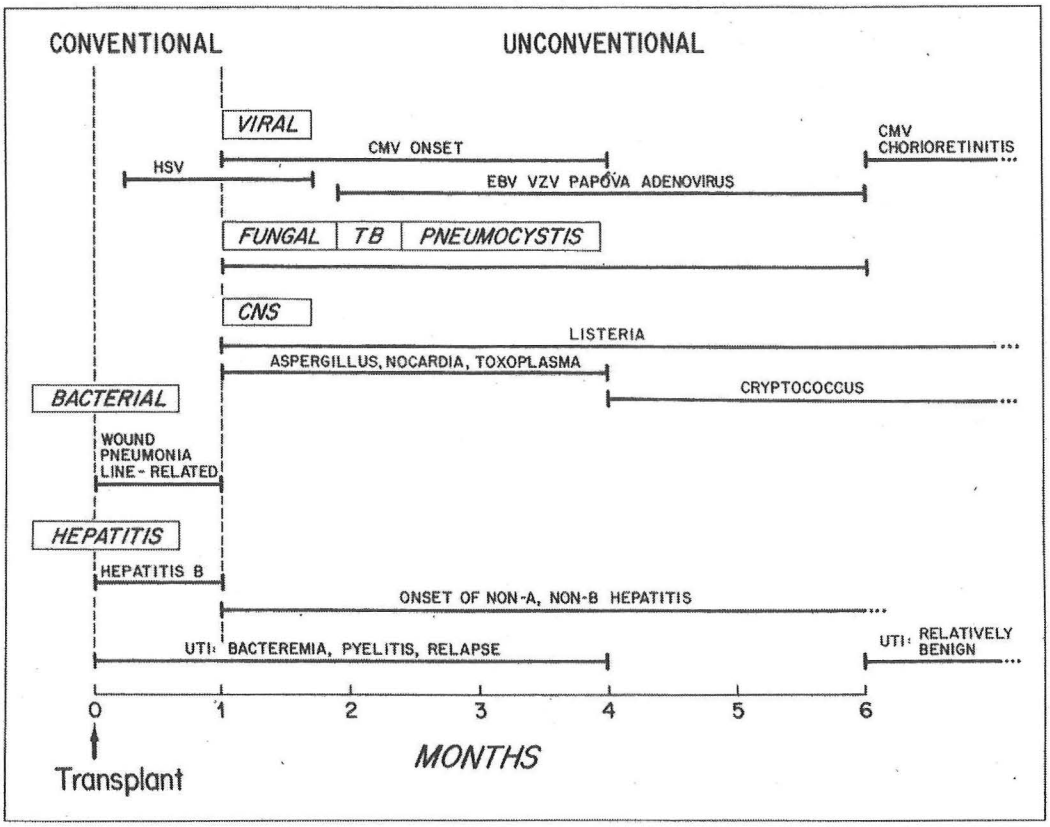

Figure 3. Timetable for the occurrence of infection in the renal transplant patient.

Kusne, et al, have demonstrated (Figure 4) (5) the increased frequency of severe infections in relation to time spent in the operating room in liver transplant surgery.

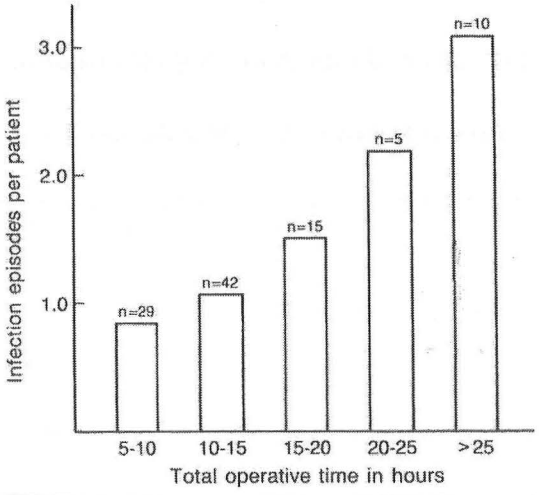

Figure 4. Frequency of severe infections in relation to total operative time per patient in hours.

A small percentage of infections are transmitted via the allograft. Cultures are obtained from both the donor and the recipient at the time of transplantation to help guide peri- and postoperative antimicrobial therapy. Bacterial or fungal infections in either donor or recipient commonly seek the allograft, especially at the vascular suture lines, leading to the formation of the mycotic aneurysm and possibly catastrophic rupture (25). In lung transplantation, the bronchi of the donor are cultured. The organisms cultured from the donor's bronchi are taken into consideration in guiding peri- and postoperative antimicrobials.

Notable during the first month posttransplantation is the usual absence of typical opportunistic pathogens. Although, the amounts of immunosuppressive drugs administered are the greatest during this period, the main determinant of the net state of immunosuppression which dictates infection risk, is the level of sustained immunosuppression rather than the short-term effects of a particular immunosuppressive regimen (25).

The Herpes Virus Family:

The human herpes viruses are listed in Table 5. Humans are infected with the majority

Human Herpes Virus Family

HERPES VIRUS	CLINICAL MANIFESTATION
Herpes Simplex type 1	Cold sores
Herpes Simplex type 2	Genital herpes
Varicella-zoster virus	Chickenpox/Shingles
Cytomegalovirus	Mononucleosis/pneumonia/hepatitis/etc.
Epstein-Barr virus	Infectious mononucleosis, PTLD
Human Herpes virus 6	Roseola infantum/exanthema subitum/sixth
Human Herpes virus 7	Febrile illness
Human Herpes virus 8	Kaposi Sarcoma

Table 5

of these viruses early in life; infections can be asymptomatic to an acute viral illness with systemic signs and symptoms. Once infection or disease occurs, the virus becomes dormant in the human body, only to reactivate and, at times, cause significant disease during states of significant stress and immunosuppression. Perhaps no organism causes as much morbidity and some mortality as the herpes virus: cytomegalovirus (=CMV). Overall, lung and heart-lung transplant recipients are at highest risk for CMV disease: liver or heart transplant recipients have an intermediate risk, and kidney transplant recipients are at the least risk (6).

