Lassa Virus Infection in Experimentally Infected Marmosets: Liver Pathology and Immunophenotypic Alterations in Target Tissues

Running head: Lassa Virus Infection in Marmosets

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ABSTRACT

Lassa virus causes thousands of deaths annually in western Africa and is considered a potential biological weapon. In an attempt to develop a small nonhuman primate model of Lassa fever, common marmosets were subcutaneously inoculated with Lassa virus strain Josiah. This inoculation resulted in a systemic disease with clinical and morphologic features mirroring those in fatal human Lassa infection: fever, weight loss, high viremia and viral RNA load in tissues, elevated liver enzymes, and severe morbidity between day 15 and 20. The most prominent histopathology findings included multifocal hepatic necrosis with mild inflammation and hepatocyte proliferation, lymphoid depletion, and interstitial nephritis. Cellular aggregates in regions of hepatocellular necrosis were largely composed of HAM56+ macrophages, devoid of CD3+ and CD20+ cells, and characterized by marked reduction in the intensity of HLA-DP,Q,R staining. A marked reduction in the MHC-II expression was also observed in lymph nodes. Immunophenotypic alterations in spleen included reduction in overall numbers of CD20+ and CD3+ cells and disruption of lymphoid follicular architecture. These findings identify the common marmoset as an appropriate model of human Lassa fever and present the first experimental evidence that replication of Lassa virus in tissues is associated with alterations that would be expected to impair adaptive immunity.
INTRODUCTION

Lassa virus is the causative agent of Lassa fever, a fatal disease affecting more than 300,000 people a year in western Africa (33). The overall instance of fatality is 1-2%, but can be as high as 15-30% in hospitalized Lassa fever patients. Lassa virus, a member of the Arenaviridae family (Old World or Lassa-LCMV serogroup) (41), is transmitted from a natural rodent reservoir, *Mastomys* ssp., to humans via contaminated rodent excreta or by close contact with infected individuals (33). Following an incubation period of 7-18 days, the disease is marked by a gradual onset of symptoms including fever, weakness, and malaise. As the disease progresses, nausea, vomiting, diarrhea, and abdominal pain are often observed. Hemorrhage on mucosal surfaces, such as conjunctival hemorrhages or gastrointestinal or vaginal bleeding, occurs in less than 20% of the cases. Late stages of the disease are marked by shock, seizures, and coma, culminating in death. Disease severity, imported cases of disease from patients that traveled to endemic areas, and the potential use of this agent as a biological weapon underscores the need to understand its viral pathogenesis as well as to develop intervention strategies (6, 27, 31, 33).

Several models have been used to study the disease, including guinea pigs and nonhuman primates (8, 17, 18, 39, 45, 46). The strain 13 guinea pig has become a useful model for the study of Lassa fever pathogenesis and shares characteristics consistent with human disease (18, 39). This model has been successfully used for the evaluation of the efficacy of Lassa fever vaccine candidates (2, 9, 25, 40). However, Lassa virus is treated differently by the immune system of guinea pigs and nonhuman primates, and vaccine studies in guinea pigs are not necessarily predictive for human vaccines or therapeutics (13). Because the immune system of nonhuman primates is similar to that of humans, nonhuman primates are a relevant model to study the pathogenesis of many human
infectious diseases and are generally good predictors of efficacy in vaccine development and intervention strategies. The rhesus macaque has been used to study Lassa virus and has proven to be a superior model in pathology. It is hypothesized to be more predictive than the guinea pig model in the development of intervention strategies (8, 17, 45, 46). Unfortunately, there is now a shortage of rhesus macaques available for biomedical research in the U.S. (38). The development of alternative nonhuman primate models in which to study potential bioterrorism agents and emerging infections is therefore warranted.

The common marmoset (Callithrix jacchus) is a small anthropoid primate that generally weighs between 320-450 g when kept in captivity. Because of the need for maximum biocontainment housing of Lassa virus-infected animals, the use of these small-bodied primates would be valuable and would allow the use of technologies already developed for large rodents and guinea pigs. Marmosets have been successfully used to characterize a number of viral diseases, including arenavirus infections caused by Junin virus, LCMV (lymphocytic choriomeningitis virus), SARS-coronavirus, GB virus B, as well as other human syndromes (3, 15, 16, 19, 30, 36).

