Abstract

Despite advances in the husbandry of nonhuman primates, natural and experimentally induced diseases continue to pose risks to animal health. These risks are particularly important when such disease results in immunodeficient states that provide an opportunity for the development of opportunistic infections. Because opportunistic agents may serve as significant confounders to research and hold potential for zoonotic transmission, knowledge of disease pathogenesis, surveillance, and risk reduction is particularly important to individuals who work closely with primates. Endogenous diseases of primates that result in blunted immune responses and thus allow for the development of opportunistic infection include simian type D retroviruses and measles. In addition, simian immunodeficiency virus is a frequently studied experimental cause of immunosuppression. This article focuses on clinical and pathological aspects of the most common opportunistic infections that occur in nonhuman primates maintained in research settings. The complete elimination of all infectious agents from primate colonies may be impossible and unwarranted, but microbial surveillance programs can help both to define the complement of agents present in a colony and to elucidate their potential impacts on colony health, zoonotic risk, and experimental research. We discuss risk reduction through the use of quarantine procedures, specific pathogen-free animals, and environmental controls.

Key Words: immune dysfunction; immunosuppression; measles; opportunistic infections; simian immunodeficiency virus; simian type D retrovirus

Introduction

Advances in the husbandry of nonhuman primates (NHPs) maintained in research facilities have led to the routine housing of such animals under controlled conditions that include complete nutrition, shelter from the elements, environmental enrichment, and restricted access to animals that may harbor disease. Nonetheless, natural and experimentally induced diseases in nonhuman primates continue to pose risks to animal health and serve as significant confounders to research. These risks are particularly important when such disease results in immunodeficient states and thus provide an opportunity for the development of opportunistic infections (OIs). In this review we discuss the most common OIs that affect New World (NWP) and Old World primates (OWP) housed in the research setting.

Causes of Immunosuppression in Nonhuman Primates

Simian Type D Retroviruses

There are a number of endogenous NHP diseases that result in blunted immune responses. Of these, simian type D retroviruses (SRV; genus Betaelement, family Retroviridae) are arguably the most important to consider both for their risk to macaque colony health and as confounders to research. These viruses can be transmitted horizontally, vertically, or sexually by symptomatic or asymptomatic animals. Clinical signs, when present, may include diarrhea, weight loss, lymphadenopathy, splenomegaly, and retroperitoneal fibromatosis (Henrickson et al. 1983; Osborn et al. 1984).

The virus has a wide cellular tropism, infecting B and T
lymphocytes, macrophages, hematopoietic progenitor cells, and epithelial cells. Such infection often results in anemia or pancytopenia (Maul et al. 1988). While the mechanism that contributes to the profound cellular depletion is not completely understood, SRV strains are reported to downregulate major histocompatibility complex (MHC) class II (van Kuyk et al. 1991), reduce mitogen-induced proliferative response in peripheral blood mononuclear cells (PBMC) (Maul et al. 1985, 1986), decrease serum immunoglobulin production (Maul et al. 1986), and contribute to functional deficits in polymorphonuclear cells (Cheung and Gardner 1991). These defects of the humoral and cell-mediated immune response may contribute to the wide range of SRV-associated opportunistic infections such as bacterial septicemia, cytomegalovirus, candidiasis, cryptosporidiosis, and noma (Maul et al. 1986; Osborn et al. 1984).

Prevention is the key to eliminating complications due to SRV. Specific pathogen-free (SPF) colonies have been developed to eliminate the virus from research animals. Because both antibody-negative/viremic and antibody-positive/nonviremic states are possible, a combination of serology and virus isolation or molecular detection is necessary to determine whether an individual animal is infected (Guzman et al. 1999; Lerche et al. 1994).

**Measles Virus**

There are reports of measles virus (genus Morbillivirus, family Paramyxoviridae) in both OWPs and NWPs. Once established in primate colonies, the virus spreads rapidly by the aerosol route (Willy et al. 1999). In contrast to the disease in OWPs, which is usually mild or asymptomatic and associated with fever, upper respiratory signs, and a maculopapular exanthema, the disease in NWPs can be severe, with gastrointestinal signs and a high morbidity and mortality (Auwaerter et al. 1999; Chen et al. 2000; Levy and Mirkovic 1971; Lorenz and Albrecht 1980).

Measles virus immunosuppression is associated with a pronounced lymphopenia as well as decreases in neutrophils and monocytes (Okada et al. 2000). The lymphopenia results from depletion of infected and noninfected B and T lymphocytes (CD4+ and CD8+). Profound lymphoid depletion may also occur in the thymus, lymph nodes, and spleen (Figure 1). While CD4+ T cell counts can drop below 500/μL, host defenses may be bolstered by a compensatory increase in natural killer cell activity (Okada et al. 2000). Contributions of measles virus to immunosuppression are likely multifactorial and include reduced delayed type hypersensitivity responses, T lymphocyte functional deficits, altered cytokine levels, inhibition of dendritic cell function, reduced immunoglobulin production, and inhibition of IFN-γ upregulation of the MHC-II antigen (Kerdiles et al. 2006). Immune defects may persist for up to 6 months after infection (McCchesney et al. 1989).

OIs after natural measles infection in macaques include disseminated CMV, adenoviral and bacterial pneumonia, and candidiasis (Choi et al. 1999). Immune dysfunction from measles virus is also known to interfere with the response to old mammalian tuberculin (Staley et al. 1995).

Measles virus is most often acquired from contagious human handlers and is preventable by vaccination of NHPs and strict adherence to personal protective equipment (PPE) requirements. Serologic assays are an effective method of periodically assessing the adequacy of vaccination programs.

**Simian Immunodeficiency Virus**

Of the experimentally induced infections, simian immunodeficiency virus (SIV; genus Lentivirus, family Retroviridae) is among the most thoroughly studied viruses that contribute to the development of OIs (Hirsch and Lifson 2000; Letvin and King 1990; Stump and Vandewoude 2007). Scientists concurrently recognized and isolated the virus in the 1980s at the New England, California, Yerkes, Tulane, and Washington Primate Research Centers (Benveniste et al. 1986; Daniel et al. 1985; Fultz et al. 1986; Letvin et al. 1985; Murphey-Corb et al. 1986).

