ASPECTS OF INFECTIOUS DISEASES IN THE TRANSPLANT PATIENT

Suzanne Wada, M.D.
University of Texas Southwestern Medical Center
Internal Medicine Grand Rounds
February 24, 2000

Biographical Information

Name: Suzanne Wada, M.D.

Rank: Assistant Professor of Internal Medicine

Division: Infectious Diseases

Interests: My 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 will be discussing "off-label: uses in her presentation."

BACKGROUND

To transplant an organ into an often critically ill patient to sustain life is a tremendous and remarkable medical feat. There is a huge investment that society makes when a transplantation occurs. Therefore, common sense mandates that meticulous care and compulsive attention to detail be maintained to ensure the very best possible outcome and success of the transplantation.

Though advances in surgical technique, transplantation biology and the use of immunosuppressive agents have furthered the science of transplantation, infection continues to cause significant morbidity and mortality. There is a constant balancing act between the immunosuppressive agents given to prevent allograft rejection and the subsequent immunosuppressed state that predisposes the transplant recipient to infection.

Immunosuppression is most intense early in the post transplantation period. The dosing of the immunosuppressive agents is gradually decreased over time until the patient is maintained on as minimal a regimen as possible to maintain allograft function and help prevent rejection. Table 1 [(1)] lists some of the commonly used immunosuppressive agents and their mechanism of action. Most of these agents depress cell-mediated immunity; however, blunted antibody responses and leukopenia may also be a result of the use of these agents.[(1)] These agents also possess noteworthy side effects. And to complicate matters further, there are significant interactions between immunosuppressive agents and commonly used antimicrobials as illustrated in Table 2 and Table 3.[(2)]

TABLE 1 IMMUNOSUPPRESSIVE AGENTS

Agent	Mechanism of action
Prednisone	Down-modulates lymphocyte and macrophage function; impedes other aspects of inflammatory response
Azathioprine	Inhibits cell proliferation, interfering with DNA synthesis
Cyclosporine	Blocks T-cell activation targeting calcineurin, inhibits cytokine production (IL-2, IFN-γ, etc.)
Tacrolimus	As for cyclosporine
Cyclophosphamide	Decreases antigen-driven lymphocyte proliferation
Mycophenolate mofetil	Inhibits IMP dehydrogenase, selectively suppressing proliferation of T and B lymphocytes
Methotrexate	Blocks proliferation of cycling cells
OKT3 monoclonal antibody	Depletes T cells; impairs T-cell function via down-regulation of T-cell receptor-CD3 complex
Antilymphocyte globulin	Depletes lymphocytes
Antithymocyte globulin	Depletes T cells; inhibits T-cell activation
Total lymphoid irradiation	Inhibits development of primary antigen-specific response

TABLE 2 CLINICALLY OBSERVED AND REPORTED INTERACTIONS BETWEEN TACROLIMUS AND ANTIMICROBIAL AGENTS.

Antimicrobial agent	Interaction
Erythromycin	Increased tacrolimus levels, with subsequent nephrotoxicity
Clarithromycin	Increased tacrolimus levels, with subsequent nephrotoxicity
Clotrimazole	Increased tacrolimus levels, with subsequent nephrotoxicity
Fluconazole	Increased tacrolimus levels, with subsequent nephrotoxicity and neurotoxicity
Ketoconazole	Increased tacrolimus levels
Rifampin	Decreased tacrolimus levels, leading to acute rejection

TABLE 3. THEORETICALLY SIGNIFICANT INTERACTIONS BETWEEN TACROLIMUS AND ANTIFUNGAL AGENTS

Antifungal agent	Potential interaction	Comments
Amphotericin B	Additive nephrotoxicity	Liposomal preparations should be less toxic
Clotrimazole	Inhibition of tacrolimus metabolism	Clinically important, even when used orally
Fluconazole	Inhibition of tacrolimus metabolism	Clinically important
Flucytosine	None suspected	Reduce dose if renal impairment develops
Griseofulvin	Neurotoxicity	May resemble that of tacrolimus
Itraconazole	Inhibition of tacrolimus metabolism	Clinically important
Ketoconazole	Inhibition of tacrolimus metabolism	Clinically important
Miconazole	Inhibition of tacrolimus metabolism	Not likely to be significant if used topically
Nystatin	None suspected	Should be safe
Terbinafine	None suspected	Do not use if renal function impaired

There is no standard immunosuppressive "cocktail". The type of solid organ transplanted and the practice of the transplant surgeons and physicians at each transplant center dictate the "cocktail" used. However, the ingredients are usually very similar.

Despite all the advances in transplantation medicine, infection remains a common life threatening complication. The optimal approach to infection is prevention; however, failing this, prompt and aggressive diagnosis and therapy is paramount. The sources of infectious agents post transplantation include endogenous organisms, the allograft itself and the environment.[1] A guiding principle to consider in evaluating a transplant patient is that the signs and symptoms of infection may be blunted by the immunosuppressive therapy, and the usual diagnostic techniques may be inadequate. Therefore, aggressive and often invasive investigations of seemingly minor symptoms may be warranted.

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[(1)] represents the pretransplantation infectious disease evaluation performed at the Mayo Clinic. Especially noteworthy are the serological testing and the various vaccines administered prior to transplantation. The serological testing reveals the possibility of past exposure or infection to the listed pathogens.

TABLE 4 PRETRANSPLANTATION INFECTIONS DISEASES 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 infections, prostatitis, vaginitis, genital herpes, genital warts, syphilis, gonorrhea, pelvic inflammatory disease, chlamydial infection
  - Cutaneous: skin and nail infections, 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 endemic 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 mycoses if history of exposure present)

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

The human herpes viruses, shown in Table 5, are classic examples of pathogens that become dormant after a primary acute infection but can reactivate and subsequently cause significant disease if the host is stressed (i.e. by immunosuppression). Perhaps no organism causes as much havoc in the transplant recipient (both solid organ and bone marrow) as cytomegalovirus. CMV will be discussed more thoroughly later.