CMV is the single most important infectious agent affecting recipients of organ transplants with at least 2/3 of these patients having CMV infection 1 to 4 months after transplantation (7). In the United States, at least 50% of adults are seropositive for CMV (i.e. CMV IgG+) and, thus, harbor latent virus. During systemic CMV disease, the virus is found in a variety of white blood cells. In clinical transplantation, CMV can be transmitted to the transplant recipient via the donor organ. CMV can be found within the cells of the allograft (i.e. hepatocytes, renal tubular/glomerular/peritubular capillary endothelial cells of a kidney) or be present in leukocytes within an allograft.

In addition to CMV's latent state, CMV is spread from cell to cell, with direct contact among the cells being of critical importance, thus rendering neutralizing antibody inefficient, and cell mediated immunity critical in controlling the infection (7).

There are 3 major clinical patterns of CMV infection in the solid organ transplant recipient: they are primary infection, reactivation and superinfection. (The newly-described

human herpes viruses; human herpes virus 6 and human herpes virus 7, may play a critical role in the pathogenesis of CMV, as will be discussed.)

Primary CMV infection occurs when a transplant recipient, who is seronegative for CMV, becomes infected with CMV carried latently in the cells from a CMV seropositive donor (7). In kidney transplantation, the source of the CMV latently infected cells in 80 to 90% of transplant recipients is the kidney from the CMV seropositive donor. In the remaining 10 to 20%, the source is viable leukocyte containing blood products from CMV seropositive donors (7). In addition, investigators believe that passenger leukocytes within an allograft are a major source of latent CMV. The risk of transmission of CMV via blood products is decreased with the use of leukocyte poor blood (i.e. leukocyte filtered blood products).

In kidney transplantation, investigators have found that transmission of CMV via CMV seropositive donors to CMV seronegative transplant recipients is increased when organs from cadaveric donors are used vs. organs from living related donors (7). Why this is so is, as yet, unclear. (As mentioned, allografts from living, related donors have greater success rates.) In addition, only a subset of CMV seropositive donor organs are capable of transmitting CMV. For instance, when two CMV seronegative kidney recipients receive a kidney from the same CMV seropositive cadaveric donor, either both recipients develop primary CMV infection or neither develops it (7).

Data from heart, heart-lung and pancreas-kidney CMV seronegative transplant recipients also identifies the allograft from CMV seropositve donors as the major source

of CMV that causes primary CMV disease. In liver transplant recipients, immense quantities of blood products contribute to the risk of CMV transmission; however, the CMV seropositive donor allograft is still the primary culprit in transmission of CMV.

In a report on 218 liver transplant recipients at the Mayo Clinic by Marin, et al, (Figure 5) (8), it was shown that CMV infection and disease incidence was directly related to the donor and recipient's CMV serological status at time of transplantation (8).

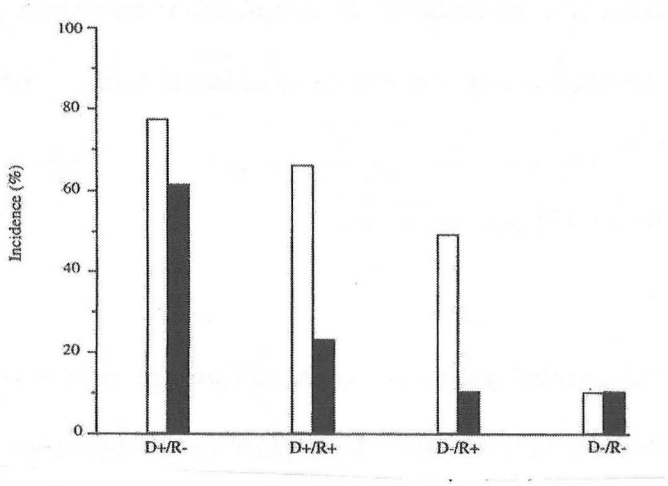

Figure 5. Incidence of CMV infection *(open bars)* and CMV disease *(solid bars)* after liver transplantation, according to pretransplantation CMV serological status of donor *(D)* and recipient (R).

In reactivation infection, the transplant recipient has been infected with CMV previously (and is seropositive for CMV prior to transplantation); reactivation of endogenous latent virus occurs (7). The degree of reactivation determines the extent of clinical manifestations due to CMV.

In CMV superinfection, a CMV seropositive individual receives an allograft from a CMV seropositive donor; however, the virus strain that is activated is of donor rather than

recipient origin (7). In as many as 50% of kidney transplant recipients who are CMV seropositive before transplantation and who receive a kidney from a CMV seropositive cadaveric donor, the virus that is activated is of donor rather than endogenous origin (7). In addition, transplant recipients are more likely to manifest clinical disease with superinfection rather than with reactivation infection.

The incidence of symptomatic clinical disease is different for these 3 forms of CMV infection. At least 2/3 of patients with primary infection will develop symptomatic disease; less than 20% of those with evidence of endogenous viral reactivation become symptomatic; and perhaps as many as 40% of those with superinfection become symptomatic (7).