SIV strains naturally infect a large number of African macaque species and, while usually asymptomatic in these primate species, experimental infection in Asian macaque species results in an immunodeficiency syndrome remarkably similar to human immunodeficiency virus (HIV; Hirsch et al. 1995). Although strain and isolate dependent, SIV not only has a similar genetic organization to HIV but also shares tropism for CD4+ T lymphocytes and monocytes.
using the CD4 and CCR5 chemokine receptors for viral entry. Genetically engineered chimeric viruses consisting of SIV internal structural proteins and the HIV-1 envelope (SHIV) are also widely used experimentally for determining the efficacy of candidate vaccines targeting the HIV-1 env glycoprotein (Lu et al. 1996).

SIV infection of Asian macaques typically has three phases (Figure 2). Primary infection occurs 1 to 3 weeks after inoculation and is characterized by viremia, transient leukopenia, and prodromal signs such as fever, lymphadenopathy, diarrhea, rash, anorexia, and malaise. It is during this phase that the SIV-specific antibody response develops.

The asymptomatic phase of infection is characterized by a stabilization of viral load, termed the viral load "set point." There is initially a slight rebound in circulating CD4+ cell numbers followed by a gradual decline. Characteristics of the third and final phase, simian acquired immune deficiency syndrome (SAIDS2), include profound CD4+ cell depletion and onset of OIs and SAIDS-associated pathologies such as giant cell disease, lymphocytic interstitial pneumonitis, myocarditis, encephalitis, nephropathy, neuropathy, and wasting disease, among others. Table 1 lists the most common AIDS2-associated OIs in HIV patients and in macaques experimentally inoculated with SIV.

The normal progression of disease averages about 18 months, but additional phenotypes such as the elite controller and rapid progressor profiles may be demonstrated over time courses. Elite controllers have a vigorous cell-mediated and humoral immune response to infection and are able to limit viral replication, whereas rapid progressors have a reduced antibody response and survival of 3 to 4 months. Influences on pathogenicity include viral strain, species of animal inoculated, virus tissue culture passage, and host characteristics.

Decreased numbers of circulating CD4+ T cells are the hallmark of SIV/HIV infections and the primary contributor...
to immunodeficiency. A number of factors likely contribute to CD4+ cell decline and include virally mediated destruction of infected cells, destruction of CD4+ cells by virus-specific cytotoxic T lymphocytes or natural killer cells, antibody-dependent cell-mediated cytoxicity, activation-induced cell death, and destruction of lymph node and thymic architecture leading to reduced regenerative capacity (McCune 2001). Onset of OIs often occurs when the absolute circulating CD4+ cell count drops below 250 to 400/μL (Hirsch et al. 2004).

Quantitative deficits in total circulating T cell counts are likely not the only factor contributing to HIV/SIV-induced immunosuppression. HIV patients who initiate antiretroviral therapy at lower CD4+ T cell nadirs demonstrate incomplete functional immune restoration despite a return to normal levels of CD4+ cell counts (Lange and Lederman 2003). Studies suggest that persistent low numbers of specific T cell subsets—such as the memory phenotype or T cells that express CD28, a co-receptor critical for T cell activation—contribute to reduced antigen-specific reactivity and subsequent incomplete immune reconstitution (Hirsch et al. 2004; Lange et al. 2002). In addition, loss of CD4+ effector memory T cells in extralymphoid interface tissues such as the gut, lung, and genital tract may increase susceptibility to OIs (Picker 2006).

Although these infectious agents are well known for their contribution to immunosuppression in NHPs, environmental and host factors such as stress, malnutrition, and age probably also contribute to reduced immune responses. A further contributor, experimental chemical/radiological immune suppression for solid organ transplant, is the subject of an article by Haustein and colleagues in this issue.

**Viral Opportunistic Infections**

**Herpesviridae Subfamily Betaherpesvirinae**

Simian *cytomegalovirus* (CMV) infections have presented in a variety of OWP and NWP species including macaques, African green monkeys, baboons, squirrel monkeys, tamarins, and chimpanzees (Eizuru et al. 1989; Elkington et al. 2004; Hendricks Hutto et al. 2004; Lorenz and Albrecht 1980; Rangan and Chaiban 1980). Although viruses in this group are thought to have a narrow host range, there are reports of cross-species transmission (Mueller et al. 2002). Seroprevalence surveys in rhesus macaques have revealed that 95% of adult animals may be infected and that seroconversion occurs primarily during the first year of life (Andrade et al. 2003; Vogel et al. 1994).

The virus is readily transmitted in oral secretions, breast milk, and urine (Barry et al. 2006). Vertical transmission, important in human CMV (HCMV) infections, has not yet been documented in the NHP (Barry et al. 2006). Exposure can be determined by enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR). Disease in the healthy, mature host is usually asymptomatic and associated with latent virus present in glandular tissue, lymphoreticular cells, kidney, PBMC, and myeloid progenitor cells (Klotman et al. 1990; Sinclair and Sissons 2006). In contrast, immunodeficient states are associated with reactivation and dissemination. Loss of CD4+ T cell-mediated immunity and subsequent reduction in the CD8+ cytotoxic T lymphocyte response are thought to play a crucial role in reactivation (Elkington et al. 2004; Kaur et al. 1996, 2002). Disseminated CMV frequently occurs in conjunction with drug-induced immunosuppression and in SIV-infected macaques (Jonker et al. 2004; Mueller et al. 2002; Sequer et al. 2002; Simon et al. 1992). Clinical signs relate to the organ system affected and may include diarrhea, respiratory compromise, or neurologic sequelae. Less frequently a proliferative arteritis, neuritis, orchitis, or hepatitis may occur (Baskin 1987). Prophylaxis can be attempted with the antiviral medication ganciclovir.

CMV is slowly cytopathic, leading to nuclear and cytoplasmic enlargement with the presence of both intranuclear (often described as “owl’s eyes” inclusion bodies) and intracytoplasmic inclusions (Figure 3). Viral replication is accompanied by extensive necrosis and neutrophilic infiltrates. Multiple sites may be affected, including the nervous system, hepatobiliary system, lung, lymph nodes, spleen, intestine, and vasculature (Baskin 1987). In situ hybridization (ISH) or immunohistochemistry (IHC) often reveal many more cells infected than initially suspected on evaluation of routine hematoxylin- and eosin-stained tissue sections.