TABLE 5 HERPES VIRUS FAMILY

Herpes Virus	Infection
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
Human Herpes virus 8	Kaposi Sarcoma

End stage liver disease secondary to hepatitis B and/or C is a relatively common reason for liver transplantation. Because the liver is probably the largest reservoir of HBV, it was hoped that by removing the infected liver (combined with blood loss and subsequent transfusion), the virus would be cleared. However, persistent HBV infection is found in 85 to 90% of patients who are positive for HBsAg.[3] Patients in whom chronic hepatitis B is the primary liver disease necessitating transplantation have 1 and 5 year survival rates of 73% and 44%.[3] Patients with either HBV DNA or HBeAg in serum are more likely to have recurrence of hepatitis B after liver transplantation than are patients negative for these markers of active viral replication. Decreasing HBV DNA levels or clearing HBsAg may prevent the recurrence of hepatitis B. Long-term anti-HBV hyperimmune globulin therapy, begun at the time of transplant and continued indefinitely, has markedly improved the outcome of liver transplantation.[4] Lamivudine and to a lesser extent, famciclovir, has significant efficacy against HBV infection but after approximately twelve months there is a high rate of relapse due to selection of drug resistant mutants.[4] Interferon is effective treatment of chronic hepatitis B in immunocompetent patients. However, chronic rejection manifested by the acute vanishing bile duct syndrome has occurred in liver transplant recipients treated with interferon.[5]

Therapy for HCV is less well developed than for HBV. A 12 month course of prophylactic treatment with interferon starting very early after transplant was found to decrease the incidence of recurrent HCV hepatitis after transplant.[6] However, in another study using a 6-month prophylactic course of interferon, there was only a delay in the occurrence of hepatitis C but no reduction in its incidence or severity.[7] Monotherapy with interferon or ribavirin has been found to be ineffective [8-10] and the only promising results have been reported with the combination of interferon and ribavirin.[11] Another confounding factor is that there exist multiple genotypes of HCV with differing biological behaviors and antigenic diversity (i.e. quasispecies).[12]

It has been well established that donor-related transmission of infectious agents occurs. Donor kidneys have transmitted cytomegalovirus, toxoplasmosis, herpes simplex virus, HIV, HBV and HCV.[13] Donor hearts have transmitted cytomegalovirus, toxoplasmosis, HIV, HBV and HCV.[13] Donor livers have transmitted cytomegalovirus, HIV, HBC and HCV.[13] Bone marrow has transmitted cytomegalovirus.[13] In addition, blood products have transmitted CMV, EBV, HIV, HBV, HAV, HDV, HCV, HTLV-1 and in rare instances, Chagas disease, malaria, babesiosis, and syphilis.[13] As part of the transplant protocol, serological studies of the donor include HIV 1, HIV 2, HTLV-1, syphilis, HBV, HCV, CMV, toxoplasmosis gondii and EBV. Allografts from donors who are HIV 1 or HIV 2 antibody positive are not used for transplantation.

The use of liver allografts from donors antibody positive for HBV or HCV is controversial. However, because of the severe organ shortage, livers from donors HBV and HCV antibody positive are used. Transmission of virus via the allograft (liver) from donors with actively replicating virus approaches 100%.[4] Therefore, most transplant centers exclude liver allografts from HBsAG positive donors. Approximately 50% of anti-HCV positive donors are RNA-positive by PCR and hence harbor transmissible virus.[4] At five years post transplant there is little difference in the outcome of liver transplantation however it is probable that chronic liver disease will be progressive as is reflected in the natural course of the disease. Each transplant center dictates their use of allografts from high risk patients, (i.e. liver allografts from HBsAg positive donors). One major transplant center restricts the use of anti-HCV positive donors to those patients who are emergently in need of a transplant, older patients, and highly sensitized patients unlikely to have access to another allograft.[4] At this center, organs from anti-HCV positive donors are not used in children.[4] One advocated strategy has been to reserve anti-HCV positive donor organs for anti-HCV positive recipients. In the case of hepatitis B, there is a small risk that HbsAg negative, HBcAb positive donors could transmit infection via an allograft.[4] However, because donors are in short supply, at risk allografts are used.

Another example of how important it is to know donor and recipient antibody status is in the setting of cardiac transplantation and toxoplasmosis. Toxoplasmosis gondii is a ubiquitous obligate intracellular parasite. Following primary infection (a mild glandular fever-like syndrome in an immunocompetent person), the organism remains dormant in the body, often within muscular tissue. The person is asymptomatic. Serological studies indicate that 3 to 70% of healthy adults have been infected with *t. gondii* in the United States.[14] The incidence of infection varies with the population group and geographic location.[14] In France, the prevalence of seropositivity is as high as 75% by the fourth decade of life.[14] Cardiac transplantation from donors seropositive for *t. gondii* into seronegative recipients is associated with the risk (greater than 50%) of severe primary toxoplasmosis, while clinically apparent disease is rarely caused by reactivation of infection in seropositive recipients after transplantation.[15] Cardiac transplant recipients who are seronegative before transplantation and who receive a heart from a seropositive donor should be followed closely for evidence of seroconversion for *t. gondii*.[16] For the high-risk transplant (i.e. seropositive heart into seronegative recipients) some advocate the use of prophylactic co-trimoxazole post transplantation. Others advocate the use of prophylactic pyrimethamine post

transplantation while still others advocate treatment with pyrimethamine and sulfadiazine upon seroconversion for *t.gondii.*

The third source of infection, besides the host itself and the allograft, is the environment. Epidemiological exposure occurs in the community and in the hospital.[17] Community exposures to respiratory viruses (influenza, parainfluenza, respiratory synctial virus, adenovirus), food-borne pathogens (salmonella, listeria monocytogenes, campylobacter jejuni) and geographically restricted systemic mycoses (blastomyces dermatitidis, coccidioides immitis, and histoplasma capsulatum) can occur. The degree of immunosuppression dictates the risk of clinical infection.

Within the hospital, environmental exposure may be domiciliary or nondomiciliary.[17] Domiciliary exposure occurs on the hospital unit where the patient is housed, as a result of contamination of the air or potable water with pathogens such as aspergillus, legionella or gram negative bacilli such as pseudomonas.[17] Hands of personnel and contaminated equipment have caused outbreaks of vancomycin-resistant enterococcus faecium, methicillin-resistant staphylococcus aureus, and clostridium difficile.

Nondomiciliary exposure occurs within the hospital when the patient is exposed to contaminated air during travel to or from clinical procedures such as surgery or radiologic imaging.[17]

THE TIMETABLES

In both solid organ and bone marrow transplant recipients, there is a well established timetable for when different infections tend to occur. (Figs. 1[18] and 2[19]). For the solid organ transplant recipient, the timetable is easily organized into 3 segments; the first month, 1-6 months, and more than 6 months post transplantation. The clinician uses this timetable to help him/her prioritize the most likely diagnosis. Obviously, there are exceptions to the rule, especially when there is ongoing rejection and subsequent increased immunosuppression.