These 3 modes of transmission account for > 90% of CMV infection in organ transplant recipients. Occasionally, a CMV seronegative recipient who received a CMV seronegative organ will develop primary CMV infection months after transplantation through sexual transmission or blood transfusions (7). The different epidemiologic patterns may also reflect the fact that human CMV isolates in nature have long been known to exhibit considerable genomic and antigenic heterogeneity and that recent studies have shown that different CMV strains are biologically and clinically important (7).

Pathogenesis of CMV in Organ Transplant Recipients:

The single most important exogenous factor in reactivation of CMV, regardless of the latent state, is the kind and intensity of the immunosuppressive therapy administered (7). Of all the immunosuppressive agents used, biologic agents (i.e. T lymphocyte

monoclonal antibody [OKT3], antithymocyte globulin [ATG], anti-lymphocyte globulin [ATG]) appear to have the greatest effect on reactivation of latent virus. Antilymphocyte preparations such as OKT3 are very potent reactivators of CMV (8).

High dose steroids, ATG, ALG, OKT3, and possibly mycophenolate mofetil delay and dampen CMV-specific, cell mediated and humoral immune responses and lead to uncontrolled CMV replication and disease (9). Cyclosporine has minimal effect on reactivation of latent CMV virus, but interferes with the ability of the host to control infection (7). Patients who receive cyclosporine alone or cyclosporine plus low-dose prednisone have significantly fewer problems with CMV infection than do patients who receive regimens that add antilymphocyte globulin to cyclosporine (7).

Other risk factors for CMV reactivation, besides the immunosuppressive and biologic agents discussed, include: allograft rejection, allogeneic stimulation, viral coinfections with human herpes virus 6 and 7, stress associated with critical illness, intra-abdominal infections, sepsis, and physiologic events (i.e. intraoperative hypothermia) (9). Characteristic of these factors and conditions include the production and secretion of high levels of cytokines, including tumor necrosis factor-alpha (9). The proinflammatory cytokine TNF-alpha strongly upregulates the CMV immediate-early (IE) enhancer and/or promoter activity via the TNF-alpha 1 receptor, with the subsequent activation of protein kinase C and nuclear factor-kB (NF-kB); the activated NF-kB translocates into the nucleus and binds within the CMV IE enhancer region, consequently stimulating CMV replication (9).

CMV interacts with the host immune response in noteworthy ways. CMV is felt to be an immunomodulating virus (7). CMV causes a metabolic abnormality in lymphocytes and monocytes that impairs their ability to produce and to respond to cytokines such as interleukin1 and interleukin 2 (7). CMV appears to suppress the functioning of antigen-specific cytotoxic T-lymphocytes (7). In addition, CMV causes a change in the circulating T cell subsets, with a decrease in CD4 cells and an increase in CD8 cells, a finding that correlates with a decrease in cell-mediated immunity (7). Thus, CMV has direct immunosuppressive effects on the host's immune system (8). Consequently, CMV is a predisposing risk factor for bacterial and fungal infections following liver transplantation (8). Donor CMV seropositivity is an independent risk factor for bacteremia in liver transplant recipients (10).

The transplant recipient's cell-mediated immune response to the virus is critical. Clinical recovery is contingent upon virus specific cytotoxic T cells to destroy CMV infected cells. (In fact, infusion of cloned CMV specific cytotoxic T-lymphocytes (CTL) is being explored in bone marrow transplantation [11]). In the bone marrow transplant literature, a series of studies has shown that specific cytotoxic T lymphocytes cloned in vitro can be given safety to patients and that cytotoxic anti-CMV activity can be detected during follow-up (6).

Clinical Manifestations of CMV:

CMV causes a multitude of clinical syndromes. In the transplant recipient, the allograft is often the initial site of CMV infection.

Clinical CMV infection can begin insidiously with a nonspecific prodome of fever, malaise, myalgias, arthralgias and anorexia (7). Prolonged fever may be the only manifestation of CMV infection (7). In the solid organ transplant recipient, CMV accounts for the majority of febrile episodes in the 1 to 4 month period posttrans-plantation.

In transplant recipients who develop CMV-related fever, 20 to 30% will develop CMV pneumonia (7). CMV pneumonia has similar signs and symptoms as Pneumocystis Carinii pneumonia (PCP). In fact, it is not uncommon to find these two diagnoses (i.e. CMV pneumonia and PCP) concomitantly in immunocompromised patients.
In CMV pneumonia, the patient has a fever and develops a nonproductive cough. Progressive respiratory distress can ensue over several days. Auscultation of the lungs can be unrevealing even in the presence of full blown pneumonia (7). There is much variability in the radiologic appearance of CMV. The most common appearance is that of a bilateral, symmetric peribronchovascular process predominantly affecting the lower lobes (7).

CMV can affect the bone marrow, sometimes dramatically. One may find an atypical lymphocytosis, leukopenia, anemia and thrombocytopenia.

Mild to moderate hepatitis with abnormal liver function is seen in 30 to 50% of solid organ transplant recipients with systemic CMV infection (7). CMV hepatitis is rarely a problem in kidney and cardiac transplant recipients (7). However, in liver transplant recipients, CMV hepatitis is a major issue. In liver transplant recipients, the only way to

distinguish rejection from viral infection as the cause of hepatocellular dysfunction is by liver biopsy (7). Presence of CMV within the liver can be demonstrated by finding typical CMV inclusions within the liver parenchyma.

