Nonhuman Primate Infections after Organ Transplantation

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Abstract

Nonhuman primates, primarily rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and baboons (*Papio* spp.), have been used extensively in research models of solid organ transplantation, mainly because the nonhuman primate (NHP) immune system closely resembles that of the human. Nonhuman primates are also frequently the model of choice for preclinical testing of new immunosuppressive strategies. But the management of post-transplant nonhuman primates is complex, because it often involves multiple immunosuppressive agents, many of which are new and have unknown effects. Additionally, the resulting immunosuppression carries a risk of infectious complications, which are challenging to diagnose. Last, because of the natural tendency of animals to hide signs of weakness, infectious complications may not be obvious until the animal becomes severely ill. For these reasons the diagnosis of infectious complications is difficult among post-transplant NHPs. Because most nonhuman primate studies in organ transplantation are quite small, there are only a few published reports concerning infections after transplantation in nonhuman primates. Based on our survey of these reports, the incidence of infection in NHP transplant models is 14%. The majority of reports suggest that many of these infections are due to reactivation of viruses endemic to the primate species, such as cytomegalovirus (CMV), polyomavirus, and Epstein-Barr virus (EBV)–related infections. In this review, we address the epidemiology, pathogenesis, role of prophylaxis, clinical presentation, and treatment of infectious complications after solid organ transplantation in nonhuman primates.

Key Words: immunosuppression; infection; nonhuman primate; transplant

Introduction

Because the nonhuman primate (NHP[1]) immune system closely resembles its human counterpart (Bontrop et al. 1989; Jonker and Nooij 1986), nonhuman primates—especially rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and baboons (*Papio* spp.)—are frequently used in models of solid organ transplantation (Bontrop et al. 1989; Jonker and Nooij 1986). These models support the development of strategies for tolerance induction, allow researchers to delineate details of the immune response to transplanted organs, and enable tests of new therapeutics before testing in human patients.

Solid organ transplant models necessarily involve immunosuppressive medications, which make the animals susceptible to various infectious complications. Furthermore, the risk for certain types of infection may vary based on the origin of the research animals as well as the choice of immunosuppression used in the study. Often, especially when new treatments are first applied to nonhuman primates, the risk of infection may be unknown initially, and unexpected signs and symptoms of infection may not become apparent until the end of the study period. But there are few reports in the NHP literature of opportunistic infections after solid organ transplantation. We have therefore, for the purpose of this review, combined the observations made in nonhuman primates with those from the human literature.

Epidemiology

Nonhuman primate solid organ transplant models have generally been limited to small numbers of animals (<30) because NHP research is quite costly. Because of these small sample sizes, we were not able to find a value for the overall incidence of post-transplant infections in the NHP literature. Therefore, to determine the incidence of infection after nonhuman primate transplantation, we performed a literature review in PubMed. The following search terms were limited to the last 5 years: "infection AND organ transplant AND macaca" (n = 21), "transplant AND baboon NOT stem cell

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[1]Abbreviations used in this article: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV or SIV, human or simian immunodeficiency virus; NHP, nonhuman primate; PTLD, post-transplant lymphoproliferative disease

NOT bone marrow" (n = 124), and "transplant AND macaca NOT stem cell NOT bone marrow" (n = 159). We screened the titles of the resulting papers to include only those applicable to solid organ transplantation (n = 94). Our review of the 94 abstracts and/or papers found 36 that commented on infectious complications. In these 36 papers, a total of 828 animals underwent transplants and there were 114 infections, thus the incidence of post-transplant infection was 14%.

Risk factors for infection after solid organ transplantation should be evaluated in the following categories: environmental exposures, the overall level of immunosuppression, and the time elapsed since transplantation (Rubin et al. 2001). It is also important to keep in mind that post-transplant infection rates vary by the type of organ transplanted (San Juan et al. 2007).

Environmental and Technical Exposures

Exposures to infectious agents may be environmental, technical, or immunosuppression-related. Environmental exposures may be room-specific or related to transportation for medical procedures (Rubin et al. 2001). Reports have described *Shigella* outbreaks in isolated rooms of primate housing where one or more asymptomatic carriers spread the infection (Arya et al. 1973; Banish et al. 1993; Wolfensohn 1998). Researchers have also documented outbreaks of simian parvovirus, Epstein-Barr virus–related lymphoproliferative disorder, BK virus, and cytomegalovirus (CMV[1]) among post-transplant NHPs (Asano et al. 2003; Borie et al. 2005; Jonker et al. 2004; Mueller et al. 2002; Pearson et al. 2002; Schmidtko et al. 2002; Schroder et al. 2006; van Gorder et al. 1999).