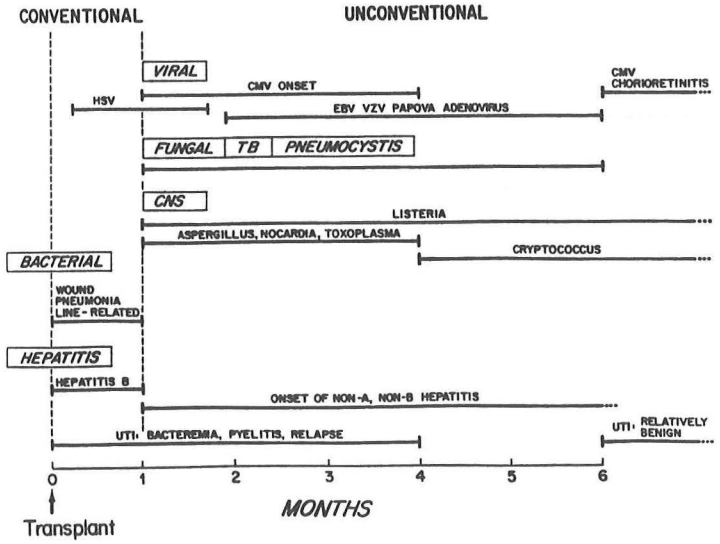


Figure 1. Timetable for the occurrence of infection in organ transplant recipients. (*Modified from* Rubin RH, Wolfson JS, Cosimi AB, et al: Infection in the renal transplant recipient. Am J Med 70:405–411, 1981; reprinted with permission from American Journal of Medicine.)

Figure 2

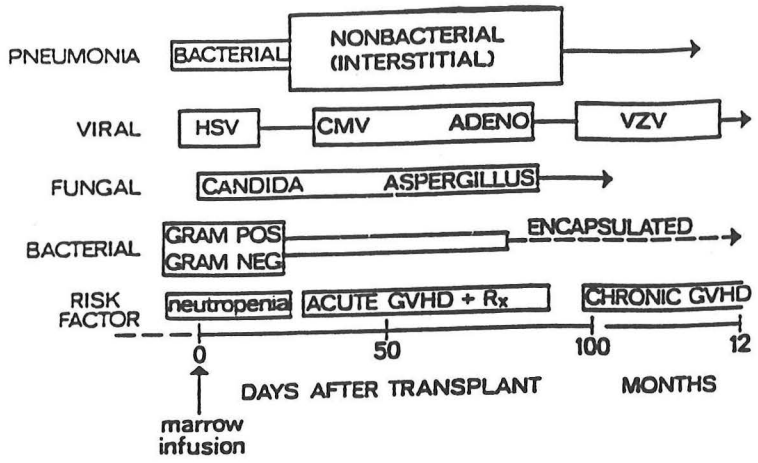
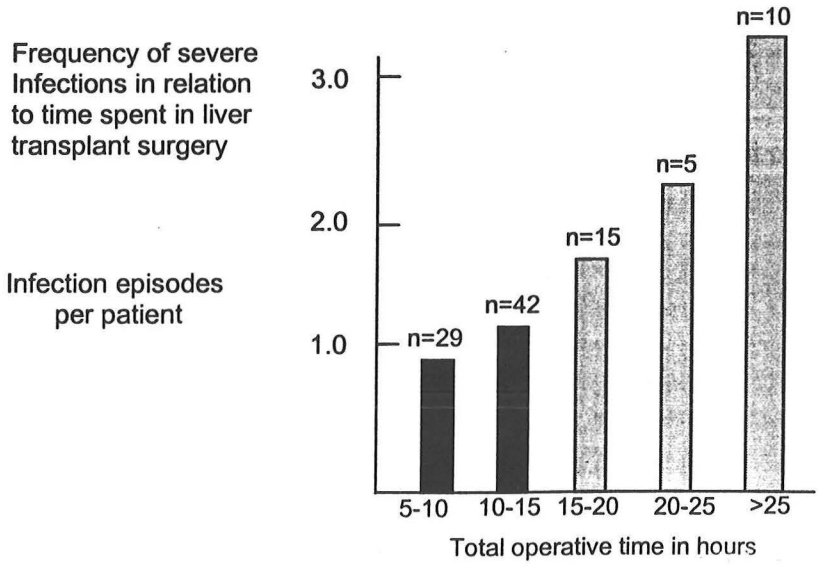


Fig. 2 Predisposing risk factors and common infections by time after hematopoietic stem cell transplant (HCT). [From Meyers JD. Infections in marrow transplant. In: Principles and Practices of Infectious Diseases. 3rd ed.. Churchill Livingston, London, pp. 2291–2294, 1990.]

Infection in the first month after transplantation (solid organ)

The patient is in many ways a typical post-operative surgical patient. More than 90% of the infections occurring in the first month are the same nosocomial, bacterial or candidal infections of the surgical wound, lungs, urinary tract, or vascular access devices that occur in surgical patients who are not in a state of immunosuppression.[17] Key factors in determining the incidence of such infections are the nature of the operation and the technical skill with which the surgery and postoperative care are accomplished.[17] The risk of postoperative infection increases with the duration of vascular access and use of drainage catheters, the duration of intubation, the presence of indwelling stents and other foreign bodies, and the presence of devitalized tissue or fluid collections.[17] Kusne et al have demonstrated (Fig. 3[20]) the increased frequency of severe infections in relation to time spent in the operating room in liver transplant surgery.

Figure 3



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.[17] 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 post transplantation 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.[17]

Infection 1-6 months post transplantation (solid organ)

As depicted in Figure 1, this is the period of typical opportunistic infections. The troublesome herpes viruses, specifically CMV and to a lesser extent EBV, as well as the hepatitis viruses begin to have clinical significance. Viral infection may contribute to the development of graft rejection and may exert systemic immunosuppressive effects that predispose to the occurrence of opportunistic infections.[21]

Infection greater than 6 months post transplantation (solid organ)

Six months post transplantation, patients can be divided into 3 categories of infectious disease problems.[17]

The majority (greater than 75%) of patients have a good result from transplantation and are maintained on minimal long-term immunosuppressive therapy. The infections are similar to those seen in the general immunocompetent population. Most of these infections are primarily respiratory. Opportunistic infections are unusual unless a particularly intense environmental exposure has occurred (i.e, aspergillosis after digging in the garden).[17]

In a small percent of patients (approximately 10%), patients may have progressive and chronic problems with viral infections, specifically the herpes viruses, CMV, EBV, and hepatitis viruses, HBV and HCV.

