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CHAPTER

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Experimental Infections of the Common Marmoset (*Callithrix jacchus*)

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Interest in the use of marmosets for experimental infectious disease has dramatically increased in the last decade. These animals are native to the Atlantic coastal forests in northeastern Brazil. The majority of experimental animals come from the National Primate Research Centers and other breeding facilities. They are advantageous because of their relative small size, weighting 350–400 g as adults, their life span is compact compared with other nonhuman primate (NHP), and they produce offspring by 3 years of age. They are free of Herpes B virus and, it is believed, to date, other dangerous human pathogens (Abbot et al., 2003) [1]. We describe here the experimental infections of marmosets to human pathogens. While it is always interesting to compare various NHPs with each other, the importance of an animal model is always in comparing its similarities to human infections.

MARMOSET AS A MODEL FOR VIRAL ENCEPHALITIS

The infection of human Eastern equine encephalitis virus (EEEV) was the first experimental encephalitis in marmosets [2]. EEEV is an alphavirus and a severe human arthropod-borne disease in North America (NA) and is genetically and antigenically distinct from a strain from South America (SA) (strain BeAr436087). South American strains have not been associated with severe human disease or mortality. To confirm this apparent phenotypic difference, the marmoset was infected using the intranasal route and monitored for weight loss, fever, anorexia, depression, and other neurological signs. The NA EEEV-infected animals died or were euthanized within 5 days due to anorexia and other neurological signs. The SA EEEV-infected animals remained healthy throughout the course of the experiment even though they developed viremia titers of $2.8–3.1^{10}$ PFU/mL by days 2–4 after infection. Interestingly, NA EEEV-infected animals showed no detectable viremia; however, virus was detected in the brain, liver, and muscle of NA EEEV-infected animals at the time of death or euthanasia. Additionally, the NA EEEV-infected animals developed meningoencephalitis in the cerebral cortex with some perivascular hemorrhages, which were consistent with brain lesions described in human infections of EEEV. These experiments confirmed previous studies that the SA strain induced a higher level of viremia compared with NA EEEV strains [3], and that NA strains, while showing undetectable or transient levels of viremia, induced encephalitis and subsequently death [4]. In summary, this study showed that the marmoset was a very useful small nonhuman primate (NHP) model of human EEEV.

West Nile virus (WNV), a flavivirus, is another common form of arthropod-borne encephalitis, which occurs worldwide and was responsible for sporadic encephalitis in parts of Africa, Asia, and Europe. In 1999, the virus was detected in New York City and spread rapidly through the United States, the Caribbean, Central America, and Canada. By 2004, there were over 7000 cases of neuroinvasive WNV disease in the United States alone [5]. The enzootic cycle occurs between mosquitoes and birds, whereas the virus can be transmitted, via mosquitoes, to equines and humans. Twenty percent of infected individuals develop a febrile syndrome; however, less than 1% of infected individuals progress to develop meningitis, encephalitis, or paralysis [6].

Experimental infection of the common marmoset with the virulent European WNV strain (WNV-Ita09) resulted in a more productive infection compared

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with rhesus monkeys, with higher rates of viral replication and a wider tissue distribution of virus [7]. Neither species showed behavioral changes or impaired kidney function, even though marmosets had detectable WNV RNA in kidney tissues. Both species developed a natural killer response followed by IgM and IgG antibody responses. The common marmoset is at least as susceptible to infection with WNV, if not more susceptible, than macaques, and due to a comparable immune response as that seen in humans, it could serve as a model for WNV disease.

MARMOSET AND DENGUE VIRUS INFECTIONS

Dengue virus (DENV) is an arthropod-borne flavivirus and a major cause of worldwide morbidity and mortality. The four serotypes (DENV 1, 2, 3, and 4) of DENVs are transmitted in tropical countries. All serotypes cause syndromes that are self-limited or severe. DENVs can cause a flu-like disease often referred to as "break-bone fever" or dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), which is characterized by sudden vascular permeability. It has been hypothesized that DHF/DSS is the result of enhancing and cross-reactive neutralizing antibodies that produce the syndrome with sequential dengue infections of a different strain [8].