CMV myocarditis can be an important cause of myocardial dysfunction in cardiac transplant recipients. The allograft, both as foreign tissue and as the site of reactivation, is the primary target for CMV-induced injury.

CMV gastroenteritis has emerged as an important manifestation of CMV in the transplant recipient. Gastrointestinal hemorrhage caused by ulcerations in the gastrointestinal mucosa occurs. Cells bearing the typical CMV inclusions can be recognized within these ulcerations. Occasionally, these ulcerations go on to perforate. The right colon, particularly at the cecal level, is the usual site for these lesions, but the stomach, esophagus and proximal small bowel can also be affected (7). CMV-associated gastrointestinal lesions can appear in the absence of other manifestations of CMV disease (7).

Other less common manifestations of CMV infection include esophagitis, encephalitis, transverse myelitis and vasculitis. The major late manifestation of CMV infection is chorioretinitis, which first becomes manifest greater than 6 months after transplantation (7).

Not only does CMV itself cause disease, but more importantly, it predisposes the transplant patient to life-threatening superinfection with a variety of microbial agents includ-

ing gram negative bacilli, Listeria monocytogenes, P. carinii, Aspergillus species, Cryptococcus neoformans and Candida species due to CMV's enhanced immunosuppressed state (7). Patients with CMV infection after solid organ transplantation have a higher incidence of invasive fungal disease (9). Investigators found the incidence of fungal infection following heart transplantation reduced among patients who received effective ganciclovir prophylaxis (9). Several studies have demonstrated that CMV infection increases the risk for other opportunistic infections (6).

The most controversial aspect of CMV infection in transplantation is whether CMV plays a role in allograft rejection (7). Early onset allograft rejection is significantly higher among CMV infected kidney transplant recipients (9). Richardson, et al, described a glomerular lesion in kidney allografts associated with CMV viremia (12). Patients with this glomerulopathy responded poorly to classic antirejection therapy. It has been proposed that CMV infection is associated with the production and release of interferons and with an upregulation in MHC antigens in the graft. This glomerolopathy may represent an unusual form of allograft injury due to cytokine-induced upregulation of MHC antigens on donor glomerular cells followed by the host immune attack (7).

Studies suggest an association between CMV and accelerated arteriosclerosis, particularly among heart transplant recipients; CMV infects endothelial cells influencing smooth muscle cell migration and growth in vitro and induces neointimal proliferation in the rat model (9). Infection with rat CMV results in endothelial activation (inflammation) and subsequent intimal thickening in aortic and cardiac allografts; ganciclovir diminishes or abolishes this intimal inflammatory process (9).

Stanford University's cardiac transplant program has reported that, with CMV infection, there is both an increase in early allograft rejection and an increase in the incidence of graft atherosclerosis (13). An association between CMV and left ventricular dysfunction has been reported in heart transplant recipients (9). In addition, CMV has been associated with bronchiolitis obliterans in patients undergoing lung transplantation (8); the incidence of which ganciclovir prophylaxis has reduced (9).

Viral coinfections are common after solid organ transplantation (9). The interactions among the betaherpesviruses (i.e. CMV, HHV-6, HHV-7) may manifest as increased severity of CMV disease (9). Some authors report that CMV accelerates the course of hepatitis C virus infection, resulting in increased cirrhosis, need for retransplantation, and mortality after liver transplantation (9). A 2002 Mayo Clinic study of 92 HCV infected liver transplant patients reported that CMV is a key pathogen that influences HCV pathogenesis; that CMV reactivation was highly predictive of mortality regardless of whether it remained subclinical or evolved into CMV disease (14).

Table 6 summarizes the direct and indirect clinical effects of CMV (9).

Clinical Effects of CMV

Direct Clinical Effects	Indirect Clinical Effects
Virus CMV Syndrome	Increased bacterial infections
End-organ diseases	Increased fungal infections
Hepatitis	Influences hepatitis C pathogenesis
Gastrointestinal Disease	Betaherpesvirus interactions
Encephalitis	Graft dysfunction and rejection
Pneumonitis	Accelerated atherosclerosis
Nephritis	Patient Mortality
Retinitis	
Betaherpesvirus interactions	
Graft dysfunction and rejection	
Patient mortality	

Table 6

CMV Diagnosis/Management/Treatment:

Table 7 lists the major diagnostic methods used in making the diagnosis of CMV.

CMV Diagnosis

Serological Testing
Viral Culture
Histopathologic Diagnosis
Antigenemia Assay
Nucleic Acid Amplification

Table 7

Serological testing is useful for pretransplantation assessment of the recipient's risk for CMV infection/disease. In general, the development of CMV IgM antibodies or a fourfold rise in IgG titers over time indicates acute infection (8). Serologies, however, are insensitive in immunocompromised patients and have limited clinical usefulness in the acute care setting because of the prolonged time for confirmation of the diagnosis (8).

Isolation of CMV from tissue or body secretions is the "gold standard" against which other tests are compared (8). Conventional viral cultures require a long time (i.e. several days) to confirm the viral cytopathic effect on fibroblast cultures, and viral recovery may be further delayed when viral replication is low (8). The shell vial assay can detect the presence of virus in 12 to 24 hours. This method uses a monoclonal antibody to detect a 72 kD antigen of CMV in urine, blood or throat cultures performed on flat monolayers on coverslips in shell vials (8). The assay is widely available, rapid and specific for CMV infection (8).

Histopathologic diagnosis of tissue invasive CMV is based on the presence of characteristic intranuclear inclusions (Cowdry type A) in enlarged cells with a prominent nuclear rim, giving the typical "owl's eye" appearance (8).

