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Lymphocytic choriomeningitis virus (LCMV) infection of macaques: a model for Lassa fever

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Abstract

Arenaviruses such as Lassa fever virus (LASV) and lymphocytic choriomeningitis virus (LCMV) are benign in their natural reservoir hosts, and can occasionally cause severe viral hemorrhagic fever (VHF) in non-human primates and in human beings. LCMV is considerably more benign for human beings than Lassa virus, however certain strains, like the LCMV-WE strain, can cause severe disease when the virus is delivered as a high-dose inoculum. Here we describe a rhesus macaque model for Lassa fever that employs a virulent strain of LCMV. Since LASV must be studied within Biosafety Level-4 (BSL-4) facilities, the LCMV-infected macaque model has the advantage that it can be used at BSL-3. LCMV-induced disease is rarely as severe as other VHF, but it is similar in cases where vascular leakage leads to lethal systemic failure. The LCMV-infected macaque has been valuable for describing the course of disease with differing viral strains, doses and routes of infection. By monitoring system-wide changes in physiology and gene expression in a controlled experimental setting, it is possible to identify events that are pathognomonic for developing VHF and potential treatment targets.

Keywords

viral hemorrhagic fever; monkey models; rhesus macaques; arenaviruses; LCMV; Lassa fever virus

1. Introduction

Viral hemorrhagic fever (VHF) can be caused by enveloped RNA viruses from four different families, Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae (Gowen and Holbrook, 2008a). The bleeding signs of VHF are more frequently associated with Marburg

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and Ebola (filoviruses), Crimean Congo hemorrhagic fever (bunyavirus), and South American hemorrhagic fever (arenaviruses), but less frequently associated with dengue (flavivirus), Rift Valley fever (bunyavirus) or Lassa fever (arenavirus). Lymphocytic choriomeningitis virus (LCMV) is related to Lassa fever virus, and some strains of LCMV can be used to model aspects of severe Lassa fever.

The Arenaviridae have the largest number of virus species known to cause VHF. At least 10 new arenaviruses have been discovered in the last 3 years as a result of both rodent surveys and disease outbreaks (Briese et al., 2009; Cajimat et al., 2007; 2008; Delgado et al., 2008; Gunther et al., 2009; Lecompte et al., 2007; Milazzo et al., 2008; Palacios et al., 2008; 2010; Vieth et al., 2007), but not all of the new viruses qualify as distinct arenavirus species (Salvato et al., 2011).

Clinical signs of VHF are fever, rash, buccal sores, thrombocytopenia, vascular leakage, bleeding, edema and elevation of hepatic enzymes. Despite these universal signs, their frequency and underlying disease mechanisms differ from one virus family to another. Our goals were to examine the initial stages of arenavirus hemorrhagic fever in order to define early treatment targets. We used lymphocytic choriomeningitis virus strain WE (LCMV-WE), a risk group 3 virus that, when introduced into rhesus macaques, causes a disease that closely resembles human infection with Lassa fever virus, a risk group 4 agent (NIH Guidelines, 2009). This article reviews our findings and may guide other researchers who want to build on our experiences with the LCMV-infected non-human primate model. We hope to convince readers that this model will be most useful for testing antiviral approaches that target host molecules.

2. Arenaviruses and arenaviral hemorrhagic fever

The first arenavirus isolated was lymphocytic choriomeningitis virus (LCMV) from cerebrospinal fluid of people with aseptic meningitis (Armstrong and Lillie, 1934; Rivers and Scott, 1935; Traub, 1936). Since these patient samples were passaged in laboratory mice, it was difficult to distinguish whether the virus came from mouse or man. Nevertheless, viruses were exchanged between three laboratories and their relationship was confirmed by serology (Rivers and Scott, 1936). In 1956, Tacaribe virus (TCRV) was found in Caribbean fruit bats (Downs WG, et al., 1963), making it the only arenavirus to have a non-rodent reservoir. Research in the 1960's identified morphological and serological characteristics of the family Arenaviridae. In 1969, Lassa fever virus was identified by its grainy morphology that was similar to electron micrographs of other arenaviruses including laboratory strains of LCMV and several South American viruses (Buckley and Casals, 1970; Rowe et al., 1970; Ruggiero et al., 1964).

The arenaviruses have since been characterized as rodent-borne enveloped viruses with a bi-segmented ambisense single-stranded RNA genome. The large (L) RNA segment encodes the virus polymerase (L protein) in the negative sense and matrix (Z protein) in the positive sense, whereas the small (S) RNA segment encodes the nucleocapsid protein (NP) in the negative sense and glycoprotein precursor (GPc) in the positive sense (Salvato and Shimomaye, 1989). LCMV and Lassa virus belong to Old World arenavirus lineages whereas the American arenaviruses belong to the New World lineages (Salvato et al., 2004, 2011). During European migrations to the Americas in the 16th century, LCMV probably arrived via murine hosts well after the New World lineages had been established amongst Sigmodontine rodents of the Americas (Albariño et al., 2010).

3. LCMV and its natural host range

The natural reservoir hosts for arenaviruses are rodents (with the exception of TCRV that was isolated from bats), whereas human beings and non-human primates are incidental hosts. It is remarkable that the reservoir hosts are unscathed by infection, aside from some decreases in fecundity, whereas primates occasionally develop severe disease. Outbreaks of VHF are related to increased contact with natural reservoir hosts. For example, the incidence of human infection with Junin virus, the agent for Argentine hemorrhagic fever, coincides with the prevalence of infections amongst local rodents (Mills et al., 1992).

The LCMV host range includes monkeys, dogs, guinea pigs, rabbits, chickens and rodents (Parker et al, 1976; Skinner and Knight, 1979), but rodents such as mice and hamsters often carry inapparent infections while they are viremic and viruric. LCMV infection of mice has been an excellent model for studying viral persistence, immunological tolerance, and immunopathogenesis (reviewed by Buchmeier et al., 1980; Lehmann-Grube, 1984; Oldstone, 2002).

4. Spectrum of LCMV infection of humans

4.1. Common forms of illness

LCMV infections in people occur infrequently and are usually related to ingestion, inhalation or direct contact with virus shed by mice, hamsters or guinea pigs. Most LCMV infections are subclinical or mild flu-like illnesses (Barton and Hyndman, 2000). Symptoms appear 6 to 20 days after exposure and include fever, nausea, stiff neck, occasional photophobia, and headache that is poorly responsive to non-steroidal anti-inflammatory drugs. Disease rarely advances from meningitis (inflammation of the meninges) to encephalitis (extensive inflammation resulting in confusion and cognitive impairment). Disease is rarely fatal and is more often transmitted to people from rodents than from other human beings; so LCMV infection is not considered an important public health threat. The commonly seen LCMV disease represents the mild end of a spectrum, the extreme end of which is occupied by severe hemorrhagic fever.

LCMV has been a frequent contaminant of tissue cultures and of experimental mice, guinea pigs and hamsters. Consequently, numerous outbreaks of aseptic meningitis in laboratory workers or animal handlers have been traced to LCMV infection (Bowen, 1975; Deibel et al., 1975; Hinman et al., 1975; Smith et al., 1984; Emonet et al., 2007).