Common marmosets developed high levels of viremia after inoculation with clinical isolates of all four serotypes of DENV, generating 1 x 10^6 genome copies/ml after inoculation with DENV-2. DENV-2 RNA was detected in lymphoid organs including lymph nodes, spleen, and thymus as well as nonlymphoid organs such as lung, liver, kidneys, pancreas, and urinary bladder. When marmosets were inoculated with DENV-2 and then reinfected, it appeared that primary inoculation with DENV-2 induced protective immunity to only DENV-2. More recently [9], it has been shown that after primary infection with DENV-1, 2, and 3, inoculation with heterologous virus produced viremia to that virus as well as high levels of neutralizing antibody titers to the challenge infection not only from serotypes with which they had been inoculated but also to serotypes to which they had not been inoculated. While the primary infection with a serotype elicited lifelong immunity to that serotype, the immunity to other serotypes was short lived. This suggested that DENV infection with any subtype does not prevent reinfection with a different serotype, and that cross-reacting antibodies develop which may complicate the immune response, as seen in human DENV infection. These data suggest that marmosets may prove to be a valuable tool to assess dengue vaccine candidates.

MARMOSET AND CORONAVIRUS INFECTIONS

Severe respiratory infections from coronaviruses (CoVs) have emerged in the last decade. The first of the severe respiratory illnesses to emerge was severe acute respiratory syndrome (SARS). The disease primarily presents with pneumonitis but later can progress to renal, gastrointestinal, and hepatic disease. When common marmosets were inoculated intratracheally with cell supernatant containing the SARS-associated pathogen, SARS-CoV, and evaluated over time up to 7 days after infection [10], all animals developed multifocal mononuclear cell interstitial pneumonitis, multinucleated syncytial cells, and some developed bronchiolitis. At 2, 4, and 7 days after infection, viral RNA was detected from pulmonary extracts and sometimes detected in tracheobronchial lymph node and myocardium. As in human disease, there was a correlation between the expression of the primary functional receptor, ACE-2, SARS-CoV-associated pathology, and the detection of viral RNA [11,12]. This study suggested that ACE-2 expression levels were similar to humans; however, SARS-CoV infection in marmosets did not present many of the severe clinical signs of respiratory disease that were observed in humans.

Middle East respiratory syndrome (MERS) emerged in 2012. The etiological agents of MERS are Middle East respiratory syndrome associated coronavirus (MERS-CoV). WHO estimated that by December 2016 there had been 1841 cases of MERS in 27 countries. The outbreak originated in Saudi Arabia, with the most recent serious outbreak being in South Korea (WHO updates). Marmosets became of interest as a model for MERS-CoV when dipeptidyl peptidase 4 (DPP4), also known as CD26, was shown to be a functional receptor for MERS-CoV [13]. Comparison of marmoset DPP4 showed that it is 96.4% identical to human DPP4 [14]. When marmosets were inoculated via combined intratracheal, intranasal, and/or ocular routes, most marmosets developed a lethal pneumonia. There were extensive lesions evident in the lungs, and viral titers of 1.5×10^6 were detected at 3 days postinfection. MERS-CoV RNA was detected in blood, which suggested a systemic dissemination, and viral RNA was detected in nearly all tissues in infected animals. In another study [15], investigators compared two isolates of MERS-CoV inoculated via the intratracheal route of exposure. This report showed that marmosets developed a mild-to-moderate nonlethal respiratory disease. Chan et al. [16] were able to improve the outcome of disease by treatment with Lopinavir/Ritonavir or interferon-B1b when marmosets were intratracheally delivered MERS-CoV. All these data support that marmosets are a valuable NHP model for MERS-CoV.

HEMORRHAGIC FEVERS AND MARMOSETS

Lassa Fever Virus

Marmosets have been recently evaluated as models for various hemorrhagic fevers. The first hemorrhagic fever examined was Lassa fever, a member of the Arenaviridae family. Lassa virus (LASV) is the causative agent of Lassa fever, with approximately 300,000 cases occurring each year in Western Africa. The fatality rates are between 1% and 2%, with as many as 100,000 hospitalized [17]. Lassa is transmitted from a rodent reservoir, *Mastomys natalensis*, via rodent excreta or by close contact with infected individuals. The disease progression includes nausea, vomiting, diarrhea, and abdominal pain, with hemorrhages occurring in about 20% of cases.

Common marmosets were subcutaneously inoculated with LASV strain Josiah [18]. The inoculation produced systemic disease with clinical features mimicking those of fatal human Lassa infection and included fever, weight loss, high levels of viremia, and viral RNA found in tissues. There were elevated liver enzymes and severe morbidity between days 15 and 20 postinfection. Importantly, there was also multifocal hepatic necrosis with mild inflammation and hepatocyte proliferation, lymphoid depletion, and nephritis. Immunophenotypic alterations in the spleen included reductions in overall numbers of CD20-positive and CD3-positive cells and disturbances with lymphoid follicular architecture. These data showed that in an animal model infected with LASV, infection impaired adaptive immunity as has been noted in LASV infections in humans [19].