![Figure 3](image-url)
There is no known zoonotic potential associated with simian CMV, but HCMV infection is common, with seroprevalence rates of 50-90% (Alford et al. 1990). Although the majority of infections do not result in disease, HCMV is clinically important in both immunodeficient individuals and newborns (Barry et al. 2006). The advent of highly active antiretroviral therapy has helped reduce the impact of this disease; nonetheless, up to 40% of HIV patients with advanced disease can present with HCMV infection (Cheung and Teich 1999). HCMV also represents the most significant pathogen associated with post-transplantation complications (Pereyra and Rubin 2004). Because infection in rhesus macaques reflects the epidemiology and natural history of disease in both immunocompromised and immunocompetent individuals, researchers have suggested the utility of simian CMV as a model of human disease (Elkington et al. 2004; Kaur et al. 1996, 2002).

Herpesviridae Subfamily Gammaherpesvirinae

Rhesus rhadinovirus (RRV 1) is a gamma-2 herpesvirus closely related to Kaposi’s sarcoma herpesvirus (KSHV 1; human herpesvirus 8). Rhadinoviruses are thought to be species specific and have been reported in rhesus, pigtail, and cynomolgus macaques as well as in African green monkeys, baboons, chimpanzees, and gorillas (Bruce et al. 2005; Lacoste et al. 2000; Schultz et al. 2000). The macaque homologues of this family are the most completely characterized. Members of this family are classified into two distinct lineages: the rhadinovirus 1 lineage, which includes KSHV and the primate retroperitoneal fibromatosis herpesvirus (RFHV), and the rhadinovirus 2 lineage, which includes rhesus rhadinovirus (RRV) (Desrosiers et al. 1997; Rose et al. 1997). Transmission likely occurs via oral secretions, with seropositivity rates for RRV approaching 90% by 1 year of age and 98% by 2 years of age (Desrosiers et al. 1997). Infection with RRV can readily be detected by ELISA.

These viruses demonstrate two phases of infection: a latent (or nonproductive) phase and a lytic (or productive) phase. RRV demonstrates a tropism for CD20+ B lymphocytes, which likely serve as the primary site of RRV latency (Bergquam et al. 1999). Animals may cycle between both phases of infection, although the factors associated with a change from latency to reactivation remain unclear. Natural RRV infection generally remains asymptomatic, whereas experimental RRV inoculation produces a mild febrile response and a lymphoproliferative disorder, with characteristics resembling multicentric Castleman’s disease (Mansfield et al. 1999; Wong et al. 1999). Reports have also suggested an association between RFHV infection and retroperitoneal fibromatosis (Bruce et al. 2006). This association has been primarily observed in SRV type D coinfected macaques, although there are limited reports of SRV-negative/SIV-positive RFHV-associated retroperitoneal fibromatosis (Bielefeldt-Ohmann et al. 2005).

Histopathologic examination of lymphoid tissue from RRV-inoculated animals demonstrates an extensive lymphoid hyperplasia (Mansfield et al. 1999). Changes are initially characterized by paracortical expansion and vascular hyperplasia progressing to follicular hyperplasia. An increase in CD20+ B lymphocytes may occur in lymphoid tissue. Upon regression of lymphadenopathy, lymph nodes acquire features typical of multicentric Castleman’s disease, including hyalinized follicles surrounded by layers of loosely concentric lymphocytes. RFHV-associated retroperitoneal fibromatosis lesions are characterized by a proliferation of spindloid cells of mesenchymal origin (Figure 4). IHC with anti-latency-associated nuclear antigen (anti-LANA) antibody demonstrates reactivity within nuclei (Bruce et al. 2006).

Although the level of zoonotic risk is thought to be low, these viruses are closely related to KSHV, the causative agent of Kaposi’s sarcoma (KS). KS is a highly vascularized neoplasm derived from cells of endothelial origin that is diagnosed in both immunocompetent and immunocompromised individuals. KSHV is also involved in the pathogenesis of the lymphoproliferative disorders multicentric Castleman’s disease and pleural effusion lymphoma seen most commonly in AIDS patients. The ability to readily propagate rhadinoviruses in culture enables their use in the study of the transcription, virus structure and assembly, and protein interactions of gammaherpesviruses. Recapitulation of aspects of human disease upon inoculation of macaques makes RRV a suitable in vivo model for the study of KSHV pathogenesis.

Figure 4 Retroperitoneal fibromatosis occurs in the context of simian type D retrovirus infection and is characterized by the proliferation of neoplastic spindloid cells. Retroperitoneal fibromatosis-associated herpesvirus can be detected in lesions and is believed to play a role in tumorigenesis.
Simian lymphocryptoviruses (LCV) are also members of the gammaherpesvirinae subfamily that have been recognized in several species of Old World and New World primates (Cho et al. 2001; Ishida and Yamamoto 1987). Studies of the biology of these viruses have focused primarily on rhesus and cynomolgus macaques. LCV is readily transmitted with serosurveys, indicating that greater than 90% of adult macaques are persistently infected (Moghaddam et al. 1997). The virus is closely related to the human Epstein-Barr virus (EBV).

Infection is clinically silent in immunocompetent animals. Macaques rendered immunodeficient by SIV infection may demonstrate LCV-associated malignant lymphomas similar to the non-Hodgkin’s lymphoma seen in HIV-infected AIDS patients (Moghaddam et al. 1997; Rivailler et al. 2004). A retrospective analysis identified a 4.4% incidence of SIV-associated lymphoma, of which 89% of the cases were associated with LCV infection (Fortgang et al. 2004). Like RRV, LCV persists in B lymphocytes and demonstrates latent and lytic phases of infection. Periodic lytic phases in immunocompetent hosts are accompanied by shedding of the virus in oral secretions. Although cellular immune responses to LCV are unable to eliminate latent infection, T cell immunity is thought to be critical to the prevention of virus-induced lymphoproliferation (Fogg et al. 2006; Johannessen and Crawford 1999; Rooney et al. 1998). Loss of cytotoxic T lymphocyte activity in addition to genetic instability may facilitate development of LCV-associated neoplastic disease (Rivailler et al. 2004).