Seroprevalence amongst residents of Birmingham, Alabama ranged from 0.3% in residents under 30 years of age to 5.4% in residents over the age of 30 (Park et al., 1997). Residents of Baltimore showed a 4.7% seroprevalence (Childs et al., 1991) at the same time that the Baltimore rodent population had a 3.9-13.4% seroprevalence (Childs et al., 1992). Serosurveys of patients hospitalized with aseptic meningitis revealed 10% positivity for LCMV, especially during winter months when mice move indoors (Adair et al., 1953; Barton and Hyndman 2000). Foci of murine LCMV infection (37% of 56 samples) have been noted in Bratislava, Slovakia (Reiserova et al., 1999), and in New South Wales, Australia where a survey identified 30-52% of the feral mice to be seropositive for LCMV (Smith et al., 1993).

4.2. Occurrence of more severe disease

During the third trimester of pregnancy, a relatively immune-suppressed state, LCMV infection can achieve high viral titers with disastrous consequences for the fetus. Congenital LCMV infections have been known to cause birth defects such as hydrocephalus, chorioretinitis and mental retardation (Barton et al., 2002). It has been suggested that the

effects of LCMV on transcription factors like PRH/HEX account for the irreversible effects of this virus on neuronal development (Djavani et al., 2005). Ribavirin, a nucleoside analog with therapeutic efficacy during early LASV or LCMV infection, is not recommended in pregnancy due to its teratogenicity.

Cases of lethal human LCMV infection also resulted from transplantation of LCMV-infected organs (Fisher et al., 2006). In one case, the donor could not be linked to any rodent exposure. In another case the donor had come in contact with a pet hamster carrying LCMV, then suffered a severe headache and died of a brainstem herniation. Transplanted organs caused fatal infections in seven of eight recipients; the sole survivor had received ribavirin. One of the recipients was a woman who died 17 days after her liver transplant with fever, rash, diarrhea, hypernatremia, thrombocytopenia, hypoxia and kidney failure (Fisher et al., 2006). It is controversial whether the severity of these deaths was more related to the immune-suppressed state of the transplant recipients, the dose of the virus, or the strain of LCMV involved. From our experience with SIV-infected monkeys, given mild and virulent strains of LCMV, the mild strain Armstrong (LCMV-ARM) remained mild in the context of SIV-infection, and the virulent strain (LCMV-WE) did not progress more rapidly due to the SIV infection (Salvato and Zapata, unpublished observation). Although SIV-infection does not provide as drastic an immune-suppression as the T-cell ablation imposed upon transplant recipients (Wallace et al., 2000), it is possible that the severity of the transplant infections was due to dose and strain of LCMV as well as to immune-suppression.

5. Spectrum of LCMV infection in laboratory animals

5.1. Rodents

LCMV infection of its natural reservoir host, the mouse, can vary widely depending on the age and strain of the mouse and the dose, strain and infection-route of the virus. In general, infection of adult immunocompetent mice elicits a vigorous cell-mediated immune response that peaks in one week and clears the infection within two weeks (Zinkernagel and Doherty, 1974). Interference with cell-mediated immunity (by pregnancy or by genetic or chemical knockout) usually results in chronic infection. From the 1970's to the 1990's LCMV infection of inbred laboratory mice was the best experimental system for defining immune responses to chronic infection. In the 1990's, AIDS superseded LCMV disease, but chronic LCMV infections continue to reveal immunological mechanisms relevant to AIDS progression (Velu et al., 2009).

Hallmarks of murine LCMV infection such as lymphocytic infiltration into the choriomeninges and breakdown of the blood-brain barrier (Doherty et al., 1986) are less pronounced in LCMV and Lassa-infected primates. The cell-mediated immune response so valued for its ability to clear infection can be dangerous when the virus is inoculated intracerebrally causing mice to die from immunopathological choriomeningitis. Over the years, immunopathology has been further defined at a molecular and cellular level. (Doherty and Zinkernagel, 1974) and others (Ahmed et al., 1984; Gilden et al., 1972) used adoptive transfer and antibody ablation to eliminate the effectors of immune pathology. Antibody treatments of mice with anti-CD8 serum eliminated both virus-specific MHC-restricted T cells and immunopathology. Knockout mice lacking molecules involved in the cytotoxic T cell response (perforin, granzymes, interferon and interferon receptors) also knocked-out the immunopathological response (Kagi et al., 1996; Nansen et al, 1999). Thus, by blunting the vigor of the cell-mediated immune response, immunopathology was alleviated and mice were able to slip into a relatively mild chronically-infected state.

The infection outcomes of LCMV strains used commonly in mice bear little resemblance to outcomes with the same viruses in primates. The LCMV-WE we used in non-human

primates as a model of Lassa fever in man is similar to the viscerotropic Docile strain in mice (Oehen et al., 1991), and the strain used as the benign strain in non-human primates (LCMV-ARM) is similar to the neurotropic Aggressive strain in mice (Oehen et al., 1991). The Zinkernagel laboratory used LCMV-Docile (LCMV-WE-like) and LCMV-Aggressive (LCMV-ARM-like) strains to show that mice infected with high doses of the Docile strain usually survived unless they had been vaccinated with LCMV GP or NP antigens (Oehen et al., 1991). In this case vaccination could accelerate the development of disease by shifting the balance slightly from an asymptomatic carrier state to a more responsive immunopathological state. Consequently, vaccines that promote cell-mediated immunity in the mouse have the potential to cause harm in mice, whereas in the primate model they usually prevent disease (Fisher-Hoch et al., 2000; Salvato and Lukashevich, 2009).

Whereas acute “immuno-pathological” LCMV disease in the mouse could be alleviated by immune suppression, arenaviral VHF of guinea pigs or primates was not alleviated by immune suppression (Peters et al., 1987). Guinea pigs are similar to primates in their susceptibility to VHF, but acute arenavirus infections of guinea pigs are accompanied by inflammatory infiltrates that are uncommon in infections of human or non-human primates (Lukashevich et al., 2005; Martinez-Peralta et al., 1990).

The NIH guidelines cryptically define risk group 3 arenaviruses as “neurotropic strains” (NIH Guidelines, 2009; Appendix B-III-D), and since the tropism in mice and primates can be dissimilar, it is best to interpret this as tropism in primates. As mentioned above, the LCMV-ARM strain is neurotropic in mice but not in primates, whereas the WE strain is viscerotropic in mice but is more frequently associated with aseptic meningitis (than is Armstrong) in primates (Buchmeier et al., 1980). A very important area of future research would be to determine how some of the infection outcomes in mice relate to outcomes in primates.

5.2 Primates

The Indian rhesus macaque (*Macaca mulatta*) is the most frequently used primate model because of its availability and its ability to recapitulate specific human diseases (Voevodin and Marx, 2009). Besides the Indian rhesus macaque, there are 6 other smaller subspecies from various regions of China that have been introduced since the 1978 ban on monkey-exports from India (Voevodin and Marx, 2009). Rhesus macaques were used in early poliovirus infection studies (Landsteiner and Popper, 1909), and in yellow fever vaccine research (Theiler and Smith, 1937); most viral hemorrhagic fevers have been amenable to direct study in the rhesus macaque model (see Gowen and Holbrook, 2008a, and Table 1). Healthy rhesus macaques from breeding colonies in Puerto Rico or the United States were used in our studies.