The marmoset model was subsequently utilized to evaluate the efficacy of the ML29 reassortant vaccine for LASV [20]. The ML29 vaccine candidate encodes the NP and GPC of LASV and the Z protein and L proteins of Mopeia virus (MOPV). MOPV is an Old World group of arenaviruses related to LASV; however, this virus is nonpathogenic in experimental animals [21]. The virulence for arenaviruses is encoded in the large genome segment, encoding the Z and L proteins. Thus this vaccine has the antigenicity of LASV with the virulence of MOPV. It was determined that a single injection of ML29 reassortant vaccine induced low, transient viremia, and low or moderate levels of ML29 replication in marmoset tissue. This result was expected for a live-attenuated vaccine. The vaccination results in immune responses and the induction of sterilizing cell-mediated immunity. These data support the notion that ML29 is a promising vaccine candidate, and that the marmoset could be used for vaccine and treatment developments.

Filoviridae

Ebola and Marburg viruses (MARV) are members of the Filoviridae and induce a serious hemorrhagic disease. The filoviruses most commonly associated with causing human disease are MARV and Ebola virus (formerly known as Ebola Zaire) (EBOV) [22]. The case fatality rates (CFR) reported, in the various outbreaks, range from 24% to 100%. The average mortality of EBOV disease is estimated at 65% and MARV disease at 54% [23]. Of the know outbreaks, the most recent outbreaks of EBOV in West Africa had a CFR of 47%, the lowest of all of the EBOV outbreaks [24]. It is generally held that the West African outbreaks resulted in lower mortality due to better supportive care that allowed time for the immune system to clear the virus.

Marmosets have been shown to be a model for filovirus-induced disease [25]. The marmosets were inoculated intramuscularly with EBOV and MARV. The infections resulted in systemic fatal disease with clinical features resembling human infections. Animals experienced weight loss, fever, high virus titers in tissues, thrombocytopenia, neutrophilia, high liver transaminases and phosphatases, and disseminated intravascular coagulation. There were some differences in the histopathology between MARV and EBOV. Ebola infection showed widespread intravascular coagulation in multiple organs, including spleen, adrenal gland, kidney, and lung. There was extensive lymphocytic necrosis in medullary and parafollicular regions. However, follicles showed no evidence of necrosis. There was extensive fibrin deposition. MARV-inoculated animals had moderate fibrin deposition in the spleen, whereas intravascular coagulation was not observed. Interestingly, the hepatic lesions were less severe in animals that received a higher dose of virus, which suggested that the disease course developed too rapidly to allow for the development of any hepatic pathology. Also, marmosets did not develop petechial rash, which suggested that rapid progression of disease prevented some symptoms from occurring. These authors suggest that marmosets have a very similar disease course to human disease, and this NHP model may be superior to others in understanding the pathogenesis of the filovirus disease [26].

Development of a vaccine against an air-borne filovirus is a priority, particularly to the military. Filoviruses are select agents and are therefore considered to be a possible biowarfare agent [23]. As filoviruses may be aerosolized, investigators developed marmoset models where MARV was delivered via the respiratory route. Smither et al. [27] showed that low doses of virus, between 4 and 28 $TCID_{50}$, were sufficient to cause a lethal infection. Animals became febrile at days 5 and 6,

succumbing to disease between 8 and 11 days post challenge. Typical clinical observations included hemorrhaging in all animals and a transient rash in a small number of animals. The authors suggested that rash may be due to a slightly longer duration of disease, or a slight genetic difference between marmosets from different backgrounds. Virus was isolated from lungs at 3 days post challenge and from liver, spleen, and blood from day 5 post challenge. There was pathology in the kidney and liver on day 3. Those animals with the highest viral titers developed the most severe clinical symptoms.

Marmosets exposed to low doses of EBOV-Kikwit (between 4 and 27 $TCID_{50}$) via the respiratory route [28] developed a lethal infection. Animals developed fever at days 5 and 6 postinfection and succumbed to infection at days 6 and 8 postinfection. Similar results to the MARV marmoset model were observed whereby EBOV was isolated in lungs at day 3 post challenge and from liver, spleen, and blood on day 5 post challenge. Animals that died of the infection had high viral titers in all organs, increased liver function enzymes, increased blood clotting times, decreased levels of platelets, and multifocal moderate-to-severe hepatitis and edema. Marmosets again proved to be excellent models of human disease for another viral family.