The 10% of patients with chronic rejection who are on higher than usual doses of immunosuppressive agents are not only prone to chronic viral infections but also may suffer from the typical opportunistic infections usually seen in the 1-6 month period post transplantation. This subgroup of patients is not only persistently at risk for a broad range of opportunistic infections but also at risk for post transplantation lymphoproliferative disease (PTLD), lymphoma and other forms of cancer.[21]

In addition

In the bone marrow transplant patient, the type of transplant (i.e. autologous vs. allogeneic), the degree and duration of neutropenia, and the presence of acute/chronic GVHD are determining risk factors for infection (Fig. 2). As alluded to earlier, CMV is

probably the most problematic infection in bone marrow transplant recipients. Fungal infections, particularly aspergillosis, also cause significant morbidity and mortality.

CMV

CMV is the single most important infectious agent affecting recipients of organ transplants with at least 2/3 of these patients having CMV infection 1-4 months after transplantation.[22] CMV is a member of the herpes family, a group of viruses that humans are usually exposed to or infected within the first few decades of life. The exposed/infected person recovers, but the virus can remain dormant in the human body with the capacity to reactivate and cause clinical disease when a person is "stressed" (i.e. immunosuppression to prevent allograft rejection, human immunodeficiency virus). In the United States, about 50% of adults are seropositive for CMV (CMV IgG+), and thus harbor latent virus. During systemic CMV infection in normal hosts, the virus is found in a variety of white blood cells. In clinical transplantation, CMV can be transmitted to the organ recipient via the donor organ. CMV may be present within the cells of the allograft (i.e, hepatocytes, renal tubular, glomerular or peritubular capillary endothelial cells of a kidney) or be within leukocytes within an allograft.

Besides the characteristic of latency, 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.[22]

Epidemiology - CMV

There are 3 major epidemiologic patterns of CMV infection in the solid organ transplant patient, each with a different propensity for causing clinical disease. They are primary infection, reactivation infection and superinfection.[22]

Primary CMV infection occurs when an individual who is seronegative for CMV becomes infected with virus carried latently in cells from a CMV seropositive donor.[22] In kidney transplantation, the source of the latently infected cells in 80-90% of allograft recipients is the kidney from the CMV seropositive donor; in the remaining 10-20%, the source is viable leukocyte containing blood products from CMV seropositive blood donors.[22] Some 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 the setting of kidney transplantation, investigators have found that transmission of CMV via CMV seropositive donors to CMV seronegative recipients is increased when organs from cadaveric donors are used vs. organs from living related donors.[22] Why this is so is as yet unclear. And to add to the mystery, 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.[22] Unfortunately, at present, no markers are available that distinguish between these two groups of CMV seropositive donors and their potential to transfer CMV.[22]

Data from heart, heart-lung and pancreas-kidney CMV seronegative transplant recipients also identify organs from CMV seropositive donors as the major source of primary CMV infection. In liver transplant recipients, immense quantities of blood products contribute to the risk of CMV transmission; however, the CMV seropositive donor allograft again is of primary importance in the CMV seronegative recipient.

In a report on 218 liver transplant recipients at the Mayo Clinic by Marin et al, Figure 4,[23] it was shown that CMV infection and disease incidence was directly related to the donor and recipient's CMV serological status at transplantation.[23]

Figure 4

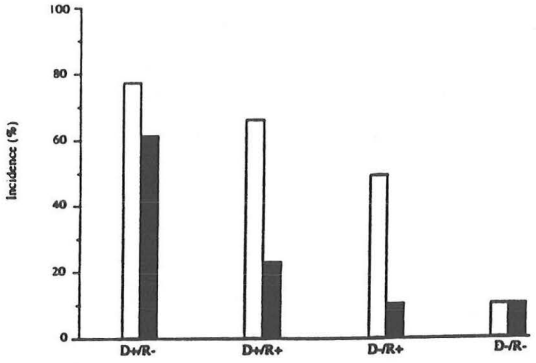


Figure 4 Incidence of CMV infection (*open bars*) and CMV disease (*solid bars*) after liver transplantation, according to pretransplantation CMV serological status of donor (*D*) and recipient (*R*).

The second major epidemiologic pattern of CMV infection is that of reactivation infection. In reactivation infection, the transplant recipient who has been infected with CMV previously (and is seropositive for CMV before transplantation) undergoes reactivation of endogenous latent virus.[22] The degree of reactivation determines the extent of clinical manifestations due to CMV.

The third major form of CMV is superinfection, in which 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.[22] 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.[22] In addition, it is believed that transplant patients are more likely to have clinical disease with superinfection rather than with reactivation infection.

The incidence of symptomatic disease is different for these 3 forms of CMV infection. At least 2/3 of patients with primary infection will develop symptoms; 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.[22]

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.[22]

In addition, while CMV disease is generally more severe in primary infection of recipients of solid organ transplants, in the bone marrow transplant population severe disease is more likely to occur in those with reactivated CMV infection.[24]

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.[22] It should be mentioned that person to person spread of CMV among patients and personnel in dialysis and transplant units does not appear to occur.[25]

Pathogenesis of CMV-Organ Transplant Recipients

The single most important exogenous factor in reactivation of CMV, regardless of the latent site, is the kind and intensity of the immunosuppressive therapy administered.[22] Of all the immunosuppressive agents used, antilymphocyte globulin, appears to have the greatest effect on reactivation of latent virus.[22] Antilymphocyte preparations such as OKT3 are very potent reactivators of CMV.[23]

Steroids appear to have minimal effect on reactivation of latent CMV.[22] Cyclosporine also has minimal effect on reactivation of latent CMV virus, but interferes with the ability of the host to control infection.[22] 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.[22] Cyclosporine, steroids and rapamycin, while poor reactivators of CMV, do increase viral replication once the virus is activated.[23]

CMV interacts with the host immune response in noteworthy ways. CMV is felt to be an immunomodulating virus.[22] CMV causes a metabolic abnormality in lymphocytes and monocytes that impairs their ability to produce and to respond to cytokines such as interleukin 1 and interleukin 2.[22] CMV appears to suppress the functioning of antigen-specific cytotoxic T-lymphocytes.[22] In addition, CMV causes a change in 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.[22] Thus, CMV has direct immunosuppressive effects on the host's immune system.[23] Consequently, CMV is a predisposing risk factor for bacterial and fungal infection following liver transplantation.[23] Donor CMV seropositivity is an independent risk factor for bacteremia in liver transplant recipients.[26]

The host'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.[24]

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.[22] Prolonged fever for several days may be the only manifestation of CMV infection.[22] In the solid organ transplant recipient,

CMV accounts for the majority of febrile episodes in the 1-4 month period post transplantation.