CMV infects leukocytes. The antigenemia assay detects late structural protein pp65 produced in peripheral blood leukocytes by the use of a monoclonal antibody against the CMV matrix protein (pp65). The number of positively stained leukocytes appears to be an indication of viral load and disease severity as well as a helpful marker for the monitoring of infection and the patient's response to therapy (8). The antigenemia assay is mostly applied to blood and cerebrospinal fluid with a sensitivity of 89% and a specificity of 100% (15).

In solid organ transplantation, a relatively small number of antigen positive cells (i.e. less than 10 antigen positive cells per 50,000 polymorphonuclear cells) generally indicates asymptomatic infection, whereas a larger number (>50 antigen positive cells per 50,000 polymorphonuclear cells) indicates a 60% likelihood of CMV disease (8). The positive predictive value of the antigenemia assay in detecting disease, however, is not absolute, meaning that some patients with low antigen levels may have severe disease and that some patients with high antigenemia levels may remain asymptomatic (11). Despite some limitations, the antigenemia assay alone, or in combination with shell vial cultures, is of significant value in detecting and monitoring CMV. Numerous studies indicate that the antigenemia assay provides a good estimate of the systemic CMV burden (16).

One can get a rapid result with PCR and the antigenemia assay. CMV dissemination in the blood occurs during active infection, and viremia has been recognized as the major virologic risk factor for the progression to clinical disease (16). Thus, quantitation of the systemic CMV load may provide a highly sensitive and specific method to predict the development of CMV disease (16). PCR is extremely sensitive and specific in detecting viral DNA. Its ability to detect very few DNA copies raises concern, however, that a positive signal may not differentiate between a replicating virus vs. a latent virus (8). Quantitation of CMV DNA in the blood and urine via PCR has been studied (9). High levels of CMV DNA in these fluids correlate with the presence of CMV disease (9) vs. low level of CMV replication may lead to complications such as acute and chronic allograft rejection (6). (An association between CMV and acute and chronic renal allograft rejection has been shown; CMV conferred a high risk of chronic rejection in recipients of hepatic allografts [17]).

There are basically 3 treatment approaches against CMV in the transplant patient: prophylactic treatment, preemptive treatment and treatment of established disease. Traditionally, prophylactic treatment is given to a large population of patients regardless of their stratification into a "high-risk" or "low-risk" group for that particular disease process (18).

Prophylactic treatment involves administration of therapy to all patients during the period that they are deemed to be at particular risk for CMV infection (usually up to 90 to 100 days after transplantation); and preemptive therapy, in which a shorter course of antiviral treatment is targeted toward a subset of patients with early viral replication in

an attempt to prevent the progression of asymptomatic infection to CMV disease (17). Antiviral (i.e. anticytomegalovirus) prophylaxis involves the administration of potentially toxic antivirals to all patients who are at risk (i.e. all patients with the exception of CMV donor negative/recipient negative) beginning at a time soon after transplantation and continuing up to day 100 after transplantation (18). (Targeted prophylaxis, is the use of drug therapy during periods associated with a high rate of CMV reactivation (i.e. during immunosuppressive therapy with a monoclonal antibody for allograft rejection) [18]). Preemptive therapy entails the administration of antiviral drug therapy only to patients with laboratory evidence of CMV replication (i.e. significant number of CMV pp65 positive cells). Table 8 (9) lists the benefits vs. risks/disadvantages with both prophylactic and preemptive antiviral therapy.

Benefits vs. Risks of Prophylaxis and Preemptive Therapy

Strategy	Benefits
Prophylaxis (universal)	May prevent reactivation of other herpes viruses (i.e. HHV-6 and HHV-7) Does not rely on a highly predictive assay for CMV detection
Preemptive	Reduces the number of patients exposed to antiviral drugs Reduces direct drug costs Reduces duration of antiviral drug use Reduces the toxicity related to prolonged antiviral drug use May decrease emergence of antiviral drug resistance Lower incidence of late-onset CMV disease

Table 8

Strategies	Risks/Disadvantages
Prophylaxis (universal)	May facilitate the emergence of antiviral drug resistance with prolonged antiviral drug exposure May expose many patients to adverse effects of drug with prolonged antiviral drug use Higher incidence of late-onset CMV disease (compared to preemptive therapy)
Preemptive	Requires a highly predictive test for early identification of patients at risk for CMV disease Requires strict compliance with stringent surveillance schedule Increases the cost of diagnostic surveillance May not identify all patients at risk of CMV Does not prevent reactivation of other herpes viruses because of its selective nature Higher risk of early-onset CMV disease (compared to universal prophylaxis)

Table 8

The antiviral drugs that are currently used for the treatment of CMV disease after solid organ transplantation are ganciclovir (valganciclovir), foscarnet and cidofovir. Ganciclovir has excellent activity against all members of the human herpes virus family. Ganciclovir is the drug of choice for the treatment of CMV disease in solid organ transplantation (9). Following are the unfortunate side effects of these very effective antiviral therapies: for ganciclovir: bone marrow suppression (i.e. neutropenia, thrombocytopenia, anemia, eosinophilia, bone marrow hypoplasia), hemolysis, nausea, infusion site reactions, diarrhea, renal toxicity, seizures, mental status changes, fever, rash, and abnormal liver function tests; for foscarnet: electrolyte abnormalities, nephrotoxicity, anemia, seizures; for cidofovir, nephrotoxicity, ocular hypotony and neutropenia (9). Antiviral drug resistance has been well documented. Resistance has

been associated with prolonged use of antiviral drugs, as well as low antiviral blood levels.