Environmental exposure may also result from building construction of primate housing facilities, during which exposure to *Aspergillus* may become an issue (Rubin 2002a). Some pathogens are spread via caregiver interactions (Fietze et al. 1994; Rubin et al. 2001); regowning and -gloving when entering a room of immunosuppressed NHPs may help to prevent potentially hazardous infections.

For all these reasons nonhuman primates involved in organ transplantation warrant particularly careful maintenance and sanitation of their housing environment.

Technical exposures include infections resulting from the operation itself, such as abscesses, urinary infections due to catheter placement or other urinary instrumentation, pneumonia due to aspiration and intubation, or local infectious complications from intravenous (or other) catheter insertions.

Level of Immunosuppression

The overall level of immunosuppression can be estimated in various ways. The amount, duration, and type of immunosuppressive drug used, the presence of comorbid illnesses, and the presence of immunomodulating viral infections are all indicators of the level of immunosuppression (Rubin 2002a). Comorbid illnesses include preexisting immune deficiencies, technical complications, neutropenia and/or thrombocytopenia (often a result of immunosuppression), metabolic derangements (e.g., diabetes, malnutrition), hypogammaglobulinemia, and advanced age. Immunomodulating viral infections include cytomegalovirus (CMV), Epstein-Barr virus (EBV[1]), varicella zoster virus (VZV), herpes simplex virus (HSV), and human herpesvirus 6 (HHV-6) (Rubin 2002a).

Post-Transplant Periods of Infection

The risks of infectious complications sort themselves into three periods, each with its own set of expected pathogens: early infections occur within 1 month of transplantation, intermediate infections occur 1 to 6 months after transplantation, and late infections occur more than 6 months after transplantation (Baden et al. 2003a; Rubin 2002a; San Juan et al. 2007).

Early Infections

Early infections include wound infections, pneumonia, central line infections, and urinary tract infections. Over 95% of these common postoperative infections are caused by bacteria or *Candida* (Rubin et al. 2001). Early infections may also include HSV, hepatitis B, human or simian immunodeficiency virus (HIV or SIV[1]), West Nile virus, or rabies; these are often unrecognized in the donor and are transmitted via the transplanted organ (Marty and Rubin 2006). Opportunistic agents are rarely the cause of infection during this period, unless there is the possibility of an intense exposure (Marty and Rubin 2006; San Juan et al. 2007).

Intermediate Infections

From 1 to 6 months after transplantation, lingering and latent infections tend to emerge. These include CMV, EBV, VZV, HSV, herpes B virus, HHV-6, and SIV. In addition, hepatitis C recurrence may surface at this time. Mycoses also begin to bud; these include fungal infections, most commonly *Candida* and *Aspergillus*, and more rarely *Nocardia*, *Toxoplasmosis*, *Listeria*, *Pneumocystis jiroveci*, *Cryptococcosis*, and endemic mycoses such as *Coccidioidomycosis*, *Histoplasmosis*, and *Blastomycosis* (Marty and Rubin 2006). It is important that primate housing be constructed and maintained appropriately to minimize exposure to sources of mold (Silveira and Husain 2007). Urinary tract infections may also occur during this period, especially after kidney or kidney-pancreas transplantation (Marty and Rubin 2006).

Late Infections

After 6 months, the main risk of infection returns to community-acquired respiratory viruses and benign urinary in-

fections (Marty and Rubin 2006). Most of these infections are uncomplicated and have a good outcome, especially if treated with appropriate antiviral or antibiotic therapy. Recurrent hepatitis B and C continue to reemerge during the late period. These diseases are tedious to manage due to the need for a fine balance of immunosuppression and immunostimulating antiviral therapies.

Human transplant recipients who have had a complicated recovery constitute an exception to these late-period risks (Rubin 2002a): earlier infectious complications, multiple rejections requiring increased immunosuppression, poor graft function, or other adverse events predispose these recipients to fungal infections, most commonly *Candida* and *Cryptococcus*, and less often *Aspergillus* or *Zygomycetes*. Cytomegalovirus chorioretinitis and other CMV-related complications may arise as well. It is uncertain whether the incidence of fungal infections or CMV infections at this time is partially due to the end of prophylaxis. Rubin and colleagues (2002a) have suggested that a subgroup of "chronic never-do-wells" may need to continue prophylaxis for 6 to 12 months or longer, depending on the level of immunosuppression.