Lymphomas observed with LCV infection are most often B cell in origin and may be classified as large cell, immunoblastic, or Burkitt-like (Baskin et al. 2001; Fortgang et al. 2004). Viral infection is also associated with a proliferative epidermal lesion termed oral hairy leukoplakia (Baskin et al. 1995; Kikutok et al. 2004). This lesion commonly occurs in the oral mucosa and esophagus and less commonly in haired skin (Figure 5). It is characterized by hyperkeratosis, parakeratosis, and enlarged acanthocytes. Brick-shaped inclusion bodies may be present. Diagnosis can be confirmed with IHC or ISH. Common markers include EBNA2, the nuclear antigen essential for B cell immortalization; BZLF1, a lytic cycle protein; LMP-1, a membrane protein expressed during the latent cycle; and viral capsid antigen. ISH can be accomplished using probes for the small EBV-encoded RNAs (EBERs) expressed in latently infected cells.

Simian LCV has no known zoonotic potential. EBV, the human correlate of the disease, is the most common cause of infectious mononucleosis and can be associated with a variety of neoplastic conditions such as Burkitt’s lymphoma, post-transplant lymphoproliferative disease, and nasopharyngeal carcinoma. Neoplasms associated with EBV infection can be aggressive, resulting in up to a 70% mortality rate (Johannessen and Crawford 1999). Rhesus LCV-associated acute and persistent infection, development of lymphoma following experimental infection, and expression of an identical repertoire of latent and lytic viral genes all make this virus an excellent in vivo model for the study of EBV pathogenesis (Fogg et al. 2005, 2006; Rivailler et al. 2002).

**Herpesviridae Subfamily Alphaherpesvirinae**

While not commonly considered an OI, a high level of suspicion should be maintained for reactivation of *Cercopithecine herpesvirus* 1 (B virus) in immunocompromised macaque species. The virus often remains latent, but reactivation and shedding can occur in times of stress such as breeding, parturition, or concurrent illness (Huff et al. 2003; Weigler et al. 1993). Viral shedding, increased antibody titers, and development of oral ulceration have been reported in macaques dosed with immunosuppressive drugs (Chellman et al. 1992; Zwartouw and Boulter 1984). Increases in viral titers suggesting reactivation have also recently been associated with shipping stress and changes in housing (Mitsunga et al. 2007). There are rare reports of dissemination (Simon et al. 1993), and the clinical presentation of disseminated B virus disease may not obviously suggest its etiology. Pathology may involve necrosis and inflammation in multiple organs including the lungs, liver, kidney, and central nervous system (CNS), with intranuclear inclusion bodies at lesion margins.

Due to the high zoonotic risk associated with B virus, every effort should be made to use SPF animals in models that are likely to result in immunosuppression. Determination of B virus status in an individual animal can be difficult as both serological assays and viral isolation have limitations. Antibody-negative status is not sufficient to ensure
that an animal is free of B virus, and SPF status can only be ascertained through knowing the virologic history of the animal under consideration and the history of all its contacts. Because of this, it is advisable to treat all macaque species as if they are potentially infected with B virus and to take appropriate precautions.

**Adenoviridae**

At least 27 serotypes of adenoviruses have been isolated from a variety of OWPs and NWPs. Many of the isolates have come from healthy animals, although there are reports of adenoviral-associated respiratory and gastrointestinal disease. The virus is readily transmitted by aerosolization or the fecal-oral route. The prevalence of exposure in macaques varies by serotype and the individual colony under evaluation.

In most cases infection is asymptomatic, although clinical illness may appear in young animals (Boyce et al. 1978; Stuker et al. 1979). Disease in immunodeficient animals may be prolonged and severe, with symptoms related to hepatic, gastrointestinal, or pancreatic involvement (Baskin and Solkie 1989; Ochs et al. 1991). Lesions consist of epithelial cell necrosis with the presence of large basophilic intranuclear inclusions. These inclusions must be distinguished from those associated with CMV and B virus. A well-characterized form of chronic active pancreatitis frequently occurs in SIV-infected macaques and is recognizable by the presence of multifocal areas of exocrine pancreatic necrosis and adenoviral inclusions in acinar cells (Martin et al. 1991). A denovirus infection of renal epithelial cells has also been identified in SIV-infected macaques, resulting in a necrohemorrhagic tubulointerstitial nephritis that must be distinguished from simian virus 40 (discussed in the next section).

The potential for zoonotic transmission of primate-associated adenovirus remains in question. Recently, humans residing in sub-Saharan Africa have shown evidence of neutralizing antibodies to chimpanzee adenovirus, suggesting cross-species transmission (Xiang et al. 2006). Human adenoviral strains are important pathogens that cause significant morbidity and mortality in pediatric bone marrow transplant recipients, AIDS patients, and individuals receiving immunosuppressive chemotherapy (Feuchtinger et al. 2005; King 1997; Walls et al. 2003). Current research of primate adenoviruses has focused on the development of adenoviral-based vector systems for gene therapy and vaccine delivery.

**Papovaviridae**

**Simian virus 40** (SV-40; genus Polyomavirus) is a common latent infection of Asian macaques that was first isolated from primary macaque kidney cell lines used in polio vaccine production. This virus has been extensively studied due to its ability to immortalize primary human cells and its oncogenic potential in newborn hamsters (Butel and Lendvicky 1999), and has consequently become a common laboratory contaminant. Because of this, identification of SV40 sequences by molecular techniques in clinical samples should be viewed with a critical eye. SV40 appears to be readily transmitted, with virtually all captive macaques demonstrating seropositivity. Exposure to the virus likely occurs when animals are in group housing environments (Minor et al. 2003).

SV40 does not appear to be associated with disease in healthy animals. The virus remains latent in renal tissue, with virions shed in the urine. In contrast, SV40 infections cause a number of clinical conditions in immunosuppressed animals. Signs are slowly progressive and relate to pulmonary, renal, or CNS involvement. Symptoms appear secondary to SIV inoculation and in animals receiving immunosuppressive drugs for solid organ transplant. Serology and/or PCR of blood or urine samples are effective ways to screen for the virus.

In the brain, SV40 causes a lesion similar to the progressive multifocal leukoencephalopathy (PML) observed in human patients infected with JC virus (Figure 6) (Axthelm et al. 2004; Chrétien et al. 2000; Holmberg et al. 1977). The lesion is characterized by multifocal to coalescing areas of demyelination and gliosis throughout the white matter and subependymal regions. Demyelination results from direct viral infection and destruction of oligodendrocytes. Large basophilic intranuclear inclusions appear in oligodendrocytes and astrocytes. A distinct form of SV40-
induced meningoencephalitis occurs in SIV-infected macaques, in which infection of astrocytes rather than oligodendrocytes predominates (Simon et al. 1999). Renal lesions are found primarily in the inner cortex and medulla (Horvath et al. 1992). Affected tubules are lined by hypertrophied and hyperplastic epithelial cells and contain intranuclear inclusions. Occasionally, affected renal tubular epithelial cells appear dysplastic, forming fronds that project into tubular lumens. Pulmonary lesions consist of a proliferative interstitial pneumonitis, with inclusions present in hypertrophied type II pneumocytes (Sheffield et al. 1980). Sequence differences between viral strains may contribute to tissue specificity (Ilyinskii et al. 1992). SV40 infection can be confirmed via IHC with antibodies targeting the large T antigen.