The arenaviruses we have used in monkey experiments are described in Fig. 1. They include Lassa virus-Josiah strain (LASV-JOS), Mopeia virus (MOPV), LCMV-WE, LCMV-ARM, and the Mopeia/Lassa reassortant vaccine candidate ML29. The bi-segmented arenavirus genome allowed us to use reassortant viruses, WE/ARM, ARM/WE, and ML29, (Fig 2) in several studies (Djavani et al., 2005; Lukashevich, 1992, 2005). The reassortant ML29 has the large segment from Mopeia virus (MOPV) and the small segment from LASV (Lukashevich, 1992). In addition, eighteen mutations distinguish the ML29 genome from the parental strains and likely contribute to its attenuated phenotype (Moshkoff et al., 2007). ML29 is highly attenuated in guinea pigs, rhesus macaques, and marmosets (Lukashevich et al., 2005; Lukashevich et al., 2008). Strains of LASV from different regions of West Africa vary so much that the most distant ones have only 88% amino acid sequence homology with each other (Bowen et al., 2000). LASV and MOPV have 68% amino acid sequence homology with each other (Moshkoff et al., 2007) and they both have almost 59% homology

with the LCMV strains (Lukashevich et al., 1997) (Fig. 1). To put this into context, SIV and HIV have approximately 40% protein sequence homology with each other (Kwong et al., 1998). The viruses we used for monkey inoculation were all sequenced, passed on Vero cells, and stored as high-titer stocks so that their inocula would be consistent from experiment to experiment (Djavani et al., 1997; Djavani et al., 1998; Moshkoff et al., 2007; Salvato and Shimomaye, 1989). Some variations in plaque morphology are evident in Fig. 3.

A virulent arenavirus strain for primates is defined operationally as one that causes acute VHF after inoculation. A virulent infection is characterized by: 1) high viremia (>10**5** pfu virus/ml plasma), 2) liver pathology with elevation of AST/ALT enzymes more than 5-fold, 3) a marked increase in vascular permeability notable by accumulation of Evans Blue dye in organs and lymph nodes (Gowen et al., 2010), 4) malaise and respiratory distress, 5) dehydration and weight loss, 6) body temperature elevation followed by a sharp drop shortly before death, and 7) meningitis that occasionally progresses to encephalitis. Transcriptome profiling of LCMV-infected rhesus macaque PBMC also revealed gene expression changes that could potentially serve as biomarkers of disease progression during LCMV infection: the day 1-3 PBMC transcriptome profile was remarkable for high CD14 expression and low COX2, VEGF, and other pro-inflammatory markers; the day 4-7 PBMC transcriptome profile had increased expression of interferon-inducible genes, the pro-apoptotic TRAIL protein, and the chemokine CXCL10 (Djavani et al., 2007, 2009) (Fig. 4b).

CNS involvement is a hallmark of LCMV disease but rarely causes visible lesions. After LASV infection, brain specimens of human and non-human primates failed to reveal specific pathological changes, despite the documentation in humans of fever, ataxia, hearing loss or cognitive impairment (Johnson et al., 1987; Walker et al, 1982). In rhesus macaques given a lethal dose of LCMV-WE, no CNS pathology was seen in 9 cases and a parietal lobe hemorrhage was seen in one high-dose case (Lukashevich et al., 2002; Rodas et al., 2004). Despite using high doses of the mild strain, LCMV-ARM in the macaque model, it remained sub-clinical and could not be found in the CNS, but viral nucleic acid could be detected transiently in spleen and blood. In spite of low viral loads, LCMV-ARM induced cell-mediated immunity and protected monkeys from a lethal dose of the virulent LCMV-WE strain (Lukashevich et al., 2004; Rodas et al., 2004). A similar situation was seen with the Mopeia/Lassa reassortant virus ML29. ML29 proved to be an excellent attenuated Lassa vaccine because it did not cause viremia, it did not enter the CNS, it induced cell-mediated immunity, and it protected guinea pigs and marmosets from lethal challenge with LASV (Lukashevich et al., 2005; 2008). Even in rhesus macaques with SIV/AIDS, ML29 did not enter the CNS (Salvato and Zapata, unpublished observation).

The accidental LCMV-infection of zoo-kept marmosets and tamarins provides an interesting contrast to our studies in the rhesus macaque model. Several outbreaks of hepatitis were traced to feeding the primates LCMV-infected mice. Liver involvement in this infection was more severe than previously observed for Lassa fever or LCMV-infection of human or non-human primates: aminotransferases and bilirubin in plasma were elevated at least 10-fold, random foci of hepatocellular degeneration were observed with inflammatory infiltrates, and acidophilic structures resembling apoptotic Councilman-like bodies were seen in sinusoids and in Kupffer cells (Montali et al., 1995). It is not clear whether the strain of LCMV or the sensitivity of the host was responsible for the extent of liver disease.

5.3. Possible reasons for differences between murine and primate models

Certain rodents serve as natural reservoir hosts for arenaviruses; so their innate immune responses have co-evolved with these viruses. Consequently, chronic LCMV infection is prevalent in rodents and has not been documented in human or non-human primates. In general, primates suppress some innate inflammatory responses, whereas rodents have more

rapid, effective innate responses. Mice resist many human pathogens due to a highly effective type I interferon response, hence, mice deficient in type I interferon responsiveness make good models for testing antivirals that target virus replication (Bray 2001; Genovesi and Peters 1987). VHF pathogenesis in primates is a progression of muted inflammatory responses: those chemokines that recruit immune surveillance (IL-8, Mip-1, TNF-) are shut down in infected cells (Baize 2002; Lukashevich et al, 1999; Mahanty et al., 2001), but systemically, possibly due to responses from uninfected bystander cells, primates with robust cytokine responses at an early stage have a better chance at survival (Baize et al., 2009; Djavani et al., 2007; Mahanty et al., 2001)

Primates are often more susceptible to infection than rodents, developing disease with more than a thousand-fold less infectious virus per gram of body weight. [Mice are often inoculated with 10^5-10^6 pfu/animal whereas macaques are usually given 10^3 pfu/animal]. We speculate that the major differences between murine and primate responses to LCMV are due to differences between T cell subsets and the genetic regions controlling the innate immune responses. Eighty percent of the T cells circulating in primates are the V 2 subset that has no counterpart in rodents. Primate V 2 cells proliferate in response to the small phosphoantigens that are byproducts of cholesterol biosynthesis and components of bacterial cell walls (Cairo et al., 2005; Li H et al., 2008), but rodent T cells do not respond to phosphoantigens (Bonneville et al., 2010). The V 2 subset disappears from the circulation within the first week after infection with HIV, SIV (Bordon et al., 2004), or LCMV (Rodas et al., 2010), yet it is restored in primates vaccinated with benign arenaviruses (Rodas et al., 2009) and in HIV elite controllers (Reidel et al., 2009). Although the T cells comprise only 1-4% of all circulating T cells, their normal role in bridging innate and acquired immunity and rapid disappearance following LCMV infection suggests their involvement in the pathogenesis of VHF. NK cells in rhesus monkeys also decreased two days after LCMV infection with a more dramatic decrease after LCMV-WE (virulent) infection than after LCMV-ARM (benign) infection (Rodas et al., 2009). Similarly, in Lassa-infected cynomolgus macaques, NK cell decreases were more dramatic in fatal outcomes (Baize et al., 2009).