Pathogenesis

There are several approaches to evaluating the pathogenesis of infections after solid organ transplantation. One option is to look at their causes—environmental, technical, or related to the immunosuppression (Rubin 2002a). A second approach is to consider the timing of the pathogenesis relative to the date of transplantation. In this section, we describe a third alternative, based on dividing the infections according to their etiology into subgroups of bacterial, viral, and fungal infections.

In addition to those discussed in the sections below, many infections are endemic to nonhuman primates (Merck 2005) and can develop into serious illness in immunosuppressed animals. When possible, these should be recognized and treated during the quarantine period. However, some of these infections may not become apparent until after immunosuppression has begun; therefore, one should keep an open mind in developing a differential diagnosis.

Bacterial Infections

Bacterial infections frequently occur in the early postoperative course, but they may also emerge later in the form of community-acquired illnesses (Marty and Rubin 2006; San Juan et al. 2007). Two common endemic bacterial gastrointestinal pathogens in NHPs that may result in infectious disease after transplantation include *Shigella* and *Campylobacter jejuni*. *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia*, *Salmonella*, and other bacteria are also potential pathogens. Pulmonary bacterial pathogens include *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Bordetella bronchiseptica*, *Haemophilus influenzae*, other *Streptococcus* species, *Staphylococcus* species, and *Pasteurella* species (Merck 2005). At least two studies have reported bacterial pneumonia after NHP transplantation (Asano et al. 2003; Kuwaki et al. 2004).

Viral Infections

Because most immunosuppressive drugs used in transplantation target T cell–mediated immunity, viral and fungal infections are common complications of immunosuppression. Viral infections after transplantation are dominated by CMV, BK polyomavirus (BK virus), and EBV. Of these, EBV can lead to post-transplant lymphoproliferative disease (PTLD[1]).

The principal investigator and the attending veterinarian should decide together which of the animals about to undergo transplantation may need screening for these viruses.

Cytomegalovirus

Cytomegalovirus (CMV) has been isolated from a number of nonhuman primate species (Barry et al. 2006). The best characterized of these is rhesus CMV (RhCMV), which has been described as having a pathogenesis similar to that seen in humans (Barry et al. 2006). And, like humans with CMV, the vast majority of rhesus macaques, whether in the wild or in captivity, are asymptomatically seropositive for RhCMV (Barry et al. 2006; Vogel et al. 1994). As such, it is not surprising that, just as CMV infection after organ transplantation plays a central role in human transplants (Rowshani et al. 2005), post-transplant RhCMV infection has been reasonably well described in nonhuman primates.

Jonker and colleagues (2004) have described RhCMV reactivation in previously immune NHPs after organ transplantation: three of six animals treated with an investigational drug (CDP855) developed reactivation of RhCMV; two of these cases were proven histologically by characteristic inclusion bodies. Asano and colleagues (2003) treated three baboons with ganciclovir when the animals developed symptomatic CMV infections after receiving cardiac xenografts from rhesus macaques.

Pearson and colleagues (2002) found that CMV infection was the cause of death or euthanasia in three rhesus macaques after the animals had undergone renal allotransplantation and treatment with anti-CD40L, with or without concomitant CTLA4-Ig therapy. The animals presented with weakness, weight loss, and diarrhea (in two of the three animals); all either died from disseminated pulmonary CMV before the diagnosis could be made or were euthanized. The researchers learned afterward that these animals were all CMV-negative recipients of a CMV-positive allograft.

Mueller and colleagues (2002) demonstrated the reactivation of baboon CMV in two animals that received a porcine thymo-kidney xenotransplant. One animal presented

with respiratory failure, the other died from cardiopulmonary failure after 5 days of intravenous ganciclovir. Kean and colleagues (2007) recently described a series of rhesus macaques that underwent stem cell transplantation; in that cohort of 21 animals, 6 developed CMV reactivation.

Kean and colleagues (2007) have described a regimen of CMV prophylaxis. In addition, based on the results of Pearson and colleagues (2002), animals that are CMV-naïve should receive their organ from a CMV-naïve donor (along with antiviral prophylaxis), whereas animals that show evidence of immunity to CMV may receive any donor organ. Because of the persistent risk of CMV reactivation, however, it is advisable to periodically monitor immune animals for this development.