Here we report that subcutaneous inoculation of common marmosets with Lassa virus resulted in a systemic viral disease with fatal outcome and histological features similar to those described in fatal disease in humans. Using this model we have demonstrated for the first time that virus induces alterations in target tissues that would be expected to impair adaptive immune responses. These findings support the observations of immunosuppression contributing to Lassa disease progression in humans (28).
MATERIALS AND METHODS

Biosafety. Lassa virus is a risk group 4 (CDC) and Category A (NIH) agent (6). All experiments with this virus were performed within a biosafety level 4 (BSL4) facility at the Southwest Foundation for Biomedical Research (SFBR, San Antonio, TX) certified by CDC. Personnel wore appropriate protective equipment (biosafety suit). Experimental animal protocols were approved by the Institutional Animal Care and Use Committee and the Institutional Biohazards Committee of SFBR.

Virus. The Josiah strain of Lassa virus was provided by Tom Ksiazek (CDC, Atlanta, GA). The Lassa virus stock was grown in Vero E6 cells within 150 mm tissue culture flask with Dulbecco’s Modified Eagle Medium (Invitrogen, Carlsbad, CA) containing 4,500 mg/L D-glucose, L-glutamine and supplemented with 10% heat-inactivated fetal calf serum (DMEM-10) at 37°C, 5% CO₂, and 85% humidity. The multiplicity of infection (MOI) was 0.002 and the virus was harvested at 7 days after infection. Infectious titer of the virus stock as determined by conventional virus plaque assay was 5×10⁶ PFU/ml. Cells and virus stocks were free of mycoplasma contamination.

Experimental animal and virus inoculation. Four adult marmosets, age 2.5 to 4 years and ranging in weight from 368 g to 425 g, were obtained from the Southwest National Primate Research Center at SFBR. Two additional animals were used as a control group. One week before the start of the study, animals were transferred to the BSL4 facility at SFBR and housed individually in caging specifically developed for marmoset work. For inoculation at day 0, two animals were subcutaneously injected with 1×10³ PFU of virus diluted in saline, while the remaining two animals received 1×10⁶ PFU.
of the virus in the same volume (0.5 ml). Two control animals received diluted conditioned medium of Vero E6 cells. At predetermined time intervals animals were sedated and blood samples were collected for hematology, chemistries, and virus titration. When animals became moribund they were euthanized and tissue was harvested for histological examination and immunohistochemistry.

**Virus quantification.** Virus titer was determined by conventional plaque titration. Briefly, serial virus dilutions were made in DMEM then transferred to 6-well tissue culture plates containing a confluent monolayer of Vero E6 cells. After a 1-hr adsorption period, the virus mixture was removed, and a 0.5% agar overlay containing Eagle’s Minimum Essential Medium with 2% fetal calf serum (EMEM-2; Biowhittaker Inc., Walkersville, MD) was transferred to each well and allowed to solidify. Plates were incubated at 37°C and 5% CO\textsubscript{2}. At day 6 after infection, a secondary overlay containing neutral red (0.2 g/L) was added, and then plates were incubated at 37°C for an additional 24 hr. Virus plaques were counted at day 7 after infection.

In addition to plaque assay, viremia and viral load in tissues were measured by quantitative RT-PCR (qRT-PCR). For detection of viral RNA in the blood of experimental animals, viral RNA was extracted according to the manufacturer’s recommendations from 500 µl EDTA treated blood using the Ribopure Blood kit (Ambion, Austin, TX). Total RNA was extracted from tissue samples that were stored in RNAlater. Briefly, 100 mg tissue was transferred to a screw-top microcentrifuge tube containing Trizol Reagent (Invitrogen) and a 5 mm stainless steel bead (Qiagen Inc., Valencia, CA). The tissue was then homogenized using the TissueLyser (Qiagen) homogenization system per manufacturer’s recommendations. RNA was then extracted from the homogenate following the Trizol
reagent protocol described by Invitrogen. Real-time qRT-PCR was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) and RNA Ultrasense one-step real-time qRT-PCR system (Invitrogen) according to manufacturer’s recommendations. The primer/probe set for Lassa virus strain Josiah RNA targeted a GPC region of the S segment using 36E2 and 80F2 primers (11). The qRT-PCR reaction contained 5 µM of each primer and 2.5 µM probe for each primer/probe set in a final reaction volume of 25 µl. The reaction conditions were as follows: 50 °C for 20 min, 95 °C for 2 min, then 40 cycles alternating between 60 °C for 1 min and 95 °C for 15 seconds. Standards used in qRT-PCR were generated from serial 10-fold dilutions of RNA isolated from Lassa virus strain Josiah virus stock (10<sup>1</sup> PFU/ml to 10<sup>-7</sup> PFU/ml) that were enumerated in triplicate by conventional plaque assay as previously described (25). Validation experiments revealed specificity and sensitivity of qRT-PCR, allowing detection of 10 PFU/ml blood or 10 PFU/g tissue. We have seen good correlation between viral RNA and infectious titers. The ratios of RNA to PFU ranges between 80 and 200 which are consistent with that which has been previously reported (1). The utility of the qRT-PCR method has been recently described in a manuscript in press (Carrion Jr. R, et al., A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity, Vaccine (2007), doi:10.1016/j.vaccine.2007.02.038).