OTHER INFECTIOUS AGENTS

As most marmosets are housed indoors and are cared for in Biosafety Level 2 conditions, primarily to protect the animal from acquiring human infections, they can be utilized for a wide variety of Koch's postulates experiments. When prescreened for all clades of enteroviruses only 1 out of 4 showed any significant seroconversion (Patterson and Shigeo, personal communication). Yu et al. [29] were able to use marmosets to test the hypothesis that a novel adenovirus (TMAdV) isolated from a titi monkey (*Callicebus cupreus*) was the cause of a fulminant pneumonia outbreak in a colony of titi monkeys at a national primate center in 2009. This outbreak became more significant when a human researcher at the facility and a household family showed the possibility of a cross-species transmission of the virus. When four marmosets were inoculated with a cell culture-adapted TMAdV three marmosets showed an acute, mild respiratory illness characterized by low-grade fever, reduced activity, anorexia, and sneezing. All monkeys with symptoms developed neutralizing antibodies. This work supported the notion that marmosets could be useful for assessing the zoonotic potential of adenoviruses as they arise in humans and NHPs. Similarly, the marmoset has been shown to be a model for investigations with orthopoxviruses (OPV). Kramski et al. [30] evaluated the infectivity and pathogenicity of calpox virus. In marmosets the minimal infectious dose was 10,000-fold lower than variola virus and monkeypox virus compared with the macaque models. Later, Mucker et al. [31] also showed that marmosets were more susceptible to lower titers of monkeypox virus delivered via the intravenous routes and had incubation periods more characteristic of human smallpox disease compared with macaques. This model overall was deemed to be a suitable primate model by overcoming the limitations of other OPV models, especially the requirement for high viral doses applied by unnatural routes.

Marmosets have been used to model respiratory *Bacillus anthracis* infection [32]. The marmoset was challenged via the respiratory route with small-particle aerosols containing *B. anthracis* Ames strain. The LD_{50} was 1.47×10^3 colony-forming units (CFU) compared with 5.5×10^4 CFU in rhesus macaque and 4.13×10^3 CFU in the cynomolgus macaque. The time to euthanasia was 40–140 h and was not statistically correlated with dosage. Onset of clinical signs, such as dyspnea, disorientation, and reduced response to external stimuli, appeared quickly. Marmosets have been challenged with *Burkholderia mallei* via the intranasal route [33]. Challenge doses of 2.5×10^4 to 2.5×10^5 CFU resulted in an acute lethal infection within 3–4 days postinfection in 83% of marmosets challenged. Those animals challenged with lower infectious doses of bacteria developed mild signs of illness and were culture negative. Those animals challenged with higher infectious doses developed more severe clinical signs of disease, which included lethargy, lack of appetite, conjunctivitis, and mucopurulent and hemorrhagic nasal discharge. Lesions, which resembled those seen in human cases of glanders, were present in tissues.

Marmosets have also been challenged with *Escherichia coli* [34]. These authors showed that *E. coli* can be the cause of diarrhea and hemorrhagic typhlocolitis, seen in captive marmosets. When marmosets were inoculated with three strains of *Mycobacterium tuberculosis*, they developed fulminant disease and it was speculated that marmosets could provide an attractive model for the human disease of *M. tuberculosis* [35].

GBV-B, A HEPATITIS C VIRUS SURROGATE IN NEW WORLD MONKEYS

History of the GB Agent

In the 1960s, studies were conducted in many NHP species in an attempt to find animal models for

human hepatitis infections, long before the development of diagnostic assays for HAV, HBV, and HCV. One such study suggested the presence of a new virus when human serum was injected into tamarins (*Saguinus* species). The infection induced hepatitis based on increases in the serum levels of liver enzymes. The serum was derived from a patient with the initials GB. The GB agent was characterized by serial passage in tamarins but was not isolated until modern techniques provided molecular clones of two viruses from tamarins [36,37]. The viral agents, termed GB virus A (GBV-A) and GB virus B (GBV-B), showed similarity to the Flaviviridae. Subsequent studies cloned a related virus from human serum, and it was designated GBV-C. Because GBV-A and GBV-C are not hepatitis viruses and cause no known disease, they will not be discussed. The original studies assumed that the GB agent originated from the serum of the surgeon; however, in retrospect, GBV-B clearly represents a tamarin virus. GBV-B has not been recovered from humans, and GBV-B has a very narrow host range for tamarins and other closely related New World monkeys [38,39]. The fact that it has not been recovered a second time from tamarins may be due to the rapid resolution of the acute infection in tamarins and the limited number of wild-caught tamarins that have been examined immediately on introduction into captivity. Sequence analysis of the GBV-B genome revealed a single ORF of 2864 amino acids [40] with 28% amino acid similarity with HCV [41]. Like HCV, the GBV-B genome encodes a single polyprotein with 10 proteins, possesses a 5′ IRES (internal ribosome entry site) [42], and a unique 3′ NTR (nontranslated region) [43]