Recurrent CMV disease may occur in up to 25% of solid organ transplant recipients with an initial episode of tissue-invasive disease (19). CMV recurrence may be related to the incomplete suppression of viral replication at the end of antiviral treatment (i.e. the duration of treatment may have been insufficient) (9). Thus, recurrent CMV disease usually responds well to retreatment with the antiviral agent (19). The optimal duration of antiviral therapy for CMV disease remains unknown (9). Most patients receive antiviral therapy for 2 to 4 weeks (9). Some authors suggest a longer treatment duration for CMV disease with end-organ involvement, such as pneumonitis, retinitis and gastrointestinal CMV disease (20). A study of liver transplant recipients demonstrated that a high-virus load at the end of therapy predicts clinical and virologic relapse (21).

The administration of specific CMV immunoglobulin or unselected immunoglobulins has been used to boost the humoral CMV immune response in an attempt to prevent CMV infection and disease in solid organ transplant recipients (9). Results from randomized trials in solid organ transplantation indicate that CMV hyperimmunoglobulin confers some degree of efficacy in preventing CMV disease (9). Hyperimmune CMV globulin (CMVIG) as sole prophylaxis cannot prevent CMV infection in solid organ transplantation but has been shown to decrease CMV disease significantly in high-risk (i.e. CMV donor positive/CMV recipient negative) renal transplant recipients (15). (CMV pneumonia causes significant morbidity and mortality in bone marrow recipients as well

as heart-lung transplant recipients. The combination of ganciclovir plus intravenous immunoglobulin was more effective than ganciclovir alone for the treatment of CMV pneumonia in bone marrow transplant recipients [22].)

Approximately 20-25% of CMV donor positive/CMV recipient negative solid organ transplant patients develop late-onset CMV disease once prophylactic antiviral therapy is discontinued, which is usually 3-6 months after transplantation (18). Late-onset CMV disease has emerged as a considerable clinical problem. Risk factors for late-onset CMV disease identified in 32 CMV donor positive/CMV recipient negative solid organ transplant recipients one year after transplantation included: CMV seronegativity in the transplant recipient, a non-renal organ transplant, allograft rejection, CMV replication, HHV-6 replication, HHV-7 replication and monoclonal antibody therapy (18). Late-onset CMV may be due to ganciclovir-resistant CMV in up to 20% of cases (17). The median duration of ganciclovir therapy before the diagnosis of resistant CMV was 10 months (range, 7-12 months) (17). It is increasingly recognized that CMV disease is occurring, not uncommonly, beyond one year after transplantation (18). Late-onset CMV disease may have very atypical features (23) such as the presence of leukocytosis and the absence of fever (18).

In the 1970s, the first attempts to immunize against CMV were pursued via the Towne vaccine. The Towne vaccine is a live attenuated virus that induces seroconversion in vaccinees by subcutaneous injection. Three randomized, controlled, double blind studies, were performed in renal transplant patients to determine the protection afforded by the Towne vaccine. Vaccination with the Towne vaccine did not prevent infection

with CMV, but did modify the severity of disease (24). A reduction of approximately 85% in severe disease was achieved (24).

In summary, Figure 6, (25), illustrates the numerous potential roles CMV plays in the solid organ transplant recipient.