Polyomavirus

Vilchez and Kusne (2006) have compiled an excellent review on polyomavirus infections after NHP organ transplantation, including a discussion of simian virus 40 (SV40; more on this below), **JC virus**, and BK polyomavirus (**BK virus**). In NHPs, both SV40 and BK virus have been associated with hemorrhagic cystitis and ureteral infections, whereas JC virus results in progressive multifocal leukoencephalopathy. In humans, the incidence of post-transplant BK virus is 5-20% (Fishman 2002; Nickeleit et al. 1999, 2000).

In reports of human transplants, Tantravahi and colleagues (2007) have described the implications of polyomavirus infections after human kidney transplantation. BK virus causes interstitial nephritis and tubular atrophy and accounts for 10% of late allograft loss (Tantravahi et al. 2007).

Light and electron microscopy is the best method of diagnosis, as viral inclusion bodies on kidney biopsy are the gold standard for BK virus diagnosis. BK virus PCR is also effective, as it can quantify the viral load and allow physicians to follow the response to treatment. Tantravahi's group recommends polyomavirus screening every 3 months for 2 years as well as any time there is concern for allograft dysfunction or a biopsy is indicated for other reasons.

Simian virus 40 (SV40) is a polyomavirus endemic to several species of macaques (Jones-Engel et al. 2006). High rates of seropositivity for anti-SV40 antibodies have been reported for both captive and free-ranging animals (Bofill-Mas et al. 2004; Jones-Engel et al. 2006; Viscidi et al. 2003); however, SV40 infections are lifelong but asymptomatic.

We are unaware of any cases of SV40-related kidney allograft dysfunction, but there have been two reports of allograft dysfunction associated with other polyomaviruses. Van Gorder and colleagues (1999) reported the reactivation of cynomolgus polyomavirus (CPV) in 12 of 57 (21%) cynomolgus macaques 3 to 11 weeks after the initiation of immunosuppression for organ transplantation. Its major presentation was that of interstitial nephritis in the allograft, xenograft, or even the native kidney. Renal dysfunction was associated with these infections, and one animal developed diarrhea. Similarly, Borie and colleagues (2005) found polyomavirus nephritis in two animals that were treated with a high dose of an experimental drug and subsequently presented clinically with rejection at days 34 and 63.

Epstein-Barr and Other Viruses

The vast majority of adult humans are chronically but asymptomatically positive for **Epstein-Barr virus (EBV)** infection. In a small fraction of the population, though, EBV is associated with the development of lymphomas, including post-transplant **lymphoproliferative disease (PTLD)**. The incidence of PTLD ranges from 1% to 10% in humans (Boyle et al. 1997; Dharnidharka et al. 2002; Finn et al. 1998) and varies with the type of organ transplanted; in small bowel transplantation, the incidence can be as high as 20%. The main risk factors for PTLD include an EBV-naïve recipient and prolonged and intense immunosuppression (Ho et al. 1988; Sokal et al. 1997).

EBV-related herpesvirus, or **lymphocryptovirus (LCV)**, is similarly associated with lymphomas in immunosuppressed NHPs. Schmidtko and colleagues (2002) have described an endogenous LCV in baboons and macaques that is capable of producing PTLD. The virus is endemic to Old World macaques and resides latently in B cells in most adult animals (Moghaddam et al. 1998). The animals in the Schmidtko study presented with lymphadenopathy, and PTLD was diagnosed by lymph node biopsy (Schmidtko et al. 2002). A similar infection has been described by Feichtinger and colleagues (1992) in a monkey model of HIV.

Other viruses known to infect nonhuman primates after transplantation include **simian parvovirus**, which Schroder and colleagues (2006) described as presenting with severe anemia, lethargy, weight loss, and anorexia in a group of cynomolgus macaques that had undergone heterotopic cardiac transplantation. **Simian T cell leukemia virus** infection has also been linked to the development of post-transplant lymphoproliferative disorders (Stevens et al. 1992) in rhesus macaques.