SV40 is a known zoonotic disease. During the 1950s and 1960s an estimated several million humans were exposed to SV40 due to an inadvertent viral contamination of polio vaccine stocks. Although SV40 DNA has been isolated from several human neoplasms, including those of brain and bone as well as lymphomas and pleural mesotheliomas, causal relationships and definitive associations with clinical signs remain unclear (Carter et al. 2003; Strickler et al. 1998; Vilchez and Butel 2004). Human correlates of disease include JCV-associated PML and BK virus-associated interstitial nephritis (Gardner et al. 1971). Researchers have studied recombinant, replication-deficient SV40 strains for their potential as vectors for gene delivery (Strayer et al. 2000).

Parvoviridae

Simian parvovirus (SPV) infections have been described in macaque species (Green et al. 2000). This virus replicates in rapidly dividing cells and demonstrates a distinct tropism for cells of the erythroid lineage. Infection in healthy animals is self-limiting and accompanied by mild anorexia, slight weight loss, and a transient decrease in erythrocyte numbers (Brown et al. 1995). Disease may be more severe in immunosuppressed animals and may include profound anemia, diarrhea, weight loss, and dehydration. SPV infections have been reported in cynomolgus macaques that have been either coinfected with SRV (O’Sullivan et al. 1994) or used in a cardiac transplant study (Schroder et al. 2006), and in association with simian AIDS (Foresman et al. 1999). Examination of bone marrow reveals marked dyserythropoiesis with loss of mature erythroid precursors. Large intranuclear inclusions may be present. Diagnosis can be confirmed via ultrastructural examination and ISH.

The zoonotic potential of SPV remains in question. Although seroreactivity to SPV has been detected in individuals with and without NHP contact, the significance of these findings is not known (Brown et al. 2004). SPV virus shares 70% homology with capsid proteins of the human parvovirus B19 (Brown et al. 1995). Like SPV, the disease in humans is generally asymptomatic, but clinical anemia may occur in patients with an underlying hemolytic disorder or in immunocompromised individuals. Investigators have suggested that SPV infection of macaques may serve as an appropriate animal model (O’Sullivan et al. 1994).

Derivation of Virus-Free Animals

It is clear that viral OIs serve as significant confounders to research and pose dangers to human handlers. In addition, a number of viral agents that result in OIs serve as experimental vectors for antigen delivery or as animal models of human disease. In such instances, infection is preferably achieved through controlled delivery of a specified inoculum, creating a need for animals free of endogenous disease.

Because viral infections can be difficult if not impossible to prevent by modifications of husbandry practices, the National Council on Research Resources (NCRR) and Office of AIDS Research (OAR) of the National Institutes of Health have taken a lead role in the funding and development of SPF macaques. Target viruses for macaque colonies supported by the NCRR/OAR program include B virus, simian T lymphotropic virus, SIV, and SRV. The strategies that form the basis for establishment of SPF NHP colonies are described in the article by Morton and colleagues in this issue. Several facilities have developed expanded SPF colonies free of agents such as RRV, LCV, CMV, simian foamy virus, and SV40 (Mansfield 2005). Formation of these expanded SPF colonies has relied on separation of animals from the day of birth and hand rearing in small peer groups (appropriate socialization is critical for normal development) in facilities that are well separated from the source colony. Strict separation from conventional colonies, judicious introduction of new animals, and exclusion of feral animals are all imperative. It is essential to avoid direct exposure to or fomite transmission of viral agents, particularly in areas of common use such as clinic or procedure rooms. Ongoing disease surveillance is necessary to ensure continued SPF status.

Bacterial Opportunistic Infections

Mycobacterium avium complex (MAC), composed of M. avium and M. intracellulare, is the most common disseminated bacterial infection in both human and simian AIDS. Because M. avium is a common environmental bacteria in soils and moist environments, animals reared outside may demonstrate a higher incidence of disease due to higher levels of exposure and resultant subclinical infection (M. aslow et al. 2003). DNA restriction profiles from human isolates suggest that waterborne MAC is the most common source of exposure among humans (von Reyn et al. 1994). Potable water has also been implicated as a source of infection for SIV-inoculated macaques (Mansfield and Lackner 1997). Access to autoclaved water may reduce animals’ environmental exposure to MAC.
Disseminated MAC rarely occurs outside the context of SIV/HIV, suggesting unique viral host interactions underlying disease pathogenesis (Ghassemi et al. 1995; Hendricks et al. 2004b). Retrospective studies in SIV-inoculated macaques have identified a prevalence rate for MAC of approximately 20%, although this can range from 2% to 31% depending on viral strain (Mansfield et al. 1995).

Clinical signs include progressive diarrhea and weight loss and may be accompanied by peripheral lymphadenopathy and hepatosplenomegaly. Disease generally occurs when CD4+ T cell count falls below 200/μL. SIV viral proteins may contribute to the ability of MAC organisms to avoid immune surveillance (Denis 1994a,b; Shiratsuchi et al. 1994). In healthy primates infections are generally subclinical and localized, with detection often resulting from observation of a positive intradermal skin test.

Pathologic lesions associated with disseminated MAC include grossly evident thickening of the small and large intestinal mucosal surfaces accompanied by enlarged mesenteric lymph nodes. Histologic features include sheets of epithelial macrophages that infiltrate and efface normal architecture of the gastrointestinal tract and lymph nodes. Microgranulomas may be present throughout the hepatic parenchyma and occasionally occur in multiple organs, including the skin, uterus, spleen, kidney, thymus, and bone marrow (Figure 7). Acid-fast stains reveal large numbers of intracellular bacilli. MAC seen outside the context of SAIDS morphologically resembles M. tuberculosis infection, with the presence of caseating granulomas that contain multinucleated giant cells and sparse numbers of acid-fast bacilli (Goodwin et al. 1988). Definitive diagnosis entails isolation of the organism or PCR sequencing of mycobacterial DNA. Immunosuppressed animals can have blunted delayed-type hypersensitivity responses, so there may be limited utility in intradermal skin testing as a screening tool for detection of MAC (Goodwin et al. 1988).