In transplant recipients who develop CMV-related fever, 20-30% will develop CMV pneumonia.[22] CMV pneumonia has similar signs and symptoms as Pneumocystis carinii pneumonia (PCP). In fact, it is not uncommon to find these two diagnoses (CMV pneumonia and PCP) concurrently in immunocompromised patients (i.e. HIV 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.[22] 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.[22]

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

Mild to moderate hepatitis with abnormal liver function is seen in 30-50% of solid organ transplant recipients with systemic CMV infection.[22] CMV hepatitis is rarely a problem in kidney and cardiac transplant recipients.[22] 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.[22]

Presence of CMV within the liver can be demonstrated by finding typical CMV inclusions within liver parenchyma. There are also molecular techniques that can help demonstrate the presence of CMV in liver tissue.

CMV myocarditis can be an important cause of myocardial dysfunction in cardiac transplant recipients. This again shows that 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.[22] CMV associated gastrointestinal lesions can appear in the absence of other manifestations of CMV disease.[22]

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.[22]

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, including gram negative bacilli, Listeria monocytogenes, P. carinii, Aspergillus species, Cryptococcus neoformans and Candida species.[22]

The most controversial aspect of CMV infection in transplantation is whether CMV plays a role in allograft rejection.[22] Richardson et al described a glomerular lesion in kidney allografts associated with CMV viremia.[27] 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 glomerulopathy 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.[22] 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.[28] CMV has also been associated with bronchiolitis obliterans in patients undergoing lung transplantation.[23]

CMV Diagnosis

Table 6 lists the major diagnostic methods used in making the diagnosis of CMV.

Table 6

CMV Diagnosis:
→ Serological Testing
→ Viral Culture
→ Histopathologic Diagnosis
→ Antigenemia Assay
→ Nucleic Acid Amplification

Serological testing is useful for pretransplantation assessment of the recipient's risk of CMV infection. In general the development of CMV IgM antibodies or a fourfold rise in IgG titers over time indicates acute infection.[23] Serologies, however, are highly insensitive in immunocompromised patients and have limited clinical usefulness in the acute care setting because of the prolonged time for confirmation of diagnosis.[23]

Isolation of CMV from tissue or body secretions is the "gold standard" against which other tests are compared.[23] Conventional viral cultures require a long time (1-2 weeks) to confirm the viral cytopathic effect on fibroblast cultures, and viral recovery may be further delayed when viral replication is low.[23] The shell vial assay can detect the presence of virus in 12-24 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.[23] The assay is widely available, rapid and specific for CMV infection.[23]

Histopathologic diagnosis of tissue invasive CMV is based on presence of characteristic intranuclear inclusions (Cowdry type A) in enlarged cells with a prominent nuclear rim, giving the typical "owl's eye" appearance.[23]

CMV infects leukocytes. The antigenemia assay detects antigens 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.[23]

In solid organ transplantation, a small number of antigen positive cells (less than 10 per 50,000 polymorphonuclear cells) generally indicates asymptomatic infection, whereas a large number (greater than 50 antigen-positive cells per 50,000 polymorphonuclear cells) indicates a 60% likelihood of CMV disease.[23] 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 patients with high antigenemia levels may remain asymptomatic.[24] Despite some limitations, the antigenemia assay alone, or in combination with shell vial cultures, is of significant value in detecting and monitoring CMV.

One can get a rapid result with PCR and the antigenemia assay. 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 and a latent virus.[23]

CMV Management and Treatment

There are basically 3 treatment approaches against CMV in the transplant patient: prophylactic treatment, preemptive treatment and treatment of disease. Traditionally, prophylactic treatment is given to a large population of patients regardless of stratification into "high risk" or "low risk" groups.[29] It was proposed that a new approach be taken, one in which the group at greatest risk for development of CMV disease receive the most potent anti-CMV medication at the time the risk is maximal (i.e. during administration of antilymphocyte globulin).[29] This new approach is termed preemptive therapy to be distinguished from nontargeted prophylactic therapy.[29]

Nationwide, there is little consensus on prophylactic, preemptive or treatment protocols, partly because of the large number of transplant centers, and because of different rates of CMV disease. This variation in protocols is illustrated in a randomized, controlled trial (Table 7)[30] looking at CMV prophylaxis regimens in liver transplant recipients. This trial found that prophylactic CMV immunoglobulin had no effect on the incidence of CMV infection and was associated with only a modest reduction in CMV disease.[30]

The preemptive approach has proven to be effective. For example, in bone marrow transplant patients detection of CMV in BAL fluid is highly predictive of subsequent development of CMV pneumonia.[29] CMV pneumonia causes significant morbidity and mortality in bone marrow transplant recipients. Administration of antiviral therapy (preemptive therapy) in bone marrow transplant recipients when CMV is detected in BAL fluid significantly reduces the occurrence of CMV pneumonitis.[29] Also noteworthy is that the combination of ganciclovir plus intravenous immunoglobulin is more effective than ganciclovir alone for treatment of CMV pneumonia in bone marrow transplant recipients.[30]

Table 7 Randomized, controlled trials of prophylaxis for infection and disease due to cytomegalovirus (CMV) in liver transplant recipients

Location/Regimen
Boston
CMVIG (150 mg/kg) q2–4w × 120 d
Placebo
Villejuif, France
Intravenous acyclovir (500 mg/m² q8h) × 10 d, followed by oral acyclovir (800 mg q.i.d.) × 80 d
Observation
Pittsburgh
Intravenous ganciclovir (5 mg/kg q12h) × 14 d, followed by oral acyclovir (800 mg q.i.d.) × 76 d
Oral acyclovir (800 mg q.i.d.) × 90 d
UCLA, Los Angeles
Intravenous ganciclovir (6 mg/kg q.d.) × 30 d, followed by iv ganciclovir (6 mg/kg q.d., Monday through Friday) × 70 d
Intravenous acyclovir (10 mg/kg q8h) from day +1 to discharge, followed by oral acyclovir (800 mg q.i.d.) from discharge to day +100

NOTE. CMVIG = cytomegalovirus immune globulin.

It was demonstrated in a multicenter randomized controlled trial that low dose preemptive ganciclovir (2.5 mg/kg iv qd) administered to CMV seropositive renal transplant recipients during antilymphocyte globulin treatment decreased incidence of symptomatic CMV disease by more than 50%.[31]

Newer diagnostic tests such as the antigenemia assay and PCR are useful in preemptive therapy. For example, in the "high risk" liver transplant patient (CMV seropositive donor/CMV seronegative recipient), the number of positively stained leukocytes can help determine when to begin preemptive therapy. The goal being preventing CMV infection from progressing to CMV disease.