The **hepatitis viruses** A, B, and C, **herpesviruses,** and **SIV** are all potential pathogens in NHPs. Baboons are susceptible to human hepatitis B virus infections (Kedda et al. 2000). Kean and colleagues (2007) have described reactivation of herpes B virus (*Cercopithecine herpesvirus* 1) infection in 3 of 21 rhesus macaques undergoing hematopoietic stem cell transplantation; two of the monkeys were euthanized due to complications that may have stemmed from the herpes B reactivation. Because the virus can be reactivated during times of intense immunosuppression, researchers and animal care staff must be careful to always use appropriate biosafety precautions when handling macaques, their secretions (e.g., urine, stool, vomit), and their tissue samples (blood or biopsies). It is also important to note that herpes B infection, while generally benign in healthy primates, is highly lethal in infected humans.

Fungal Infections

In humans, fungal infections occur as primary infections, reactivations that then disseminate, or reinfections with dissemination in patients who were previously immune. Ninety-five percent of fungal infections enter the host via the respiratory tract.

Candida and *Aspergillus* are the most common fungal infections after organ transplantation in humans. *Candida* infections are often due to the presence of central lines and the use of broad-spectrum antibiotics; *Aspergillus* is ubiquitous, but spores are released at especially high levels when construction is taking place either at the facility or in the vicinity (Rubin 2002a). *Aspergillus* may also be introduced due to aspiration during the placement or in the presence of an endotracheal tube. Because both *Candida* and *Aspergillus* are ubiquitous, infections with these organisms may occur early after transplantation, especially when there is high-level exposure.

Case reports describe the presence of endemic mycoses—including blastomycosis, coccidioidomycosis, histoplasmosis, and cryptococcosis—in baboons and macaques (Baskin 1991; Breznock et al. 1975; Graybill et al. 1990; Migaki et al. 1982; Pal et al. 1984; Rosenberg et al. 1984; Wilkinson et al. 1999). Zygomycosis, mucormycosis, and other rare fungal infections may also present in immunosuppressed nonhuman primates.

The Role of Prophylaxis

In humans, prophylaxis is concomitant with any immunosuppressive protocol to prevent infection and thus make immunosuppression relatively "safe" (Marty and Rubin 2006). There are no guidelines for the prophylactic regimen to use with nonhuman primate transplantation.

Prophylaxis in NHP transplantation is of two types: for wounds and for opportunistic infections. Wound prophylaxis is most effective when given intravenously within 2 hours before the initial surgical incision (Classen et al. 1992). No further wound prophylaxis is necessary, but our practice has been to cover the incision postoperatively with antibiotic ointment.

Prophylaxis against opportunistic infections includes the use of trimethoprim/sulfamethoxazole (TMP/SMX) to prevent urosepsis, *Pneumocystis carinii* pneumonia, listeriosis, and toxoplasmosis (Baden et al. 2003b; Fox et al. 1990; Tolkoff-Rubin et al. 1982; Tolkoff-Rubin and Rubin 1992, 1997; Torre-Cisneros et al. 1999). TMP/SMX prophylaxis is usually continued for 6 months after transplantation in humans. We have used it to various extents in our animal research, usually in consultation with the attending veterinarian. In animals with poor renal function after transplantation, the dose of TMP/SMX may need to be adjusted to avoid toxicity.

Similarly, human patients in many transplant centers receive CMV prophylaxis (valganciclovir) for 3 to 6 months. CMV-positive recipients may not require prophylaxis against CMV but may undergo regular CMV-DNA capture assays and begin treatment only if CMV-DNA is found (Paya et al. 2004; Razonable et al. 2003). We have not given routine antiviral prophylaxis to our animals, but Kean and colleagues (2007) have described a regimen of CMV prophylaxis in rhesus macaques.

Clinical Presentation and Diagnosis

Immunosuppression in animals alters the clinical presentation of post-transplant infections. Because any sign of weakness may be detrimental to feral animals by making them subject to predators, nonhuman primates are especially talented at hiding signs of disease, thus making diagnosis exceedingly difficult. Indeed, diagnosis of post-transplant infections may be almost impossible until the animal is near death. Thus, to successfully diagnose infection in an immunosuppressed monkey, a high index of suspicion is crucial. Animal care staff must be trained to watch closely for possible indications of illness. If an immunosuppressed animal demonstrates any signs that are even slightly out of the ordinary, these should be documented by animal care staff and communicated to the attending veterinarian and associated researchers immediately. Because indications of post-transplant infection are often subtle and vague, we have listed the signs of common bacterial, viral, and fungal infections after transplantation in Tables 1, 2, and 3 respectively.