Because disease in primates recapitulates that in humans, scientists have used SIV-inoculated macaques to study coinfections with both MAC and M. bovis (as a model for M. tuberculosis) (Hendricks et al. 2004a,b; Shen et al. 2002; Zhou et al. 1999). Although HIV-seropositive individuals are at higher risk of M. tuberculosis infection and reactivation (Chen 2004), the strict exclusion of tuberculosis from NHP colonies limits observation of OI due to this organism.

Rhodococcus equi is a gram-positive facultative anaerobe closely related to mycobacteria. The organism is a common environmental contaminant, with soil serving as an important reservoir. Disease associated with R. equi appears in immunodeficient animals and may occur in association with SRV infection. Clinical signs are nonspecific and include anorexia, weight loss, and diarrhea. Lesions, characterized by pyogranulomatous inflammation, appear in the large intestine, lung, and draining lymph nodes. With severe immunosuppression, histologic features may resemble those of MAC. The most effective treatment involves combination antibiotic therapy based on sensitivity testing. Attention to sanitation procedures may reduce exposure to organisms in animal facilities.

Enteropathogenic Escherichia coli (EPEC) has been associated with acute onset and persistent nonhemorrhagic diarrhea primarily in neonatal macaques. Disease in NWPs can occur in animals of any age and presents as acute hemorrhagic diarrhea (Mansfield et al. 2001b). Transmission is via the fecal-oral route.

While serologic studies suggest that a majority of colony animals are exposed, clinical signs are self-limiting and often go unrecognized. Contributions of EPEC to OI may be underestimated particularly in immunodeficient animals that present with multiple other opportunists. A retrospective analysis of animals with SAIDS euthanized at the NEPRC revealed that 28% had features of EPEC infection (Mansfield et al. 2001a).

Diagnosis requires speciation of lactose-fermenting organisms from fecal specimens followed by use of cellular adhesion assays and molecular identification of virulence genes. PCR amplification of the intimin (eaeA) gene may contribute to a definitive diagnosis. Premortem diagnosis is also possible through the use of colon biopsies and identification of characteristic lesions. On histopathology, the colonic surface epithelium appears irregular, with bacilli intimately associated with the apical cytoplasmic membrane. Lesions are accompanied by varying degrees of colonic crypt hyperplasia and mild neutrophilic infiltrates. The distribution of bacilli varies from a diffuse to locally extensive or focal pattern.

Sequencing of the intimin gene from both NWP and

Figure 7 Mycobacterium avium may be acquired from environmental sources and cause disease during progressive immunodeficiency. Disseminated disease may be recognized in a variety of organs; shown here are microgranulomas in the liver.
Pneumocystis carinii is an extracellular obligate fungal pathogen. The organism has a high host specificity, with isolates from varied hosts considered distinct species of pneumocystis (Furuta et al. 1993; Gigliotti et al. 1993; Norris et al. 2003; Stringer et al. 2002). Colonization has been reported in a variety of NWPs and OWPs but appears most commonly in immunodeficient macaques (Demanche et al. 2001). Infection of neonatal animals is likely uniform and asymptomatic, with infected animals either clearing the organism or becoming carriers (Demanche et al. 2005). Transmission is via aerosolization. Due to host specificity there is no zoonotic potential.

Clinical signs in immunosuppressed animals are associated with pneumonitis and include dyspnea and tachypnea; the presence of a cough is uncommon. Disease is slowly progressive with a protracted asymptomatic period (Board et al. 2003). Evidence suggests that rather than reactivation of latent infection, disease is associated with acquisition during periods of immunosuppression (Vogel et al. 1993). Clinical diagnosis may be aided by PCR amplification of DNA and cytology of cytospin preparations collected by bronchoalveolar lavage (Board et al. 2003). Pneumocystis DNA can also be detected from deep nasal cavity swabs although it is unclear if this represents pulmonary colonization (Demanche et al. 2005).

Lesions vary from a multifocal to coalescing interstitial pneumonitis to multifocal grey to white nodules. The organism can be recognized on hematoxylin- and eosin-stained sections as pale pink foamy material in alveolar spaces. Inflammatory infiltrates are mild, consisting of lymphocytes, plasma cells, and macrophages. There is often pronounced type II pneumocyte hyperplasia. A second, angiogenic form of disease may occur in severe cases. Organisms proliferate in the periarterial spaces in addition to the alveoli and may disseminate to regional lymph nodes and the thoracic wall (Yanai et al. 1999). Gomori methanamine silver (GMS) staining and IHC may be useful for diagnosis.

Prevention of pneumocystis pneumonia in NHPs is difficult. Because juvenile carriers may play a role in propagation (Demanche et al. 2005), separation of adult immunodeficient animals may be of some benefit. Reports have described significant differences in incidence of infection among animals housed in adjacent rooms, suggesting that controlled air handling and reduction of animal numbers per room may be useful in limiting spread of the organism (Vogel et al. 1993). SIV macaques held in isolators have a reduced incidence of disease (Vogel et al. 1993).

Candida albicans is a common saprophytic agent that colonizes mucosal surfaces shortly after birth. In most animals it exists as a commensal agent and does not produce clinical signs. Localized and systemic infections occur in young, immunocompromised, or debilitated animals. The localized form, also known as thrush, appears as pale tan to white plaques adhered to the epithelium of the oral mucosa and esophagus. These plaques comprise masses of pseudohyphae and blastospores mixed with sloughed epithelial cells and neutrophils. Organisms can be identified with GMS or periodic acid Schiff (PAS) stains. Candida overgrowth along the length of the gastrointestinal tract with associated diarrhea and anorexia is less common. Systemic infection accompanied by fever and hypotensive shock occurs rarely in debilitated animals treated with antibiotics or in association with concurrent disease. A gent may be cultured on Sabouraud and blood agar. Due to the ubiquitous nature of the organism prevention is difficult.