Besides differing in T cell subsets, primates differ from rodents in the way they activate pathogen response genes. For example, Toll-like receptors (TLR) and the steroid hormone pathways are prominently activated during VHF (Djavani et al., 2007, 2009; Rubins et al., 2007; Hoang et al., 2010). TLR proteins via signaling cascades indirectly activate inflammatory responses, whereas the receptors for steroid hormones, thyroid hormone, retinoic acid, and Vitamin D directly bind to promoter regions containing SINE and LINE (short and long interspersed nuclear elements). These elements comprise 42% of the primate genome, are primate-specific and are thought to dampen expression of inflammatory pathogen-response pathways via the production of microRNA (Gombart et al., 2009; Piriyapongsa et al., 2007).

6. LCMV infection of rhesus macaques

6.1. General features of illness

Current work with monkey models for Lassa fever relies heavily on the fieldwork, vaccine trials, and published clinical observations of others (Cummins et al., 1989a; Fisher Hoch et al., 1987; Fisher-Hoch and McCormick, 2004; McCormick et al., 1986a; Peters et al., 1987; Roberts et al., 1989). Czechoslovakian studies in the early 1960's described aerosol inoculation of rhesus and cynomolgous macaques with LCMV-WE, a virulent strain, and LCMV-ARM, a benign strain, in which both cynomolgous and rhesus macaques succumbed to LCMV-WE-mediated VHF within 13 days. The rhesus macaques appeared more similar

to man than the cynomolgus macaques in terms of pathology (Danes et al., 1963), so we used rhesus macaques for our studies.

Disease signs in the LCMV-WE infected macaque include severe dehydration, erythematous skin, submucosal edema, necrotic foci in the buccal cavity, and respiratory distress due to pulmonary edema. Capillary leakage appears as petechia, red mottling or occasional hemorrhaging of organs and mucosal surfaces. Histopathology reveals cellular depletion of bone marrow, thymus and other lymphoid tissues, and, in liver, scattered hepatocellular necrosis with minor inflammatory infiltrate (Lukashevich et al., 2002) (Fig. 4a).

Whether the infection is delivered intravenously or intragastrically, LCMV-WE has a major effect on liver, with several of the signs being similar to surgical or toxic liver injury: 5- to 10-fold elevation of plasma aminotransferase levels, an increase of mitotic liver nuclei from <5% to 25-40%, and increases in plasma levels of IL-6, sIL6R, sTnfRI and sTnfRII (Lukashevich et al., 2003). An increase in AST/ALT enzymes and IL-6 was also seen in cynomolgus monkeys infected subcutaneously with LASV (Baize et al., 2009). The hepatic damage seen in acutely-infected LCMV-WE rhesus macaques is more severe than that seen in human Lassa fever cases (Igor Lukashevich, personal observation). The absence of pronounced tissue inflammation/infiltration observed in human/primate infections with Lassa and LCMV-WE contrasts with the extensive PMN infiltrates observed in LCMV-WE-infected guinea pigs (Martinez-Peralta et al., 1990). In the guinea pig model, PMN are initially essential for antiviral defense; however, late in infection, PMN cause massive tissue destruction, primarily in spleen and lung (Gonzalez et al., 1987). Morbidity in the rhesus LCMV-WE model, as well as death in Lassa fever, Argentine hemorrhagic fever, and Bolivian hemorrhagic fever is due to vascular dysfunction and subsequent circulatory failure (Fisher-Hoch, 1993; Ruggiero et al., 1964), though South American hemorrhagic fevers usually have more extreme neutropenia, thrombocytopenia, neurological signs and bleeding than do fatal infections with LCMV or Lassa (Peters et al, 1987).

Transcriptome profiling of monkey PBMC during the course of disease revealed gene expression changes that could supplement the known disease signs. Several recent profiling studies on acute viral diseases show that major changes in gene expression can occur immediately after infection and prior to the outward manifestation of disease (Baas et al., 2006; Carroll et al., 2006; Djavani et al., 2007). In human beings exposed to respiratory disease viruses, changes in gene expression occur within hours of exposure whereas febrile symptoms occur approximately 3-5 days later. Analysis of the early gene expression changes reveals biochemical pathways that may be contributing to the disease process; so pharmaceutical intervention to suppress some of these pathways may serve as early and effective treatments for disease. On the other hand, it is also possible that these early pathways are essential for defense against the disease process and that blocking these pathways may actually accelerate the disease process. We noted that both acute and mild LCMV-WE liver infections drastically affected glucose metabolism (Djavani et al., 2009), thereby putting these transcriptome changes in the category of essential defenses that contribute little to pathogenesis. In contrast, interferon-inducible genes in liver tissue were more dramatically up-regulated in virulently-infected livers than in mildly-infected livers, specifically, the pro-inflammatory PPP1R3C; putting these genes in the category of reasonable treatment targets (see section 7.3).

6.2. Viremia and virus distribution

For most VHF, the virus inoculum first infects subdermal dendritic cells and macrophages. Virus-infected cells then migrate to the regional lymph nodes, where further replication results in viremia and dissemination to distal sites in the body. The clinical signs and symptoms of fever, rash, thrombocytopenia, plasma leakage, bleeding and elevation of AST/

ALT enzymes that are the hallmarks of all VHF are then manifest as part of a multi-organ syndrome.

LCMV disease in the primate is highly dose-dependent. For infection with LCMV, macaques have received subcutaneous or intravenous doses of up to 10^{8} pfu LCMV-ARM with no ill effect, but 10^{3} pfu LCMV-WE was lethal (lower doses have not been tried). Table 1 compares arenavirus infections in different primate models and corroborates the fact that iv, im, or sc infections need the lowest doses to see effects. Aerosol inoculations can also be effective at low dose, and, along with ingestion of contaminated food, constitute a natural route for infections with LCMV and Lassa (Peters et al, 1987; Rai et al, 1997). Since arenaviruses are pH sensitive (DiSimone et al., 1994), oral and i.g. inoculations require large doses, rarely result in viremia, and often protect animals from lethal challenge (Lukashevich et al., 2002, 2003, 2004; Rai et al., 1996; Rai et al., 1997). As much as 10^{7} pfu LCMV-WE introduced by intragastric gavage can be either uneventful, cause brief disease, or be fatal; with a non-fatal infection usually resulting in both cell-mediated and humoral immune responses and protection from lethal challenge (Lukashevich et al., 2002, 2003, 2004; Rodas et al., 2004). Table 2 summarizes our results in rhesus macaques.

Healthy animals inoculated with LCMV-WE (10^{3}-10^{6} iv or 10^{7}-10^{8} ig) became viremic by day 4-6, showed disease signs by day 7 and died within 10-14 days (Lukashevich et al., 2004; Rodas et al., 2004). Monkeys that had been “vaccinated” by pre-exposure to LCMV-ARM or low-dose LCMV-WE mucosal exposure survived without disease signs and resisted high dose challenges with LCMV-WE (Lukashevich et al 2004; Rodas et al, 2004)

Healthy monkeys infected with virulent virus, like LCMV-WE or Lassa, developed plasma viremia, whereas monkeys infected with mild viruses, such as LCMV-ARM or ML29 did not develop viremia (meaning that virus was not detectable in blood by plaque assay or by second-round viral amplification as described by Lukashevich et al., 2008). In sub-clinical infections, viral RNA was usually detected by RT-PCR in tissues and monkeys developed virus-binding antibodies, cell-mediated immune responses, and immunity to lethal challenge. This situation was notably different for monkeys that were SIV-infected, in which case ML29 vaccination resulted in a transient, low-level but detectable plasma viremia but no additional pathology (Salvato and Zapata, unpublished observation).