An experienced veterinarian should conduct a thorough physical exam as soon as an animal shows suspicious symptoms. Soon after transplantation, this exam should focus on possible wound infections, pneumonia, urinary tract infections, or surgical site infections, whether intra-abdominal, pulmonary, or cardiac. Fungal infections and viral infections such as CMV, BK virus, EBV, and hepatitis develop and present between 1 and 6 months after transplantation. Symptoms may include mucosal lesions, lymphadenopathy, renal dysfunction, hemorrhagic cystitis, or disseminated infections with end-organ involvement. Abnormalities should be noted in the animal's chart and followed daily.

The workup of infectious disease in nonhuman primates is limited mainly by cost. It is important to consider how the workup and therapeutic treatment might affect the usefulness of future data to be gained from the animal. If the use of antimicrobial therapy will impair the quality of the data, the most cost-effective and humane step to consider is euthanasia. If data will remain useful during therapy, the cost of the workup and treatment still must be weighed against the value of the data, the ease of repeatability of the experiment, and, most importantly, animal welfare.

Depending on the presenting signs and the veterinarian's assessment, the following diagnostic tests may be useful:

Table 1 Bacterial infections after NHP transplantation[a]

Infectious cause	Timing	Presentation	Diagnosis	Treatment
Gram-positive and -negative skin flora	First month	Erythema Edema Purulent drainage from or near wound	Physical exam, culture	Incision and drainage Antibiotics
S. pneumoniae *K. pneumoniae* *B. bronchiseptica* *H. influenzae* *Streptococcus* spp. *Staphylococcus* spp. *Pasteurella* spp. *M. tuberculosis*	First month	Cough Rhinitis Fever Lethargy Anorexia Weight loss Dyspnea	Blood or sputum culture Chest x-ray	TMP/SMX (4/20 mg/kg) for *Shigella* Penicillin/aminoglycoside Cephalosporin/aminoglycoside Euthanasia for tuberculosis
Gram-negative bacteria	First month	Difficulty urinating	Urinalysis Urine culture	TMP/SMX Enrofloxacin
Shigella *C. jejuni* *E. coli* *P. aeruginosa* *Yersinia* *Lawsonia intracellularis* *Salmonella* *A. aerogenes* *Helicobacter*	Any time	Watery or bloody feces Dehydration Emaciation Prostration Gastritis Anorexia Vomiting	Stool culture Ova and parasites *C. difficile* toxin *H. pylori* screen	Enrofloxacin 5 mg/kg SID TMP/SMX (4/20 mg/kg) for *Shigella* Erythromycin for *Campylobacter*

[a]NHP, nonhuman primate; SID, once daily; TMP/SMX, trimethoprim/sulfamethoxazole

- Simple laboratory evaluations, such as serum chemistries, can be helpful in determining renal function and hydration status.
- Liver function tests may indicate hepatitis if aspartate transaminase (AST) and alanine aminotransferase (ALT) are elevated.
- Chest x-rays may reveal pulmonary infections, including pneumonia, aspergillosis, and tuberculosis.
- A urine dipstick, preferably done on a suprapubic tap or catheterized specimen, may reveal a urinary tract infection.
- Blood and urine cultures obtained from a sterile specimen will not only determine whether there is a bacterial infection but also identify the bacterium and thus guide its treatment by sensitivity analysis.

Other blood tests, such as CMV-DNA capture, EBV-DNA capture, and HCV-PCR, may identify the presence and active replication of these viruses. CMV-DNA capture has a high sensitivity of >95%, but specificity is low (positive predictive value 54%, negative predictive value 100%). This test is nonetheless useful for screening as it can detect replication before signs of disease appear (Geddes et al. 2003). For CMV, tissue cultures can be obtained, or animals can be tested for pp65 antigenemia in blood and in cerebrospinal fluid (Fishman et al. 2000; Murray et al. 1997; Razonable et al. 2003; Rowshani et al. 2005; Rubin 2002b; Rubin et al. 1981; Storch et al. 1994; Tanabe et al. 1997). If diarrhea is present, a stool sample should be screened for ova and parasites, culture, and also for *Clostridium difficile* toxin, especially if the animal recently received an antibiotic.