**Hematology and blood chemistries.** The biochemical analysis of plasma samples was performed using mammalian liver enzyme profile rotor on VetScan analyzer (Abaxis, Inc. Union City, CA). Complete blood counts were performed using a VetScan HMT machine (Abaxis, Inc., Union City, CA).
Histology (H&E) and immunohistochemistry. Samples of aseptically removed tissues were fixed in phosphate-buffered (pH 7.2) 4% paraformaldehyde and embedded in paraffin for histology. Paraffin-embedded tissues were cut in 5-µm sections, deparaffinized and stained with H&E. For immunohistochemistry analysis, deparaffinized tissue sections were stained for CD3, CD20, HAM56, Ki67, and the MHC-II antigen HLA-DQ,P,R (catalog no. M0775, DAKO, Carpenteria, CA) and visualization of positive cells was completed by an avidin-biotin-horseradish peroxidase complex (ABC) technique with diaminobenzidine (DAB) chromogen as previously described (16).

RESULTS

Clinical observations. All animals appeared normal until 8 days after infection, when weight loss was observed. After day 10, animals lost nearly 10% of body weight. Coinciding with weight loss, behavioral changes were observed. Animals became depressed, had reduced stool production, became partially anorexic, and developed a low-grade fever just prior to euthanasia. Beginning at 8 days after infection, blood chemistries were marked by elevated serum alanine and aspartate aminotransferases (AST, ALT), markers of hepatocellular necrosis, and these parameters peaked by day of euthanasia (Fig. 1). Total bilirubin, GGT (gamma-glutamyltransferase), and ALP (alkaline phosphatase), commonly used measures for evaluation of excretory liver functions (26), were in normal ranges in plasma samples collected on day -7 to day 14 after infection. However, on day 18 plasma levels of ALP in monkeys infected with high dose of Lassa virus was significantly elevated (average 447 U/L). No jaundice was observed in the infected monkeys. Levels of albumin in plasma, one of the markers of synthetic capacity of
liver (10), decreased after day 8 and dropped almost 2-fold on day of euthanasia (Fig. 1).

We did not see significant differences in red or white blood cell counts or hemoglobin levels in infected marmosets. However, there was a gradual reduction in platelet numbers in both the low-dose and high-dose animals over the course of infection (not shown).

**Viremia and viral burden in tissues.** The virulence of Lassa virus in humans and experimental animals is related to the level of viremia, suggesting that level of virus replication is primary factor of pathogenesis. In humans, viremia of ≥10^{3.6} TCID\textsubscript{50}/ml in Lassa fever patient admission was associated with a case-fatality rate of 76% (34). In different species of nonhuman primates experimentally infected with Lassa virus, fatal infection was associated with levels of viremia greater than or equal to 4 log\textsubscript{10} PFU/ml (39).

In Lassa virus-infected marmosets we detected the virus by conventional plaque assay and by RT-PCR by day 8. The virus replicated exponentially in marmoset tissues, resulting in viremia >5-7 log\textsubscript{10} PFU/ml on day 14 and on the day of necropsy. Using a real-time qRT-PCR assay we measured Lassa virus S RNA in tissues and expressed viral burden as PFU-equivalents (Fig. 1). All tested tissues contained significant amounts of viral RNA comparable with levels of viremia. On the day of necropsy we did not see significant differences in viral RNA loads in the tissues of animals infected with low (1×10\textsuperscript{3} PFU) or high (1×10\textsuperscript{6} PFU) doses of Lassa virus. In reticuloendothelial tissue, such as the liver, spleen, and lymph nodes, viral loads exceeded viremia, suggesting that virus targets these tissues.

**Gross pathology.** Upon necropsy, an enlarged liver was observed in all experimentally inoculated marmosets. In those animals that received a high dose of virus,
pale foci were observed throughout the liver. Another finding consistent with all animals
was lung abnormalities, primarily hemorrhage, in most lobes. The spleens of three of four
animals were enlarged, while the kidney on one of the animals given a high dose was pale
red in appearance. No significant pathologic lesions were observed in other tested tissues
(Table 1).