6.3. Thrombocytopenia and coagulation defects

Rhesus macaques given LCMV-WE suffer a more acute thrombocytopenia than was described for Lassa-infected human beings or rhesus macaques given Lassa virus (Fisher-Hoch et al., 1988). It is unlikely that this is due to increased sequestration in tissues since few inflammatory infiltrates have been observed in diseased tissues. It is also unlikely that this is due to increased megakaryocyte/platelet destruction since we see little increase in pro-apoptotic activities (Rodas et al., 2009). The decrease in thrombocytes is most likely linked to the severe cellular depletion in nodes, spleen and bone marrow seen in virulent infection (Lukashevich et al., 2002). Similar depletion has been noted for Lassa fever (Fisher-Hoch, 1988), Argentine hemorrhagic fever of rhesus macaques (McKee et al., 1985) and guinea pigs (Gonzalez et al., 1987), but it has not been concluded whether this depletion is less severe during Lassa infection. Such depletion has been used to explain the impaired lymphocyte function described for Lassa infection of macaques (Fisher-Hoch et al., 1987). Thus the most likely reason for thrombocytopenia is a decrease in bone marrow megakaryocyte production, but it is not clear how this differs between LCMV-infected macaques and Lassa-infected human beings.

In general, hypovolemic shock and hemorrhage result either from directly damaged vasculature or from inadequate repair of damaged vasculature. In LCMV and Lassa disease,

vascular leakage is most likely from inadequate repair. Unlike the filoviruses Marburg and Ebola, that destroy vascular endothelial cells in culture (Baize et al., 1999; Feldmann et al., 1996; Schnittler et al., 1998; Yang et al., 1998), LASV and LCMV do not destroy cultured endothelial cells, dendritic cells or macrophages, but do diminish their capacity to express chemokines (Fisher-Hoch et al., 1987; Lukashevich et al., 1999; Schnittler et al., 1998; Baize et al., 2004). Also unlike Ebola and Marburg hemorrhagic disease, Lassa fever disease does not cause an increase in fibrinogen breakdown products or in disseminated intravascular coagulation (DIC) [Fisher-Hoch et al., 1988]. With Lassa, there is a defect in platelet aggregation that corresponds to severity of disease (Cummins et al., 1989a, b); however, platelet and fibrinogen turnover rates are normal in experimental primate infections (Fisher-Hoch et al., 1987). Thus, the bleeding disorder in Lassa-infected animals has been attributed to an unknown soluble factor, possibly from infected monocytes, that inhibits platelet function and arrests megakaryocyte maturation (Roberts et al., 1989). Our observation of LCMV-infected rhesus macaques revealed a drop in platelets from 400000-500000/mm^3 to 40000-90000/ mm^3 that directly explains decreased coagulation and inadequate vascular repair. Thus, the vascular damage observed in the i.v.-infected monkeys is most likely due to defects in vascular repair.

Transcriptome data from liver during virulent LCMV infection of macaques reveals an over-expression of complement component 1S (C1s), an anti-thrombin peptide, and high expression of fibrinogen beta chain (Djavani et al., 2009). In corroboration with the over-expressed fibrinogen, increased levels of IL-6 are observed during virulent LCMV infection (Lukashevich et al., 2003) and IL-6 stimulates the production of fibrinogen (Ernst and Koenig, 1997). An extrapolation from the gene expression data would predict an accumulation of fibrinogen due to the increased mRNA and the expression of a protein that inhibits fibrinogen cleavage. Further studies are needed to determine how the elevated levels of fibrinogen, pro-inflammatory cytokines, thrombocytopenia, and platelet function abnormalities all contribute to the coagulation defects that are a hallmark of VHF.

7. Use of the LCMV-WE-infected macaque for antiviral research

7.1 Ribavirin is a useful broad spectrum antiviral in primates

Despite the threat of VHF for human health and the evaluation of several different types of compounds for VHF treatment (Leyssen DeClercq, Neyts, 2008), no therapy for any VHF is currently licensed for clinical use. Treatment of rhesus macaques with the guanosine analog, ribavirin can prevent Lassa fever (Jahrling et al., 1980), and in cynomolgus macaques ribavirin showed an additive positive effect with immune serum (Jahrling et al., 1984). Subsequently, people given ribavirin within the first 6 days of illness experienced reduced Lassa fever mortality (McCormick et al., 1986). However this broad spectrum antiviral, has several drawbacks due to its teratogenic, hemolytic and thrombocytic properties. Ribavirin also has demonstrated potential in the pursuit of “lethal mutagenesis”, a strategy to extinguish LCMV replication by combining a mutagen, like 5’fluorouracil with an antiviral such as ribavirin (Martin et al., 2010).

7.2 New virus-specific antivirals have not been tested in primates

A number of cell culture studies and high throughput screens have identified compounds with antiviral potential, but none have yet been tested in non-human primates. The LCMV primate model would be useful for testing antivirals that target LCMV directly, however, efficacy against LASV, JUNV, or other VHF agents would have to employ those viruses to satisfy the two animal rule.

A broad-spectrum lipophilic entry-inhibitor, LJ001, was found to inhibit before the fusion step (Wolf et al., 2010). A screen for entry inhibitors found ST-294 (Bolken et al., 2006) and

ST-193 (Larson et al., 2008). The specificity of these molecules is illustrated by the fact that ST-193 blocks entry of several hemorrhagic arenaviruses but not LCMV (Larson et al., 2008), and both ST-193 and ST-294 stabilize interaction between GP2 and the arenavirus signal peptide, thereby inhibiting membrane fusion (York et al., 2008). ST-294 was effective in a TCRV newborn mouse model (Bolken et al., 2006) and ST-193 was effective against LASV in guinea pigs (Cashman et al., 2011). This entry/fusion step could also be inhibited for Junin virus by a monoclonal antibody, F100G5 (York et al., 2010). Screens also found small peptidomimetics and heterocyclic compounds that blocked the arenaviral GP2-mediated membrane fusion (Lee et al., 2008a; Whitby et al., 2009). Finally, phosphorothioate oligonucleotides that were shown to block HIV entry were also successful against a series of LCMV isolates (Lee et al., 2008b).

To target the arenavirus Z matrix protein, several aromatic disufide compounds were tried. These agents eject the essential zinc ions with the consequent loss of native structure, thereby destroying the ability of Z proteins to oligomerize (Garcia et al., 2006; Sepulveda et al., 2010). Another successful approach to block Z employed small interfering RNA (siRNA) against the Junin virus Z protein (Artuso et al., 2009). However, the Z protein can inhibit arenavirus replication (Cornu and De la Torre, 2001; Lopez et al., 2001); hence, anti-Z compounds could potentially have the detrimental effect of actually increasing viral loads *in vivo*.