Fungal infections are primarily diagnosed by culture (Silveira and Husain 2007). Ultrasound exams may reveal abscesses and fluid collections, which may harbor infections but may be amenable to drainage. Surgical drainage is the treatment of choice, but in centers where animals are already trained to cope with intravenous lines, interventional drainage may be possible, with a drainage catheter left in place. If computed tomography (CT) is available and affordable, scans of the brain, lungs, and abdomen may reveal other causes of infection (Kong et al. 1990; Lewinsohn et al. 2006; Said et al. 2004).

Primary (de novo) CMV infection in NHPs is often asymptomatic. If symptoms are present, they are generally vague, and include fever, myalgia, pharyngitis, cervical lymphadenopathy, mild hepatitis, and/or splenomegaly. In a study by Pearson and colleagues (2002), animals that underwent renal transplantation and immunosuppression presented with weakness, weight loss, and (in two of the three animals) diarrhea. All either died from disseminated pulmonary CMV before the diagnosis could be made or were euthanized.

Most human transplant recipient/donor pairs are screened for CMV before the transplant and are risk-

Table 2 Viral infections after NHP transplantation[a]

Infectious cause	Timing	Presentation	Diagnosis	Treatment
Cytomegalovirus (CMV)	Months 1-6	Weakness Weight loss Diarrhea Fever Myalgia Cervical lymphadenopathy Hepatitis Splenomegaly Pneumonitis Respiratory failure Esophagitis Colitis	Blood, CSF, sputum, urine, or tissue culture CMV-DNA quantitative PCR CMV pp65 antigenemia Viral inclusion bodies on biopsy	SQ ganciclovir (5-6 mg/kg/dose BID) PO valganciclovir (12 mg/kg) Foscarnet for resistant CMV Prophylaxis with cidofovir (5 mg/kg IV) or valganciclovir
Polyomaviruses: BK virus JC virus Simian virus 40 (SV40) Cynomolgus polyomavirus (CPV)	Months 1-6	Persistent anemia Interstitial nephritis Renal dysfunction Hemorrhagic cystitis Ureteritis Ureteral stenosis Lethargy Anorexia Pancytopenia Desquamative pneumonitis Upper respiratory infection Enteritis Meningoencephalitis Progressive multifocal leukoencephalopathy	Viral inclusion bodies on biopsy Electron microscopy Enlarged atypical nuclei SV40 large T antigen immunostaining PCR	Reduction of immunosuppression Cidofovir Leflunomide Retinoic acid Interferon IVIg
Epstein-Barr virus (EBV) Epstein-Barr-related virus Lymphocryptoviruses	Months 1-6	Lymphadenopathy Multiorgan dysfunction Tumor masses	Lymph node biopsy: distorted architecture, atypical $DC20^{+}$ cells	Discontinue immunosuppression Rituximab Chemotherapy
Simian T cell leukemia virus (STLV)	First year	Death	Post-transplant lymphoproliferative disorder	
Simian parvovirus	Month 1-6	Severe anemia Lethargy Weight loss Anorexia	Complete blood count Bone marrow biopsy with viral inclusions in red cell precursors Parvovirus PCR	Supportive Reduction in immunosuppression

[a]BID, twice daily; CSF, cerebrospinal fluid; IV, intravenous; IVIg, intravenous immunoglobulins; NHP, nonhuman primate; PCR, polymerase chain reaction; PO, per oral; SQ, subcutaneous

stratified based on the recipient results in the presence of a CMV-positive donor. Most recipients have been exposed to CMV prior to transplantation, so reactivation is fairly common after the surgery. Manifestations of CMV in humans include mild fever, myalgia, leukopenia, thrombocytopenia, transaminase elevations, pneumonitis, esophagitis, colitis, or infection of the transplanted organ (Bronsther et al. 1988; Speich and van der Bij 2001).

Management and Treatment

Whereas in humans failed therapy is reason for retransplantation, this recourse is likely not applicable to NHP research. Diagnosis of a specific infection in a nonhuman primate calls for treatment with the appropriate antimicrobial therapy. More often, however, an infection is suspected before an accurate diagnosis is made; if so, the most likely

Table 3 Fungal infections after NHP transplantation[a]