Less commonly, infections caused by saprophytic soil fungi such as Histoplasma capsulatum, Cryptococcus neoformans, and Aspergillus fumigatus have been identified as opportunistic agents in NHPs (Baskin 1991; Pal et al. 1984).
years of age. Transmission is via the fecal-oral route. Although the infectious dose is thought to be low (about 10 to 50 oocysts), animals with diarrhea may shed as many as $6 \times 10^5$ oocysts per gram of feces (Miller et al. 1990b). Resilience to environmental conditions contributes to high environmental burdens and the potential for cross contamination.

Clinical disease generally goes unrecognized in healthy animals, although infections can be associated with protracted diarrhea, anorexia, and weight loss. Juvenile animals are more commonly affected (Miller et al. 1990a; Wilson et al. 1984). Immunodeficient animals often present with severe symptoms as well as with dissemination to the liver, pancreas, or respiratory tract (Baskerville et al. 1991; K aup et al. 1994; Yanai et al. 2000). Colonization of the conjunctiva has been reported (Baskin 1996). Diagnosis is made by examination of feces or colon biopsy sections. Commercially available fecal antigen capture tests are sensitive diagnostic assays that detect Cryptosporidium parvum as well as Giardia and Entamoeba.

The organism can be identified on histological sections as spherical 3- to 4-μm diameter basophilic bodies adherent to the apical surface of epithelial cells. In severe cases there may be villous atrophy and epithelial cell hyperplasia in the gastrointestinal tract. Inflammatory infiltrates are typically mild. Ultrastructurally, the organism resides in a parasitophorous vacuole attached to host cells. Invasion of the organism into the biliary tree is associated with cholangitis, cholecystitis, and choledochitis. Neutrophilic infiltrates are evident surrounding and infiltrating bile ducts. Concentric fibrosis may surround the biliary epithelium, with involved epithelial cells assuming a squamous morphology. Organisms are best identified at the margins of inflammation. Dissemination to the trachea and lungs may result in a necrotizing bronchitis.

Control may be difficult in OWP colonies. Conventional disinfectants such as chlorine and quaternary ammonium are not effective at inactivating oocysts even after prolonged contact (Rose et al. 2002; Weber and Rutala 2001). Chlorine dioxide, hydrogen peroxide, ethylene oxide, and steam are effective means to reduce the organism's viability (Weber and Rutala 2001). Ultraviolet (UV) light and ozone exposure are the most effective methods of neutralizing the infectivity of oocysts in water (Rose et al. 2002). Husbandry practices that reduce organic waste and limit standing water may help minimize environmental contamination (Robertson et al. 1992). Efforts should be made to avoid introduction from OWP colonies, where the organism is endemic, to NWP colonies via contaminated food, water, or fomites. Although Cryptosporidium is a known zoonotic agent, strict adherence to PPE use and hand-washing practices should limit potential for exposure.

**Entercytozoon bieneusi**

Entercytozoon bieneusi is a microsporidian parasite of a number of mammals. Microsporidia are obligate intracellular parasites with no metabolically active stage outside the host. Analysis has detected the presence of chitin, suggesting that this organism is more closely related to fungi (Weiss et al. 1999). Infection has occurred in several species of macaques, although it is likely that a number of NHP species are susceptible. A symptomatic infection is common, with 15-20% of breeding animals shedding spores at any given time (Mansfield et al. 1998). The organism is transmitted by the fecal-oral route. Like cryptosporidium, spores are resistant to environmental degradation and persist in soil or water for years.

E. bieneusi resides in low numbers in the biliary and intestinal epithelium of immunocompetent animals and is infrequently associated with clinical disease (Mansfield et al. 1998). Clinical disease in SIV-infected, immunodeficient macaques results in diarrhea and wasting. The organism commonly invades the hepatobiliary system, causing jaundice and hepatomegaly. Scientists have linked increased shedding of spores to decreased CD4+ T cell counts and increases in SIV viral load (Sestak et al. 2003; Singh et al. 2006). Diagnosis is difficult but can be made based on PCR, indirect immunofluorescence assay, or fecal flotation followed by a modified chromotrope stain (Singh et al. 2005). It is also possible to identify organisms in bile samples collected via ultrasound-guided cholecystocentesis.

Pathology associated with E. bieneusi includes villous atrophy of the small intestine, peritonitis, cholecystitis, choledochitis, and cholangiohepatitis (Chalifoux et al. 2000; Mansfield et al. 1997). Grossly thickened bile ducts and gall bladder mucosa may be present. Histopathology demonstrates a characteristic marked bile duct hyperplasia accompanied by bridging fibrosis (Figure 8). Presence of lymphoplasmacytic and neutrophilic infiltrates should prompt evaluation for concurrent C. parvum infection. Identification of E. bieneusi is possible upon close examination of exfoliated biliary epithelial cells containing small ringlike structures that exclude hematoxylin and eosin staining. Application of a Weber’s modified trichrome stain may facilitate recognition.

Prevention of E. bieneusi is difficult because of widespread infection in macaque colonies. Procedures to limit environmental burden of this organism may be similar to those aimed at reducing C. parvum exposure. E. bieneusi is a known human infection. Although there are no reports of transmission from NHPs to humans, phylogenetic analysis of animal and human isolates suggests that it should be considered possible (Dengjel et al. 2001; Drosten et al. 2005).

**Acanthamoeba**

A moebae of the genus Acanthamoeba can infect both humans and animals as opportunistic pathogens. In recent years amoebic infections such as Acanthamoeba sp., Naegleria fowleri, and Balamuthia mandrillaris have been reported in Immunocompromised human patients. Cyst and...
trophozoite stages of the organism are free living in soil and water. This organism is not a frequent cause of OI in NHPs, but animals in outdoor housing may be at increased risk. Transmission occurs via inhalation of the organisms or contamination of skin wounds followed by hematogenous spread (Schuster and Visvesvara 2004).

Amoebae can cause two types of CNS disease in human and animal hosts. The first, a rapidly progressive infection called primary amoebic meningoencephalitis (PAM), has been associated with N. fowleri (Schuster and Visvesvara 2004). The second, a chronic, more slowly progressive CNS disease called granulomatous amoebic encephalitis (GAE), can be caused by either Acanthamoeba sp. or Balamuthia mandrillaris (Schuster and Visvesvara 2004). PAM occurs in healthy individuals whereas GAE generally occurs in immunocompromised hosts where the organism replicates in an extraintestinal phase. Intermediate hosts may transmit the organism if eaten as prey.