One can open any medical textbook or journal and find a wide range of prophylactic, preemptive and treatment protocols for CMV infection/CMV disease. Obviously, antivirals (ganciclovir or foscarnet) +/- immunoglobulin preparations (IVIG or CMV IVIG) are readily used in CMV disease.

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.[32] A reduction of approximately 85% in severe disease was achieved.[32] Research continues in this area.

In summary

Figure 5 [17] shows all the potential roles CMV plays in the transplant recipient.

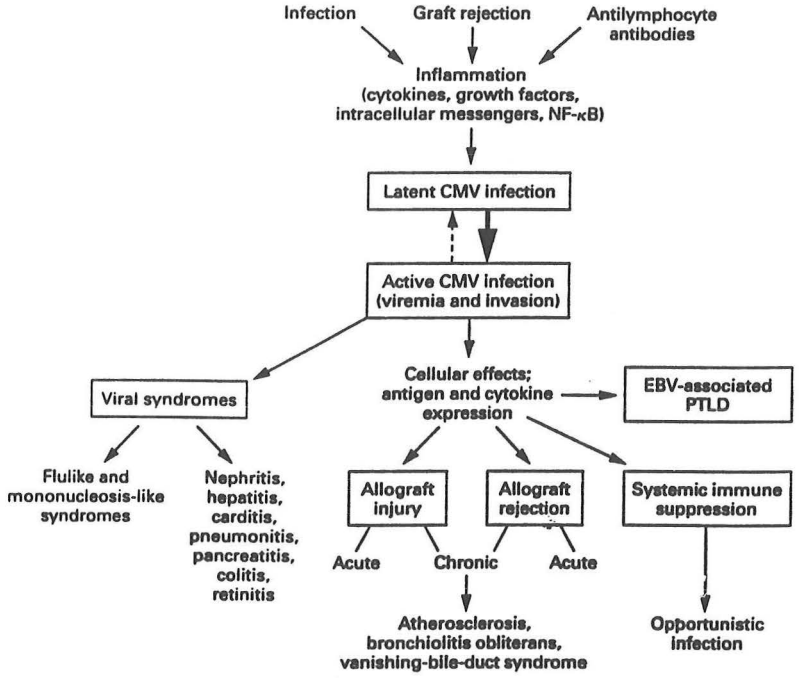


Figure 5 Role of Cytomegalovirus (CMV) Infection in Transplant Recipients.

ANTIMICROBIAL PROPHYLAXIS

Because of the known risks of certain infections occurring at certain times post transplantation, the use of prophylactic antimicrobials is an established practice. Table 8[13] lists antimicrobials used in prophylaxis for solid organ and bone marrow transplants. Each transplant center dictates their use of antimicrobials. In general, in the early post transplantation period, patients are on acyclovir to prevent herpes simplex infections, bactrim to prevent Pneumocystis carinii pneumonia (PCP) and nystatin to prevent fungal infections. The duration of prophylaxis is not well established, but in general if a patient has ongoing rejection and increased immunosuppression, prophylaxis is maintained indefinitely.

TABLE 8 Antimicrobial Prophylactic Regimens in Transplantation

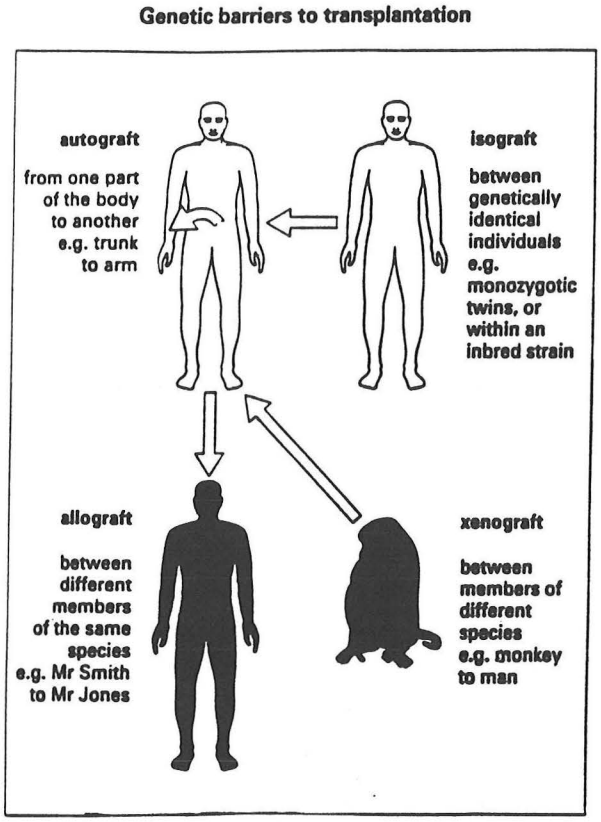
Pathogen	Prophylactic Agents
Protozoa	
Toxoplasmosis	Pyrimethamine
Viral	
Herpes simplex	Acyclovir
Cytomegalovirus	Acyclovir
	Immunoglobulin
	Ganciclovir
	Foscarnet*
Fungal	
Candida	Fluconazole
	Nystatin
	Clotrimazole
Aspergillus	Amphotericin B
	Liposomal amphotericin
Pneumocystis	Trimethoprim-sulfamethoxazole (TMP-SMX)
Bacterial	
Wound infection	Variable
Urinary tract infection	TMP-SMX
Neutropenic infection	Quinolones
Tuberculosis	Isoniazid
Pneumococcus	Penicillin (bone marrow transplants)

*Foscarnet is mostly used in bone marrow recipients who have low blood counts.

THE FUTURE

There are four potential genetic relationships between donor and recipient Figure 7.[33] Grafts can be categorized as autografts, isografts, allografts and xenografts.[33] The genetic diversity between donor and recipient dictates the degree of rejection.

Figure 7



An autograft is tissue taken from one part of the body and transplanted to another part of the same body (i.e. skin grafts, vascular structures). Since the material is not foreign, there is no rejection. Isografts are between isogeneic (genetically identical) individuals, such as monozygotic (identical) twins. With an isograft, there are no foreign antigens between donor and recipient so there is no rejection. Currently, the allograft is the common clinical transplant, where an individual donates an organ to a genetically different individual. The graft is allogeneic (i.e. between members of the same species). The cells of the allograft do express antigen which are recognized as foreign by the recipient. The degree of rejection that occurs is dependent upon the disparity of the histocompatibility antigens between donor and recipient. The histocompatibility antigens are encoded by the major histocompatibility complex (MHC). In man, the MHC, is the human leukocyte antigen (HLA) system. The maximal genetic disparity is between members of different species (i.e. a xenograft).