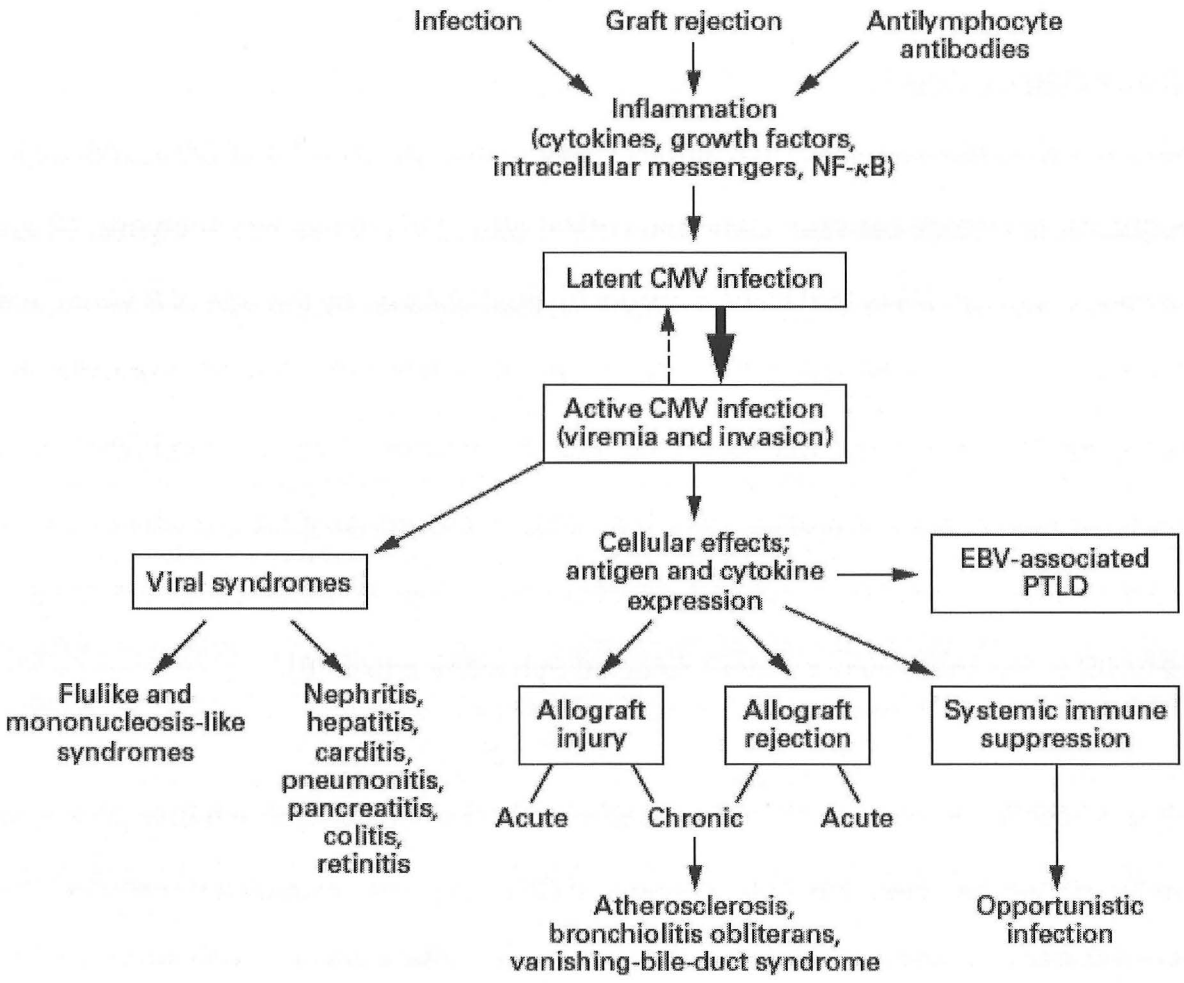

Figure 6. Role of CMV Infection in Transplant Recipients

HUMAN HERPES VIRUSES 6, 7 AND 8:

Since 1986, three novel herpes viruses, human herpes virus 6, 7 and 8 have been discovered. HHV-8, also known as Kaposi sarcoma herpes virus is causally associated with all forms of Kaposi sarcoma, including the type seen after transplantation (26). The role of HHV-6 and HHV-7 as pathogens in transplant recipients is less well understood; however, emerging data suggest that these viruses are potentially pathogenic and clinically relevant in transplant recipients (26).

Human Herpes Virus 6:

HHV-6 is a double-stranded DNA virus; closely related to HHV-7 and CMV, with 66% DNA sequence homology between CMV and HHV-6 (26). HHV-6 has two subtypes, (A and B). Serologic surveys show that HHV-6 occurs in most children by the age of 3 years, and that the prevalence in adults is very high (i.e. greater than 90%) (6). HHV-6 is usually acquired during the first year of life, with saliva the most likely mode of transmission (26). As with the other human herpes viruses, HHV-6 persists in the human host in a latent form, only to reactivate and potentially cause and contribute to disease in the stressed individual. HHV-6B is the predominant variant detected in healthy adults (6).

HHV-6 infection in early childhood causes exanthema subitum (i.e. a febrile illness with skin rash). HHV-6 has been linked to a variety of CNS diseases, including meningitis, encephalitis, and more recently, multiple sclerosis. (The evidence suggesting an association between HHV-6 and infection of the CNS is predicated on the detection of HHV-6 DNA in the cerebral spinal fluid.) The most serious clinical manifestations associated with HHV-6 infection or reactivation occurs in solid organ transplant and bone

marrow transplant recipients (6). Most HHV-6 infections occur between 2 and 4 weeks after transplantation; this characteristic timing of onset distinguishes HHV-6 from CMV, which usually occurs later (i.e. 6 to 12 weeks after transplantation) (26). HHV-6 antigenemia proceeded CMV antigenemia by about 7 days in 15 of 21 cases of concurrent CMV and HHV-6 infection in liver transplant patients (27). (Symptomatic infection due to HHV-6 occurs more frequently in hemopoietic stem cell transplant recipients than it does in solid organ transplant recipients [26]). A nonspecific febrile syndrome with bone marrow suppression with or without a skin rash is attributed to HHV-6 in both bone marrow and solid organ transplant recipients (26). HHV-6 is a neurotropic virus (26). There are well-documented cases of HHV-6 encephalitis in immunocompetent patients as well as in bone marrow transplant recipients. Seen in HHV-6 encephalitis are: mental status changes ranging from confusion to coma, seizure and headache without focal neurologic findings and the presence of csf pleocytosis. There is supportive evidence from cohort studies in transplant recipients for an association between HHV-6 and clinical sequelae including: encephalitis, bone marrow suppression with significant cytopenias, concurrent fungal infection, concurrent cytomegalovirus infection, and more aggressive recurrence of hepatits C after liver transplantation (26). There is evidence from case reports or case series for an association between HHV-6 and clinical sequelae including: pneumonitis, exanthem, hepatitis, gastroduodenitis and leukocytoclastic vasculitis (26). A febrile mononucleosis syndrome attributable to CMV in transplant recipients may be related to concurrent infection with HHV-6 and HHV-7, rather than just CMV alone (26).

Desjardin and colleagues evaluated stored serum samples from 139 orthotopic liver transplant patients (28). HHV-6 reactivation was more likely to occur among those who

received OKT3 treatment for rejection, in older persons and among persons who received prednisone, azathioprine and cyclosporine as initial immunosuppression (28). In addition, there was a striking association between CMV disease and HHV-6 reactivation, as well as severe CMV associated disease and HHV-6 reactivation (28). (Of note, this was serological study.)