Antivirals that target arenavirus replication include siRNA and nucleotide analogs that interfere with RNA synthesis. The siRNA against the conserved RNA termini upstream of NP and L genes were able to reduce expression of a Lassa virus replicon (Muller and Gunther, 2007), but this type of interference demands an exact match between siRNA and target sequences (Sanchez et al., 2005). A pyrazine derivative targeting RNA synthesis, T-705, was found to be active against influenza, JUNV, Pichinde (PICV), and TCRV and to confer protection in the PICV/hamster model for VHF (Furuta et al., 2009; Gowen et al., 2008).

7.3 Antivirals targeting host molecules show promise for testing in the VHF monkey model

One of the most powerful tools in murine studies has been the use of genetic knock-outs to determine which pathways are essential for infection. Every day more tools are becoming available to knock-out primate responses. Our own studies using antibodies to knock-out FasL (Poonia et al., 2009, Salvato et al., 2007) showed that pathogenesis and destruction of memory immunity could be attenuated by a single brief treatment with this antibody in the SIV-infected rhesus model. More recently, shRNA inhibitors of host mRNA and an array of enzyme inhibitors have become available to target host responses during VHF.

Cholesterol-depleting drugs such as methyl-beta-cyclodextrin can reduce entry of PICV, LASV, and LCMV (Shah et al., 2006). The antibody, “bavituximab”, recognizes a plasma protein, B2-glycoprotein, known to bind phosphatidylserine that is more abundant on viral membranes than on the outer leaflet of host cell membranes (Soares et al., 2008). Bavituximab protected PICV-infected guinea pigs and its efficacy was increased when used with ribavirin (Soares et al., 2008).

Genistein, a plant-derived isoflavone and host cell kinase inhibitor, has anti-PICV effects in Syrian hamsters (Vela et al., 2010). Peptide-derived inhibitors of the cellular protease needed for arenavirus maturation, S1P/SK1-1 have also shown promise against LCMV and Lassa (Rojek et al., 2010) and since the same protease is essential for other viruses these antivirals may have broader potential. Cellular protein TSG101 is known to bind arenavirus Z proteins and mediate budding, and anti-TSG101 siRNA can reduce the production of virus-like particles, VLP (Perez et al., 2003). Over-expression of tetherin, another broadly-

acting antiviral, can inhibit the production of Lassa virus (Radoshitzky et al., 2010). The AKT pathway is an important control center for cell cycling and inhibition of AKT phosphorylation with Ly294002 has recently been shown to slow production of JUNV (Linero et al., 2009).

One way to discover host molecules needed for virus replication is by genomic, proteomic, and other biochemical profiling of infected tissues. Transcriptome profiling of rhesus macaque PBMC RNA was performed at the early stages of LCMV infection (Djavani et al., 2007). By comparing the profiles of macaques given virulent LCMV-WE and macaques given LCMV-ARM, it was revealed that lethal challenge induced an extreme repression of COX2 / leukotriene pathways and several signaling pathways resembling the toxicity (hemorrhage and cardiac arrest) caused by drugs like VIOXX that block COX2 function. According to our analyses, drugs that stimulate prostacyclin biosynthesis and drugs that diminish the activity of MLCK, a kinase that erodes the blood brain barrier, would be reasonable treatments to reverse the earliest signs of arenaviral hemorrhagic fever. A rational approach to treatment studies would be to systematically target the products of differential gene expression using drugs known to modulate these proteins. To that end, we matched the products of the most extreme transcriptome changes associated with virulent infection to a library of FDA-approved drugs to find drugs that could be used to treat arenaviral HF (see Table 3).

The LCMV-infected macaque model for VHF is most powerful for revealing host response networks during the earliest stages of disease progression. When a physiological system is severely perturbed, drugs should be found to help dampen the extreme responses. Computational methods can find multiple target intervention solutions with maximum therapeutic effects (Yang et al., 2008). In the first stage a healthy and diseased network state are defined, then network perturbations expected from each drug are considered, and the drug combinations expected to come closest to a healthy state are optimized. Since the simplest primate study to test one drug costs around $70,000, testing drug combinations *in vivo* would benefit from preliminary *in silico* modeling of the expected network perturbations.

8. Conclusions

Non-human primate studies have advanced our knowledge of VHF pathogenesis. The most valuable lessons from our studies of arenaviruses in primate models are: 1) The route of infection greatly influences disease progression and immunity, 2) Primates are usually more susceptible to infection than rodents, 3) Infection outcomes in rodents do not predict disease outcomes in primates or man, and 4) new ways to analyze host responses to infection point out many new targets for antivirals.

Abbreviations

AST/ALT	ratio of the enzymes, alanine aminotransferase and aspartate aminotransferase
pfu	infectious units or plaque forming units
ffu	focus forming units
VHF	Viral hemorrhagic fever
LCMV-WE	Lymphocytic choriomeningitis virus (virulent) strain WE
LCMV-ARM	lymphocytic choriomeningitis (benign) strain Armstrong

PICV	pichinde virus
TACV	Tacaribe virus
LASV	Lassa fever virus
MOPV	Mopeia Virus
ML29	a Mopeia/Lassa reassortant vaccine candidate
iv	intravenous
sc	subcutaneous
ig	intragastric
im	intramuscular
IL-6	Interleukin-6
sIL6R	soluble interleukin-6 receptor
sTnfRI	soluble TNF-receptor 1
sTNFRII	soluble TNF-receptor 2
PMN	polymorphonuclear leukocytes

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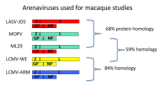
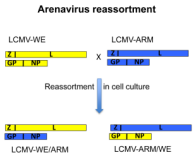
Highlights

- History of LCMV infections of primates (from mice to monkeys and man)
- Comparison of outcomes in murine and primate infections
- Description of findings from LCMV-infected rhesus macaques
- Utility of the model for testing host-response antivirals over virus-specific antivirals



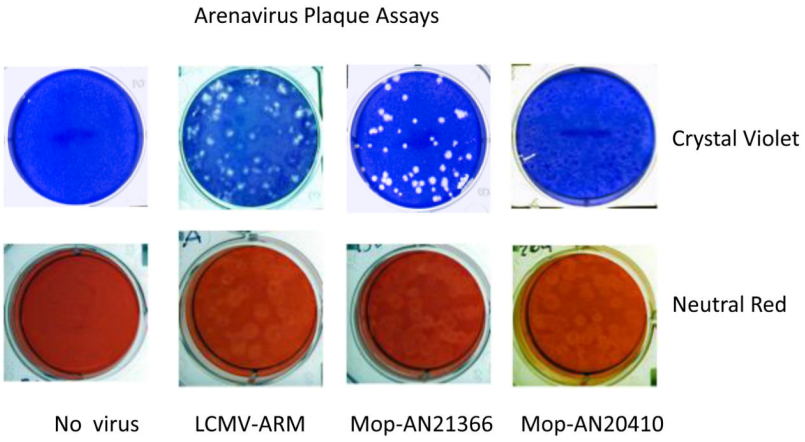
1.