Xenotransplantation, the transplantation of cells, organs or tissues between species, has been proposed as a solution to the shortage of human organs that are available for the treatment of end stage organ failure[21]. Zoonosis refers to a disease mediated by an animal pathogen and transmitted to humans under natural

conditions[34]. There are many zoonotic diseases. Xenosis/xenozoonosis has been proposed to describe infectious illnesses introduced into humans through procedures involving xenogeneic tissue[34]. Since the transfer of infectious agents from the allograft to the recipient is a well-known complication of conventional transplantation, a potential and justifiable concern with xenotransplantation is the transfer of a known or unknown pathogen of non-human origin into the human population[35]. In addition, not only are there major ethical issues (including animal rights, organ allocation, etc.) with xenotransplantation, but the risk of xenotransplantation poses challenges for obtaining informed consent since not only are there possible hazards for the patient but also for the family and other close contacts.[36]

Currently, xenotransplantation is limited to 3 main areas: 1) tissue xenografting, 2) extracorporeal perfusion with xenografts, and 3) whole-organ xenografting.

Tissue xenografting with pig to human skin grafts and pig heart valve implants has been used successfully for many years.[36] Pig neural cells have shown promise as treatment for Parkinson's disease.[36]

Extracorporeal perfusion with xenografts has been used as a temporary measure to allow time for the organ to recover function or for the allograft to become available. For example, in the setting of liver failure, a perfusion circuit is established that carries blood from the patient through the hepatic artery and portal vein of the ex-vivo organ and returns the detoxified blood to the patient.[36] Two of five patients described in recent literature were successfully managed by this technique until conventional allotransplantation could be performed.[36]

In the early 1960s, Reemtsma and colleagues transplanted chimpanzee kidneys into human recipients before dialysis was widely available.[36] Initially some of the grafts functioned; however, eventually all of the recipients succumbed to uncontrollable rejection or infection.[36] In 1985 Bailey and associates transplanted a baboon heart into a newborn infant who survived for 3 weeks until the graft was lost to antibody mediated damage.[36] In 1993, Starzl and colleagues reported 2 cases of baboon to human liver xenotransplantation in patients with end stage liver disease secondary to chronic active hepatitis B (one patient was HIV+).[36] The first patient lived for 70 days, but the second patient died 26 days post-operatively. Neither graft had evidence of rejection, but both patients died from sepsis secondary to profound immunosuppression.[36]

It is not primates but pigs that are currently the most promising source of donor organs. Pigs have large litters with a short maturation period, they are easy to breed, and their organ size and physiology are remarkably similar to that of humans.[36] Pig herds, free of known bacterial and viral pathogens can be developed. A family of porcine endogenous retroviruses (PERV or PoEV) has been identified, some of which induce productive infection of human cells in vitro.[21] It is not known whether these viruses can cause disease in humans.[21]

Table 9[21] lists the hypothesized microbiologic advantages of xenotransplantation. In addition, there is the possibility that a xenograft might, through genetic manipulation, be used as a vehicle for introducing novel genes or biochemical processes which could be of therapeutic value for the transplant recipient.[37]

Table 9 ***The Microbiologic Advantages of Xenotransplantation***

Xenotransplantation may provide significant benefits in regard to the risk of infection to the recipient. The patient suffering organ failure can receive a transplant on a semi-elective basis. Thus:

1. The "donor" can be carefully screened for the presence of infection without exposure to the nosocomial pathogens that are found in many cadaver donors.
2. In-hospital waiting times are reduced, avoiding nosocomial exposures that accompany intubation, IV lines and catheters as well as clinical "deconditioning."
3. The recipient can be "optimized" physically, nutritionally, and in terms of vaccinations and antimicrobial therapies.
4. The xenogeneic organs *may* be resistant to infection with viral pathogens Of humans including HIV (1 and 2), HTLV, hepatitis viruses, and herpes Viruses including human CMV.[21]

Suffice it to say, though research on xenotransplantation continues quietly at major transplant centers, it is in it's infancy as far as being accepted as a viable clinical alternative to conventional transplantation.

Conclusion:

In the USA, there are 261 transplant centers with 853 transplant programs covering kidney, liver, pancreas, intestine, heart, heart-lung and lung transplants. Table 10[36], Table 11[36] and Table 12[36] are recent statistics regarding number of solid organ transplants performed, number of donors recovered and number of patients on waiting lists for solid organ transplants.

Table 10 **Number of Transplants Performed in 1998**

Type of Transplant	Number
Kidney alone transplants (4,153 were living donors)	12,166
Liver transplants	4,487
Pancreas alone transplants	248
Kidney-pancreas transplants	973
Intestine transplants	69
Heart transplants	2,345
Heart-lung transplants	47
Lung transplants	862
Total	21,197

Table 11 **Number of Donors Recovered, 1998**

Type of Donation	Number
Cadaveric	5,799
Living	4,274
Total	10,073

Table 12 **The UNOS national patient waiting list (1/2000)**

Type of Transplant	Patients Waiting For Transplant
Kidney transplant	44,117
Liver transplant	14,554
Pancreas transplant	829
Pancreas islet cell transplant	182
Kidney-pancreas transplant	2,148
Intestine transplant	124
Heart transplant	4,076
Heart-lung transplant	221
Lung transplant	3,600
Total	**Total Patients: 67,491**

Table 13[39] shows survival rates for major solid organ transplants. (1997)

Table 13 Overall Graft and Patient Survival Rates by Organ Transplanted

	Graft Survival (%)		Patient Survival (%)	
Organ	1 yr	3 yr	1 yr	3 yr
Kidney	83	76	94	89
Pancreas	74	67	91	84
Heart	82	74	83	75
Liver	79	62	79	71
Lung	70	53	72	55
Heart-Lung	62	50	62	50

Transplantation is a complex medical science. The success of the transplant endeavor is contigent upon "the team approach" of scientists as well as clinicians versed in transplantation medicine. It is only when each component is in place, that one achieves the ultimate goal - sustaining a reasonable quality of life with a functional allograft.