Infectious cause	Timing	Presentation	Diagnosis	Treatment
Candida	Any time	Oral candidiasis Esophagitis Anorexia Peritonitis Candidemia Fever Candiduria	Blood, CSF, sputum, urine, or tissue culture	Oral prophylaxis with nystatin or clotrimazole Fluconazole
Aspergillus	Any time	Tracheobronchitis: lung transplant anastomosis shows necrosis, ulceration, and pseudomembranes Pulmonary aspergillosis: dry cough and dyspnea, low-grade fever, hemoptysis Disseminated aspergillosis	Galactomannan antigen testing Blood, urine, or tissue culture Histopathology Chest CT	Amphotericin Voriconazole Itraconazole Caspofungin Anidulafungin Micafungin
Cryptococcus	>6 months	CNS cryptococcosis: headache, fever, mental status changes Pulmonary cryptococcosis: asymptomatic with nodular infiltrates to acute respiratory failure Cutaneous cryptococcosis	Culture Direct microscopic examination Polysaccharide cryptococcal antigen test (serum); may have false negative results	Amphotericin B with 5-flucytosine for 2 weeks for disseminated or CNS disease Fluconazole for isolated pulmonary disease 6-12 months suppressive therapy after treatment
Mycoses: blastomycosis, histoplasmosis, coccidioidomycosis	First year or later	Fever Dyspnea Cough Depression Fatigue Anorexia Tachypnea Draining cutaneous abscesses	Histology of lesions: broad-based budding organisms Enzyme immunoassay Immunodiffusion assay Complement fixation assay Histoplasmosis antigen assay Blastomyces antigen assay	Fluconazole Amphotericin B Itraconazole Ketaconazole Voriconazole Caspofungin Anidulafungin Micafungin

[a]CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; NHP, nonhuman primate

causative agents should be addressed with anti-infective therapy.

Infected fluid collections require drainage for cure unless the accumulation is small. The treatment for polyomavirus is to reduce immunosuppression (Brennan et al. 2005), especially antimetabolites (azathioprine and MMF), but this approach may not be an option in some animal research protocols. Other treatment options have included the use of cidofovir (which is nephrotoxic), leflunomide, and intravenous immunoglobulins.

Cytomegalovirus infections are treated with intravenous ganciclovir or valganciclovir, the dosing of which is limited by myelotoxicity (Fishman et al. 2000; Marty and Rubin 2006). If resistant CMV arises, foscarnet is a treatment option but it carries the risk of nephrotoxicity (Paya et al. 2004).

Treatment of infections must take into account the regimen of immunosuppression (Marty and Rubin 2006). Calcineurin inhibitors, such as cyclosporine and tacrolimus, use the cytochrome P450 metabolic pathway in the liver and thus lead to multiple drug interactions. Failure to monitor drug levels during antibiotic therapy may result in underdosing and transplant rejection, or overdosing and drug toxicity. Among the most common interactions between antimicrobials and calcineurin inhibitors, rifampin, isoniazid, and nafcillin induce calcineurin inhibitor metabolism,

thus an increase in calcineurin inhibitor dose is likely warranted. On the other hand, macrolides (e.g., erythromycin, clarithromycin, azithromycin) and antifungal azoles (e.g., fluconazole, ketoconazole, voriconazole) inhibit calcineurin inhibitor metabolism, requiring a decreased dose of calcineurin inhibitor to prevent drug toxicity (Marty and Rubin 2006).

Certain combinations of antibiotics can be harmful independent of immunosuppressive regimen. Trimethoprim/sulfamethoxazole prophylaxis has caused renal toxicity, and the combination of gentamycin, amphotericin, or vancomycin with a calcineurin inhibitor has occasionally resulted in renal failure (Marty and Rubin 2006).

Last, the treatment options available in nonhuman primate research differ in at least one key aspect from the treatment of human patients: in research, one must always consider whether the most ethical treatment for a suffering animal may be euthanasia, rather than the prolonged use of antimicrobials and other interventions. This decision must be balanced with the value of the research data from each animal, and should depend on teamwork among the animal care staff, the attending veterinarian, the researchers, and the principal investigator.

Conclusion

Infections in nonhuman primates are difficult to diagnose after transplantation. Because animals hide pain well and do not communicate complaints, infections often go unnoticed until the animal is near death. If infection is suspected, the extent of the workup will depend on how vital it is to keep the animal alive for further data collection and on whether the data to be gained will be useful in the presence of infection and/or therapy. If data will be useful and the animal is not suffering unduly, diagnosis and treatment of the infection may be possible. On the other hand, euthanasia may in some cases be the most humane treatment. Samples from infected animals should be used with caution in laboratory research, due to the risk of contamination not only of research experiments but also of personnel.

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