Arenaviruses used for macaque studies. Old world arenaviruses Lassa virus-Josiah strain (LASV-Jos), Mopeia virus (MOPV), the Mopeia/Lassa reassortant (ML29), and the LCMV strains WE and Armstrong (LCMV-WE, LCMV-Arm) have been studied in rhesus macaques (Fisher Hoch et al., 1987; Jahrling et al., 1980; Lukashevich et al., 2002, 2003, 2004, 2005; Rodas et al., 2004). They are mentioned in this review by way of comparison to the LCMV macaque model for Lassa fever.



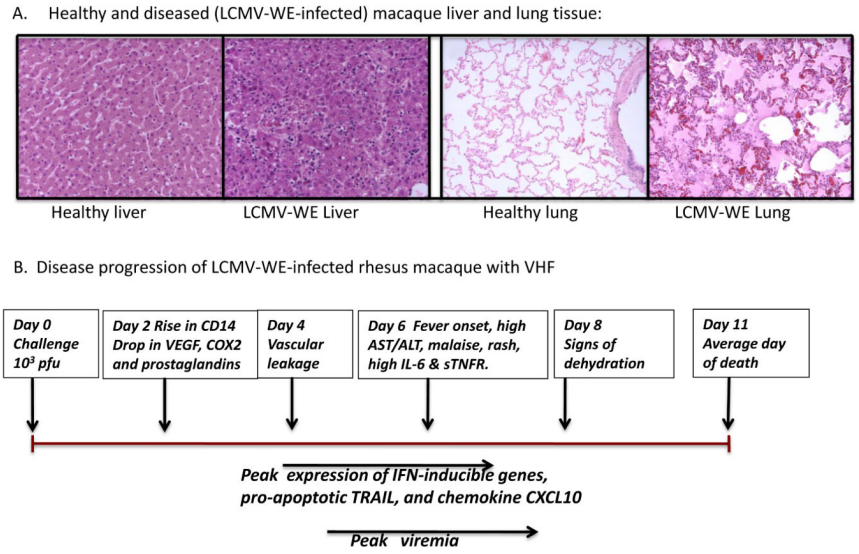
2.

Arenavirus reassortment. The two RNA segments of the arenavirus genome can be derived from two parental virus strains that co-infected the same host cell. The ML29 Lassa vaccine candidate was obtained by reassortment between LASV and MOPV parental strains (Lukashevich et al., 1992), and the LCMV-WE/Arm and Arm/WE strains were obtained by reassortment of WE and Arm strains of LCMV (Riviere et al., 1986).



3.

Arenavirus plaque morphologies. A couple of examples of different plaque morphologies for the LCMV-Arm and Mopeia (strains AN21366 and AN20410) are shown. The blue stain with crystal violet and formalin fixation is more distinct than the red stain with neutral red without fixation, but the latter enables the isolation of infectious plaques. Arenaviruses tend to bud rather than lyse the host cell, so a plaque is due to poor cell growth and adherence rather than to cell lysis (as it is for vesicular stomatitis or polio viruses).



4.

Healthy and diseased (LCMV-WE-infected) macaque liver and lung tissue (A). A timeline of disease progression for a typical LCMV-WE infected rhesus macaque with viral hemorrhagic fever (B).

Table 1

Non-human primate models for arenaviral hemorrhagic fevers.

Monkey model[a]	**Disease modeled**	**Arenavirus species**	**Dose, Route**	**Outcomes**	**References**
Rhesus macaque	Lassa fever	Lassa fever virus	2.4×10^6 ffu, sc 3,000 ID50, sc 10^4 pfu, sc $10^{1.95}$ pfu, sc	6 out of 10 died. All animals developed VHF and died. 9 out of 17 died or were euthanized due to severe disease. All 3 animals died of VHF.	Stephen and Jahrling, 1979; Kiley et al 1979; Jahrling et al, 1980 Walker et al, 1982 Callis et al, 1982 Fisher-Hoch et al, 1987
Cynomolg us macaque	Lassa fever	Lassa fever virus	1.2×10^6 pfu, sc $10^{2.7}$-$10^{4.5}$ pfu, aerosol 10^3 pfu, sc 10^7 pfu, sc	13 out of 14 died and 19 out of 20 died 10 of 10 died 2 out of 3 low-dose animals died. 3 out of 3 high-dose animals survived. All animals developed VHF signs	Jahrling et al 1984, Jahrling and Peters 1984 Stephenson et al, 1984 Baize S et al 2009
Common marmoset	Lassa fever	Lassa fever virus	10^3 pfu, sc 10^6 pfu, sc 10^3 pfu, sc challenge with Lassa. 10^3 pfu, sc ML29 vaccine, then at day 30, challenge with 10^3 pfu, sc LASV	2 out of 2 died. 2 out of 2 died. 2 out of 2 control animals were sacrificed due to severe VHF disease. 6 animals vaccinated with ML29 and then challenged with LASV survived without signs of disease.	Carrion et al, 2007 Lukashevich et al, 2008
Rhesus macaque	Lassa fever vaccinatio	Mopeia (Mozambique) virus	10^3 pfu, sc	Complete protection against Lassa virus challenge	Stephen and Jahrling, 1979; Kiley et al 1979; Jahrling et al, 1980; Walker et al, 1982
Rhesus macaque	Lassa fever vaccination	MOP/LAS reassortant (ML29)	10^3 pfu, sc	Robust cell-mediated immunity, animals not challenged with Lassa	Lukashevich et al, 2005
Rhesus macaque	VHF	LCMV-WE	10^3pfu, iv; 10^6 pfu, iv 10^6 pfu, ig 10^3 pfu, iv 10^7 pfu, ig; 10^8 pfu, ig	2 diseased animals were euthanized. 2 animals survived high ig dose without signs of VHF 1 out of 4 animals died 1 high-dosed (ig) animal developed VHF signs but recovered 1 high-dosed (ig) animal died of VHF.	Danes et al 1963, Lukashevich et al, 2002, 2003, 2004 Rodas et al 2004, Djavani et al, 2005, 2007, 2009

Monkey model[a]	Disease modeled	Arenavirus species	Dose, Route	Outcomes	References
Cynomolgus macaque	VHF	LCMV-WE	10^6 pfu sc	Lethal for all animals inoculated.	Fort Detrick (MD) work cited by Lukashevich et al 2002
Rhesus macaque	VHF, vaccination	LCMV-Armstrong	10^3 sc to 10^8 ig	Complete protection against LCMV WE virus challenge of 10^3 sc	Rodas et al 2004, Djavani et al, 2005, 2007, 2009
Common marmoset	callitrichid hepatitis	LCMV-CH	Animals ingested infected mice	All animals developed VHF signs	Montali et al 1995
Rhesus macaque	Argentine HF	Junin virus, (neurotropic and viscerotropic strains)	10^4 pfu, sc. aerosol route	18 animals infected with 4 different strains. 3 out of 6 animals died. Strain-dependent disease severity.	McKee et al, 1985, 1987; Green et al, 1987 Kenyon et 1992
Common marmoset	Argentine HF	Junin virus	10^3 TCID50, im. 105TCID50, im Tacaribe, then 10^3TCID50, im Junin challenge 10^3TCID50, im.	4 of 4 died after severe disease. 5 of 5 Tacaribe vaccinated had sterilizing immunity to Junin. 3 out of 3 died after VHF signs. 3 of 3 died with VHF signs.	Weissenbacher et al 1982 Weissenbacher et al 1986 Avila et al, 1987
Common marmoset	Argentine HF Vaccination	Tacaribe virus	10^5TCID50, im Tacaribe vaccination, 1,000 TCID50, im Junin challenge	Complete cross-protection against Junin virus challenge	Weissenbacher et al 1982, Samoilovich et al 1984, 1988
Rhesus macaque	Argentine HF Vaccination	Junin virus (Candid #1 vaccine strain)	Candid#1 =10^1 to 10^5 pfu, sc. Junin virus challenge =10^5 pfu , sc.	Complete protection by Candid#1 vaccine against Junin virus challenge.	McKee KT et al 1992, 1993
Rhesus macaque	Bolivian HF	Machupo virus	10^5 pfu, sc; 10^3pfu, sc; 10^1 pfu, sc.	9 out of 12 animals died, all developed VHF. 4 monkeys died, 1 from a non-related disease. 4 monkeys died of VHF. 2 of 4 monkeys survived the low dose.	Terrell et al 1973, Gonder E et al 1986
African green monkey	Bolivian HF	Machupo virus	10^3 pfu, sc	6 of 6 animals died. 5 animals developed VHF signs and 1 died with CNS disease	Wagner FS et al 1977, McLeod CG et al 1978