References

1. Patel R, Paya CV. Infections in Solid-Organ Transplant Recipients. *Clinical Microbiology Reviews*. 1997;10 (1): 86-124.
2. Paterson DL, Singh N. Interactions Between Tacrolimus and Antimicrobial Agents. *Clinical Infectious Diseases*. 1997;25:1430-40.
3. Poterucha JJ, Wiesner RH. Liver Transplantation and Hepatitis B. *Ann Intern Med*. 1997;126(8): 805-807.
4. Tolkoff-Rubin NE, Rubin RH. Viral Infections in Organ Transplantation. *Transplantation Proceedings*. 1998;30: 2060-2063.
5. Dousset B, Conti F, et al. Acute Vanishing Bile Duct Syndrome after Interferon Therapy for Recurrent HCV Infection in Liver-Transplant Recipients. *N. Engl. J. Med*. 1994;330(16): 1160-61.
6. Sheiner PA, Boros P, et al. The Efficacy of Prophylactic Interferon Alfa-2b in Preventing Recurrent Hepatitis C after Liver Transplantation. *Hepatology*. 1998;28:831-838.
7. Singh N, Gayowski T, et al. Interferon-α for Prophylaxis of Recurrent Viral Hepatitis C In Liver Transplant Recipients. *Transplantation*. 1998;65(1):82-86.
8. Feray C, Samuel D, et al. An Open Trial of Interferon Alfa Recombinant for Hepatitis C after Liver Transplantation: Antiviral Effects and Risk of Rejection. *Hepatology*. 1995;22:1084-1089.
9. Wright TL, Combs C, et al. Interferon-α Therapy for Hepatitis C Virus Infection after Liver Transplantation. *Hepatology*. 1994;20:773-779.
10. Cattral MS, Krajden M, et al. A Pilot Study of Ribavirin Therapy for Recurrent Hepatitis C Virus Infection After Liver Transplantation. *Transplantation*. 1996;61(10): 1483-1488.
11. Bizollon T, Palazzo, et al. Pilot Study of the Combination of Interferon Alfa and Ribavirin as Therapy of Recurrent Hepatitis C After Liver Transplantation. *Hepatology*. 1997; 26:500-504.
12. Fishman J, Rubin RH, et al. Hepatitis C Virus and Organ Transplantation. *Transplantation*. 1996;62(2):147-154.
13. Dummer J, Ho M. Risk factors and Approaches to Infections in Transplant Recipients. *Mandell Principles and Practice of Infectious Diseases* 5th edition, 2000;vol. 2; chap. 302:3126-3136.
14. Montoya J, Remington J. Toxoplasma gondii. *Mandell Principles and Practice of Infectious Diseases*. 5th edition, 2000; vol. 2; chap. 268:2858-2888.
15. Orr KE, Gould FK, et al. Outcome of *Toxoplasma gondii* Mismatches in Heart Transplant Recipients over a Period of 8 Years. *J. of Infection*. 1994; 29:249-253.

16. Luft BJ, Naot Y, et al. Primary and Reactivated Toxoplasma Infection in Patients with Cardiac Transplants: Clinical Spectrum and Problems in Diagnosis in a Defined Population. *Ann. of Int Med.* 1983;99:27-31.

17. Fishman JA, Rubin RH. Infection in Organ-Transplant Recipients. *N Eng J. Med.* 1998; 338(24):1741-1751.

18. Kontoyiannis DP, Rubin RH. Infection in the Organ Transplant Recipient: An Overview. *Inf Dis Clinics of North America: Infection in Transplantation.* 1995;9(4): 811-822.

19. Meyers JD. Infections in Marrow Transplant. *Principles and Practices of Infectious Diseases.* 3rd ed., Churchill Livingston, London. 1990:2291-2294.

20. Kusne S, Dummer JS et al. Infections after Liver Transplantation: An analysis of 101 consecutive cases. Medicine [Baltimore]. 1988;67:132.

21. Fishman JA. Infection and Xenotransplantation: Developing Strategies to Minimize Risk. *Ann NY Acad Sci.* 1998;862:52-66.

22. Rubin RH. Impact of Cytomegalovirus Infection on Organ Transplant Recipients. *Reviews of Infectious Diseases.* 1990;12, sup. 7:S754-766.

23. Kanj SS, Sharara AI, et al. Cytomegalovirus Infection Following Liver Transplantation: Review of the Literature. *Clinical Infectious Diseases.* 1996;22:537-549.

24. Zaia JA, Forman SJ. Cytomegalovirus Infection in the Bone Marrow Transplant Recipient. *Infectious Disease Clinics of North America.* 1995;9(4):879-900.

25. Tolkoff-Rubin NE, Rubin RH, et al. Cytomegalovirus Infection in Dialysis Patients and Personnel. *Ann Intern Med.* 1978;89:625-628.

26. Falagas ME, Snydman DR, et al. Exposure to Cytomegalovirus from the Donated Organ Is a Risk Factor for Bacteremia in Orthotopic Liver Transplant Recipients. *Clinical Infectious Diseases.* 1996;23:468-474.

27. Richardson WP, Colvin RB, et al. Glomerulopathy Associated with Cytomegalovirus Viremia in Renal Allografts. *N Eng J of Med.* 1981;305(2):57-63.

28. Grattan MT, Moreno-Cabral CE, et al. Cytomegalovirus Infection is Associated With Cardiac Allograft Rejection and Atherosclerosis. *JAMA.* 1989;261:3561-3566.

29. Hibberd PL, Snydman DR. Cytomegalovirus Infection in Organ Transplant Recipients. *Infectious Diseases Clinics of North America.* 1995;9(4):863-877.

30. Winston DJ, Emmanouilides C, et al. Infections in Liver Transplant Recipients. *Clinical Infectious Diseases.* 1995;21:1077-1091.

31. Hibberd PL, Tolkoff-Rubin NE, et al. Preemptive Ganciclovir Therapy To Prevent Cytomegalovirus Disease in Cytomegalovirus Antibody-Positive Renal Transplant Recipients. *Ann Intern Med.* 1995;123:18-26.

32. Plotkin SA. Vaccination Against Cytomegalovirus, The Changeling Demon. *Ped Inf Dis J.* 1999;18:313-326.

33. Roitt I, Brostoff J, et al. Transplantation and Rejection. 5th edition, *Immunology* 1998;Mosby; pp. 353-366.

34. Chapman LE, Salomon D, et al. Xenotransplantation and Xenogeneic Infections. *N Eng J Med.* 1995;333(22):1498-1501.

35. Stoye JP, Tissier PL, et al. Endogenous Retroviruses: A Potential Problem for Xenotransplantation? *Ann NY Acad Sci.*;1998;862:67-74.

36. Bigam D, Zhong R, et al. Xenotransplantation. *JCC.* 1999;42(1):12-16.

37. Platt JL, Lin SS. The Future Promises of Xenotransplantation. *Ann NY Acad Sci.* 1998;862:5-18.

38. United Network for Organ Sharing Scientific Registry 2000.

39. Infections in Solid Organ Transplant Recipients. *Mandell Principles and Practice for Infectious Diseases*, 5th edition, 2000. vol 2. Chap 304:3148-3159.