Studies have reported HHV-6 infection in 38% to 55% of renal transplant recipients, in 38% to 55% of renal transplant recipients and in 22% to 40% of liver transplant recipients (26). One study documented HHV-6 infection in 57% of heart-lung and lung transplants recipients (26). In 1995, investigators from University of Pittsburgh, described a case of a critically ill liver transplant recipient with HHV-6 variant B associated febrile dermatosis with thrombocytopenia and encephalopathy who recovered after a course of ganciclovir and reduced immunosuppression (29). The number of organ transplants recipients with HHV-6 encephalitis exhibiting amnesia and abnormal radiological findings within the temporal lobe has been recently increasing (30). Detection of viral DNA by PCR is a reliable tool for the diagnosis of HHV-6 encephalitis (31). Though there are no controlled trials assessing the efficacy of antiviral treatment against HHV-6 (30); HHV-6 is susceptible in vitro to ganciclovir and foscarnet, and both drugs have been used successfully in the treatment of HHV-6 encephalitis (31).

HHV-6 has been described as an immunomodulatory and immunosuppressive virus that may facilitate superinfections with other opportunistic infections in transplant recipients, particularly CMV (26). In 1997, Dockrell and investigators at the Mayo Clinic followed 247 liver transplant patients, and found that HHV-6 seroconversion was identified as a

significant risk factor for development of symptomatic CMV infection, including CMV organ involvement in the first 90 days after transplantation (32). (Because this study was based on serologic markers; and that serology is a suboptimal diagnostic method for the detection of herpes virus infections in transplant recipients, it was hoped that more sensitive markers of infection, such as pcr amplication or culture techniques, would be used to confirm infection [32]). In 1999, this same group of investigators at the Mayo Clinic, reported that pretransplant HHV-6 seronegativity was an independent and significant risk factor for subsequent development of fungal infection in the first 90 days posttransplantation (33). University of Pittsburgh investigators found that HHV-6 viremia was an independently significant predictor of invasive fungal infections and was associated with late mortality in liver transplant recipients (34).

In a retrospective, cohort study of 53 kidney transplant recipients, DesJardin and investigators found that HHV-6 reactivation in renal transplant recipients at risk for primary CMV infection is associated with CMV infection and disease (35). Emery reported in a study of 60 consecutive liver transplant recipients, that HHV 6 (detected by PCR) was independently associated with biopsy-proven graft rejection (CMV was also associated with biopsy proven graft rejection that remained independent of the HHV-6 association) (36). The principle effect of HHV-6 infection in association with solid organ transplantation may result from its potential to exacerbate CMV disease (37); and, in unexplained graft dysfunction, we should be more active in obtaining specimens to diagnose possible HHV-6 reactivation (38).

HHV-6 is associated with greater severity of recurrent hepatitis C virus hepatitis (26). Some have proposed that the greater propensity for fibrosis in patients with recurrent hepatitis C virus hepatitis is likely medicated via HHV-6 induced TNF-alpha production, which is known to play a role in the development of hepatic fibrosis (26). HHV-6 infection in the liver has also been shown to increase the expression of adhesion molecules and the number of human leukocyte antigen class II positive T cells (36).

Diagnosis of HHV-6 include: histopathologic findings (i.e. viral inclusion-bearing cells, lack of inflammatory response, enveloped virions with a prominent tegument seen by EM [26]), serologic studies, culture, antigenemia, qualitative polymerase chain reaction, immunohistochemical staining for detecting HHV-6 in formalin-fixed, paraffin-embedded tissue, etc.

The antiviral susceptibilities of HHV-6 resemble those of CMV (i.e. ganciclovir, foscarnet and cidofovir). Successful outcomes using ganciclovir and foscarnet have been published in case reports and case series of patients with HHV-6-associated disease (26).

Human Herpes Virus 7:

Like HHV-6, infection due to HHV-7 is ubiquitous. Primary infection with HHV-7 occurs during childhood, probably through salivary transmission. HHV-7 has also been associated with febrile illness in children and is another etiologic agent of exanthema subitum (36). (HHV-7 infection and neurologic manifestations such as acute hemiplegia in childhood and febrile convulsions has been reported in nontransplant settings [26].)

In comparison to CMV and HHV-6, less is known in regards to HHV-7 and solid organ transplantation. However, viremia due to HHV-7 has been documented by the detection of HHV-7 DNA via PCR in the blood of bone marrow and renal transplant recipients. HHV-7 may be a cofactor in the pathogenesis of CMV in transplant recipients (26); Kidd and colleagues performed viral loads (via pcr) for CMV, HHV-6 and HHV-7 in 52 renal transplant patients and found that patients coinfected with CMV and HHV-7 were more likely to have CMV disease, as compared to those with CMV infection only (39). Tong and colleagues monitored 37 renal transplant patients and also found an association between HHV-7 and CMV disease (40). Ona and colleagues evaluated 42 heart transplant recipients and found that HHV-6 and HHV-7 were detected in a higher percentage in patients with CMV infection/disease that in those patients without CMV (41). Ross and colleagues monitored 19 lung transplant recipients and found a possible association with HHV-7 and bronchiolitis obliterans with organizing pneumonia (BOOP)-like reaction, after lung transplantation (42).

IN CONCLUSION:

We have discussed: solid organ transplant statistics, some basic transplant infectious disease issues, and the rather complex issues and interactions of the human herpes viruses, specifically CMV, HHV-6 and HHV-7. The interrelationship between viral pathogens has become increasingly more relevant and its scope wider as new or previously unrecognized viruses continue to emerge as pathogens in transplant recipients (43). We must continue to monitor the activity of these viruses in this patient population to better understand the phenomenon of viral coinfection and its repercussions in these rather complex patients.

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