[a]Monkey models Rhesus macaques = Macaca mulatta, Cynomolgus macaques = Macaca fascicularis, African green monkeys = Cercopithecus aethiops; Common marmoset = Catlithrix jacchus

[b] Abbreviations: ffu = focus forming unit, pfu = plaque forming unit, sc = subcutaneous, im = intramuscular, iv = intravenous, ig = intragastric, TCID50 = 50% of the Tissue culture infectious dose, ID50 = 50% of the infectious dose.

Table 2

Rhesus macaques used for arenavirus studies. Data for Rh-iv3 to Rh-ig6c were from monkey infections described in (Lukashevich et al 2002). Data for Rh-ig7a to Rh-iv3b were from monkey infections described in (Rodas et al, 2004). Data from transcriptome profiling was from (Djavani et al, 2007, 2009). Viral loads above 10 pfu/ml of plasma were detectable by plaque assay or by co-cultivation. When infectious virus was not detectable, it was sometimes possible to detect viral RNA by Reverse Transcription-PCR. For Rh-ig7b, plasma viral RNA was detectable only on week 3, not weeks 1, 2, or 4. Abbreviations used: Rh = rhesus monkey; pi = post initial infection; pc = post challenge; lc = lethal challenge; Euth. = euthanized while healthy at the end of the experiment.

Monkey	Inoculum	Infection	Viremia c	Death
Rh-iv3	WE 10^3 iv	Acute disease	Plasma virus d7	d11 pi
Rh-iv6	WE 10^6 iv	Acute disease	Plasma virus d6	d12 pi
Rh-ig6a	WE 10^6 ig	No disease	RNA in plasma d14	Euth.
Rh-ig6b	WE 10^6 ig	No disease	RNA in plasma d14	Euth.
Rh-ig6c	ARM10^6 ig	No disease	RNA in tissues d3	Euth.
Rh-ig7a	WE 10^7 ig	No disease/failed lethal challenge (lc)	RNA not detected	d14 pc
Rh-ig7b	WE 10^7 ig	Acute disease/recovered/ survived lethal challenge	RNA in plasma day 20	Euth.
Rh-ig8	ARM10^8 ig	No disease/survived lc	RNA not detected	Euth.
Rh-ig8a	WE 10^8 ig	Acute disease	Plasma virus d6	d10 pi
Rh-ig8b	WE 10^8 ig	No disease/ failed lc	RNA not detected	d13 pc
Rh-iv3b	WE 10^3 iv	Acute disease	Plasma virus d6	d14
Rhesus macaques used for transcriptome profiling of blood, liver, other tissues				
8 -Rh	WE 10^3 iv	Acute disease	Plasma RNA d4-7	Euth.
3 -Rh	ARM 10^3 iv	No disease	RNA not detected	Euth.
5 -Rh	uninfected	No disease	RNA not detected	Euth.

Table 3

Identification of gene products (by transcriptome analysis) with drug-interactive domains

Gene / Description (Protein)	Similar Drug Domain[b]	Drug or Candidate[c]	References
CXCR4 / Chemokine (C-X-C motif) receptor 4 isoform a	Angiotensin II type 1 receptor (AT1) (P30556); ID 59%	**Agonists, e.g. Telmisartan** ®	Wienen et al, 1993
DHRS9 / Dehydrogenase/reductase (SDR family) member 9 NADP-dependent **retinol dehydrogenase/reductase**	Enoyl acyl carrier protein reductase (InhA) (P46533) ID 18%	**Inhibitors, e.g. Isoniazid** ®	Vilcheze et al, 2000
EBI2 / EBV-induced G-protein coupled receptor 2	Somatostatin receptor 2 (SSTr2) (P30874); ID 59%	**Agonists, e.g. Octreotide**®; **Lanreotide**®	Patel, 1999
GPR109B / G-protein coupled receptor 109B **(G-protein coupled receptor HM74)**	Somatostatin receptor 5 (SSTr5) (P35346); ID 15%	**Agonists, e.g. Octreotide**®; **Lanreotide**®	Patel, 1999
MYLK / Myosin light chain kinase isoform 1 **(MLCK)**	rho-associated kinase (ROCK1) (Q13464); ID 30%	**MLCK inhibitors,**[d] **e.g. Compound 6; ML-9; Wortmannin** **ROCK inhibitors,**[e] **e.g. Y-27632**[d]	Behanna et al, 2006, Weber et al, 2000 Fu et al, 1998, Ishizaki et al, 2000
NR4A2 / Nuclear receptor subfamily 4, group A, member 2 Orphan nuclear receptor **(NURR1)**	RAR beta receptor (P10826); ID 26%	**NURR1 agonists,**[d] **e.g. Benzimidazoles Experimental** **RARbeta agonists**[d]	Dubois et al, 2006 Johnson et al, 1999a, Johnson et al, 1999b
PTGS2 / Prostaglandin-endoperoxide synthase 2 **(Cyclooxygenase 2)**	Cyclooxygenase-2 (COX-2) (P35354); ID 100%	**Cox2 activators, e.g. Bryostatin**	De Lorenzo et al, 2003
TNFSF10 / Tumor necrosis factor ligand superfamily member 10 (TNF-related apoptosis inducing ligand) or **TRAIL protein**	Tumor necrosis factor alpha (P01375); ID 21%	**TNFα antagonists, e.g. Etanercept** ® **Thalidomide** ®	Taylor, 2003 Radomsky and Levine, 2001
TNFRSF21 / Tumor necrosis factor receptor superfamily member 21 precursor (TNFR-related death receptor-6) or **Death receptor 6**	ERBB-2 receptor tyrosine kinase (HER2/neu) (P04626) ID 11%	**TNFα antagonists, e.g. Etanercept** ®	Taylor, 2003

[a] Recommended treatments for virulent virus infection.

[b] Numbers in parentheses indicate Swiss-Prot accession numbers. ID means identity.

[c] "Drug" means FDA-approved and "Candidate" means in clinical trial.

[d] Not approved drug, a variety of experimental compounds or derivatives are in development.

[e] Many other ROCK inhibitors are in biological testing.