Development of the Animal Models for GBV-B

The major advantage of GBV-B as a surrogate for HCV is the small size of the animal model. Although tamarins (*Saguinus* sp.) appear to be the natural host, the virus has been adapted to the closely related marmoset (*Callithrix jacchus*) (see below). An additional advantage is the increased level of viremia, which often reaches 10^9 GE/mL, 1000-fold higher than what is observed in most HCV-infected humans. The basis for higher viremia is not known, but it may involve an increased ability to avoid the innate immune response. The one disadvantage to the GBV-B model is the low frequency of persistent infection. Infection normally resolves within 3 months, but at least four tamarins [44–47] and one marmoset (Lanford, unpublished data) progressed to chronic infection. Insight into the differences between GBV-B and HCV in the development of persistent infections may provide critical insight into vaccine strategies.

Although the natural host for GBV-B appears to be the tamarin, several New World primates are susceptible to infection [38,39]. Marmosets exhibit two distinctive viral profiles when infected with GBV-B, susceptible with rapid escalation of viremia or partially resistant with a clear delay in maximum viremia [48,49]. The different phenotypes are likely to be due to a genetic polymorphism in the marmoset population that affects a critical viral–host interaction. Serial passage of wild-type GBV-B after reaching maximum viremia in resistant animals resulted in adaptation and a uniform susceptible profile in all subsequent infections. The adapted virus contained 21 nucleotide changes and 6 amino acid changes [49]. However, it should be noted that even the adapted virus replicates at levels 10-fold lower in marmosets compared with tamarins.

In Vitro Culture Model

Unlike HCV, GBV-B can be readily cultured in primary hepatocytes from both marmosets and tamarins [50]. Decades of research were conducted on HCV before a single strain was isolated that could replicate in a unique liver cancer cell line [51,52]. Although in vitro models for HCV have become common tools for HCV, the ability to infect primary hepatocytes or to use patient-derived serum remains challenging. GBV-B can move from animal to culture and back to animals without requiring adaptive mutations. The primary hepatocyte culture system for GBV-B [50] has an intact innate immune response [53,54], providing a more authentic environment to evaluate the ability of viral proteins to subvert the innate response.

Molecular Clones and Chimeric Viruses

Infectious cDNA clones of GBV-B have been produced that induced hepatitis on intrahepatic inoculation of tamarins and marmosets with in vitro transcribed RNA [43,55,56]. Chimeric viruses between GBV-B and HCV have been developed with mixed success. Replacing a portion of the GBV-B IRES with domain III of the HCV IRES [57] induced infection in tamarins but only after a prolonged period with no viremia. The virus that emerged had two compensatory mutations in the opposite end of the genome (3′ NTR region) that allowed the chimeric RNA to replicate efficiently [58]. Unlike WT GBV-B, this chimeric virus could not be adapted for efficient infection in marmosets [59], highlighting another difference between the tamarin and marmoset models. Chimeric viruses have also been produced with HCV p7 replacing GBV-B p13 [60] and the HCV E2 hypervariable region [61]. Deletion studies of the 3′ NTR mapped the essential nature of this structure but, surprisingly, deletion of the poly U stretch resulted in one of the few documented chronic infections [45].

ANTIVIRAL STUDIES

The NS3 protease of HCV and GBV-B are interchangeable with regard to processing of the polyprotein [62,63]. This high level of functional homology led to the expectation that antiviral compounds developed for HCV could be studied in the GBV-B animal model. HCV protease inhibitors inhibit GBV-B replication in infected marmosets [48] and infected primary marmoset hepatocytes [39]. The antiviral nature of ribavirin was explored using the GBV-B model as well [64]. The data suggested that incorporation of ribavirin triphosphate by the RNA polymerase induces error-prone replication with a concomitant reduction in infectivity. The induction of error-prone replication with ribavirin has been demonstrated in the HCV replicon model [54].