Toxoplasma infection has been diagnosed in a number of OWP and NWP species. Callitrichids and owl monkeys are particularly sensitive to severe disease (Epiphanio et al. 2000, 2003; Seibold and Wolf 1971). Toxoplasmosis remains a clinically important OI in patients with HIV/AIDS, occurring when CD4 T cell counts fall below 100/L (Hoffmann et al. 2007). There are limited published reports of toxoplasmosis in SIV-inoculated macaques (Sasseville et al. 1995).

Clinical signs are nonspecific and include lethargy, anorexia, and weakness. Disease in NWPs can progress to include sudden death, neurologic manifestations, diarrhea, fever, cough, and abortion (Epiphanio et al. 2000, 2003). Infection of OWP is often mild or asymptomatic (Wong and Kozek 1974). Serum antibody titers aid in diagnosis, although they may not be elevated in acute or peracute cases (Gyimesi et al. 2006). Clindamycin and trimethoprim sulfa antibiotics have been used therapeutically in zoologic settings (Fiorello et al. 2006).

Infection, which is associated with necrosis and a mixed inflammatory infiltrate, commonly affects the lungs, liver, and CNS, and may also affect the gastrointestinal tract, skeletal muscle, and lymphoid tissue. Organisms appear as small oval or crescent-shaped bodies occasionally sur-
rounded by an artifactual clear halo. Special stains and IHC may aid in identification of organisms.

It is important to ensure the exclusion of feral rodents from animal facilities as they may serve as intermediate hosts when eaten as prey by larger primates. Cockroaches may also carry infectious oocysts. Although largely a historical practice, the feeding of uncooked meat or neonatal mice to NHPs is not advisable. Animal facilities that house various species should have standard operating procedures in place to avoid cross contamination of feed and bedding with feline fecal material.

**Plasmodium**

Malarial parasites in the genus Plasmodium are one of the most frequent parasitic diseases of NHPs originating from tropical environments and as such represent a significant OI and research confounder for experimental protocols that require immunosuppression. Infection is nearly universal for all primate species with the exception of rhesus, Callitrichinae, and Aotus. Reported prevalence of infection ranges as high as 43% in imported cynomolgus macaques (Donovan et al. 1983).

Infection is not often associated with clinical signs in the natural host, although stress, illness, or experimental manipulation can precipitate severe disease (Stokes et al. 1983). Splenectomy and chemical immunosuppression may exacerbate a previously asymptomatic infection (Schofield et al. 1985). Clinical signs of infection include anorexia, cyclic fever, and weight loss. Thrombocytopenia, leukopenia, and progressive anemia may also be present. With high parasite burdens, erythrocytic stages of the malarial organisms (trophozoites, schizonts, and gametocytes) are evident in blood smears. Pathology includes hepatosplenomegaly, erythroid hyperplasia, and presence of macrophages containing malarial pigment (hemazoin). ELISA and a fluorescent antibody test have been developed to diagnose infection, with positive antibody status suggesting underlying infection (Duarte et al. 2006; Volney et al. 2002). Treatment includes administration of antimalarial drugs (chloroquine hydrochloride), blood transfusion, and supportive care.

Plasmodium infection can be transmitted as a blood-borne pathogen and represents a serious zoonotic risk (Singh et al. 2004). Because of the indirect life cycle, requiring mosquitoes of the genus A. nophales as a vector, risk of transmission within animal facilities is low. Investigators may opt to screen at-risk species imported from malaria-endemic areas before inclusion in certain experimental protocols. Multiple use of needles among animals should be avoided (Schofield et al. 1985).

**Microbial Quality Control for Immunosuppressed Primates**

There are three critical components to effective measures for microbial quality control among immunosuppressed primates: microbial surveillance, quarantine facilities and procedures, and environmental monitoring.

It may be difficult— and unwarranted— to attempt to eliminate all infectious agents from primate colonies, but facilities that house NHPs should have a microbial surveillance program in place as a critical component to quality control. Such a program will assist in defining the complement of agents present in a given colony and help elucidate their potential impact on colony health, zoonotic risk, and experimental research. The nature of the program will vary depending on the species, animal use, and housing characteristics but should include:

- vigorous diagnostic investigation of the nature of disease in ill animals;
- necropsy and histologic examination of all animals that die (or are euthanized) for spontaneously occurring disease;
- periodic survey by culture or other means for fecal pathogens;
- periodic serologic surveys for known viral agents; and
- routine testing of all animals for *M. tuberculosis*.

Periodic surveys should supplement existing SPF testing programs, target agents of relevance, and be performed on a representative subpopulation of the animals at risk.

Appropriate quarantine facilities and procedures are critical to microbial quality control and should be designed with knowledge of the potential risks associated with incoming shipments of animals. Designated quarantine facilities should be widely separated from the existing colony, with standard operating procedures in place to prevent cross contamination. Every effort should be made to house animals arriving on different days and from different source colonies in separate holding rooms. Close communication with representatives from the source colony is beneficial to determining the types of infectious agents that may or may not be present. Quarantine procedures will vary by facility and by the particular infectious agents targeted for exclusion, but screening should include a review of medical records and complete physical examination. A minimum database is routinely collected and may include CBC, serum chemistry, fecal culture and flotation, viral screening, and tuberculin testing (intradermal skin test and in vitro lymphocyte stimulation assays). Banking of serum is useful should disease be identified in a particular animal or group of animals after introduction to the colony. For a complete discussion of quarantine procedures we refer readers to the Roberts and Andrews article in this issue.

Environmental monitoring is a third key component to a microbial quality control program. While a discussion of facilities maintenance is beyond the scope of this review, important considerations include attention to air balancing, temperature and humidity controls, and rodent and insect control, as well as proper autoclave temperature and function. The layout of traffic patterns should prevent cross contamination between various levels of SPF status. Atten-
tion to sanitation control is critical to the continued exclusion of target agents from primate colonies.

Conclusion

Individuals responsible for the clinical care of NHPs should maintain a high level of suspicion for disease associated with OIs, particularly as a result of experimental immunosuppression with infectious, chemical, or radiological agents. Although frequently associated with experimental protocols, identification of OI may also call attention to the presence of endogenous diseases (e.g., SRV, measles) or other causes of debilitation (e.g., shipping stress, malnutrition). The use of SPF animals and rigorous prescreening protocols can reduce complications due to OI. Attention to sanitation controls and biosecurity will further reduce the impact of OI on animal health and limit research confounds.

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