Marmoset and tamarin interferon alpha (IFNα) and interferon gamma (IFNγ) have potent antiviral activity against GBV-B in vitro [53], similar to what was observed for HCV in vitro [54]. New studies have focused on interferon lambda (IFNλ). The focus on the IFNλ pathway is based on the finding that SNPs in IL28B (IFNλ3) locus in the human population exert an enormous effect on viral clearance during acute infection and during IFNα therapy, yet the mechanism involved is not understood [65]. The IFNλs in humans include IFNλ1 (IL29), IFNλ2 (IL28A), and IFNλ3 (IL28B). The genomic area of the marmoset encoding interferon lambdas was sequenced. Marmosets lack IFNλ2 (IL28A in humans). The transcriptional orientation of IFNλ3 suggests that the human IL28B homologue is present but the human IL28A homologue is absent. Thus, the IL28B gene duplication giving rise to IL28A may have occurred after segregation of the New and Old World primates. In vitro and in vivo studies were conducted to characterize IFNλ3 (IL28B) antiviral activity for GBV-B infection in the marmoset. Exceptionally potent antiviral activity was demonstrated for this class of IFNs in marmosets (Lanford, unpublished data). IFNλ3 is clearly a more potent antiviral agent than IFNα in this model. One animal had undetectable viremia after 3 doses (1 week of therapy). This represents a decline of 7−9 logs of viremia and is greater than the decline observed with potent direct-acting antivirals for HCV.

ANTISENSE KNOCKDOWN OF GBV-B IN MARMOSETS

The marmoset is increasingly becoming the chosen primate model for transgenics with newer technologies that are emerging for efficient gene disruption (e.g., CRISPRs). Methods will be needed to transiently examine phenotypes of gene knockdown in this model before the investment in a transgenic approach. The marmoset model of GBV-B infection has been used to examine the potential for gene knockdown using systemic injection of antisense using the novel Locked Nucleic Acid (LNA) antisense technology. The LNA technology was first used in antiviral studies to demonstrate that sequestration of miR-122 could knockdown HCV replication in chimpanzees [66]. miR-122 is a highly abundant, liver-specific microRNA. HCV has two binding sites for miR-122 in the 5′ NCR, and both are essential for maintaining HCV replication [67,68]. The miR-122 LNA antisense progressed into phase II clinical trials for HCV as Miravirsen and showed highly potent antiviral activity [69]. As a proof of concept that the LNA technology worked as expected in the marmoset model, Miravirsen was used for GBV-B antiviral studies in the marmoset. GBV-B has two binding sites for miR-122 that have identical spacing as the two sites in HCV. In vitro studies confirmed the antiviral effect of Miravirsen using primary marmoset hepatocytes infected with GBV-B. In vivo, pretreatment with Miravirsen rendered marmosets completely resistant to GBV-B infection. These data clearly indicate that miR-122 is a valid antiviral target for GBV-B and LNA technology can be used for gene knockdown in marmosets (Lanford, unpublished data). The marmoset as a small nonhuman model for gene knockdown may be of value in a number of different research fields.

ZIKA VIRUS

The recent emergence of a member of the flavivirus family Zika virus (ZIKV) in the Americas and its role in fetal abnormalities has placed an urgency on the development of animal models for ZIKV. These mosquito-borne infections have been associated with neurological complications, the most severe being microcephaly. The full extent of the adverse events associated with ZIKV infections in pregnant women will not be known for years as we follow infants born to ZIKV-infected pregnant women. Recently, male marmosets experimentally infected with ZIKV reproduced some of the key features of the human disease, an asymptomatic infection, and a short window of detectable virus in the serum of less than a week. There was also virus detected in urine, saliva, semen, and stool for at least 2 weeks following acute infection, and there was a 1-month postinfection persistence in lymph nodes. Only saliva and serum samples produced infectious virus in cell culture. All marmosets developed neutralizing antibody responses with activation of NK cell and B cell subsets. Only circulating cytokines associated with type II interferon signaling were increased. A transcriptome

profile showed enrichment of immune responses to an active viral infection and upregulation of type I and II interferon signaling pathway. Studies to examine the role of ZIKV in pregnant marmosets are underway. These authors believe that marmosets may prove to be a valuable model for drug testing, vaccine development, and understanding the pathogenesis of ZIKV [70].

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