Histology. In general, histological changes were mild compared with the extensive
viral burden of visceral tissues and located predominantly in tissues of the
reticuloendothelial system, liver, spleen, and lymph nodes (Table 1, Fig. 2, A&B). Hepatic
lesions were observed in all animals and characterized by multifocal random hepatic
necrosis. Areas of necrosis varied from individual hepatocytes, which appeared rounded
with eosinophilic cytoplasm and pyknotic nuclei (Councilman bodies) to small clusters of
necrotic hepatocytes infiltrated by macrophages and lymphocytes. Degenerative
hepatocytes often contained single or multiple rounded eosinophilic to amphophilic
inclusions 3-8 µm in diameter (not shown). A green to brown granular pigment indicative of
bile and biliary stasis was evident within many hepatocytes throughout the evaluated
hepatic sections and within some macrophages found in necrotic foci. Portal infiltrates
were observed in two animals and were mild.

A mild to moderate multifocal interstitial pneumonitis was evident in lungs of two
animals infected with a high dose of Lassa virus (Fig. 3). In these animals, septal
thickening was accompanied by the presence of proteinaceous exudates within the
alveolar spaces and increased numbers of macrophages and lymphocytes. Vascular
changes were also noted in these animals and characterized by multifocal edema and
thickening of the periarterial space to 5 to 10 times the normal diameter. Occasionally,
these edematous areas contained increased numbers of lymphocytes and macrophages. Finally, in the affected lungs multifocally the pleurae contained hypertrophied and eosinophilic mesothelial cells.

A mild to moderate lymphoid depletion was observed in the spleen in the periarteriolar sheath in all animals. The normal dense outer mantle surrounding the sheath was absent, but evidence of ongoing necrosis was minimal. A lymphadenopathy was observed in all animals and characterized by marked enlargement of lymph nodes to 2 to 3 times the normal size with concurrent lymphoid depletion. Lymph nodes were infiltrated by increased number of histiocytes that were present within dilated sinuses, medullary cords, and paracortical spaces, giving the tissues an overall eosinophilic and depleted appearance. Well developed follicles were rarely observed. In several nodes, extensive ongoing necrosis of lymphocytes was evidenced by the presence of extensive foci of pyknotic nuclei within the mantle region of B cell follicular areas. Rare multinucleated syncytial cells were observed in some sections. Lymph node capsules were multifocally thickened and contained infiltrating inflammatory cells.

A mild multifocal lymphocytic interstitial nephritis was observed in all animals. In addition to these changes, mesangial thickening was observed and was accompanied by proteinaceous material within Bowman’s space and dilated cortical tubules. However, rare sclerotic glomeruli were evident, suggesting that this represents a preexisting condition.

The adrenal glands were available only from two marmosets. In these animals, mild to moderate multifocal necrosis was observed within the adrenal cortex. In one animal, larger areas of degeneration within the adrenal cortex were observed and characterized by vacuolization of cells, individual cell necrosis, and infiltration by histiocytes.
Immunophenotyping of Cells in Target Tissues. Immunohistochemical analysis revealed that cellular aggregates noted by H&E stains in areas of liver necrosis were composed primarily of HAM56-positive macrophages (Fig. 2C). These areas recruited just a few CD3- (Fig. 2D) or CD20-positive cells (not shown) and overall there were decreased numbers of both T and B cells within the hepatic parenchyma of Lassa virus-inoculated animals. Immunostaining for the MHC-II antigen HLA-DP,Q,R (Fig. 2E) revealed that in spite of macrophage infiltration there was marked, decreased MHC-II antigen expression in areas of necrosis and throughout the hepatic parenchyma and portal areas. We have shown previously that in Lassa virus-like hepatitis in rhesus macaques, necrotic foci were associated with areas of strong hepatocyte proliferation (22, 23). For detection of proliferating cells in the liver of Lassa virus-infected marmosets, tissue sections were stained for Ki67 nuclear antigen. This staining revealed increased numbers of Ki67-positive cells (Fig. 2F), both within areas of necrosis and the adjacent hepatic parenchyma. These cells were most frequently observed in hepatic sinusoids, perisinusoidal spaces, or portal regions.

As in the liver, in the lymph nodes of infected marmosets there was marked, decreased expression of HLA-DP,Q,R (Fig. 3). Staining on HAM56 antigen revealed a marked increase in macrophages in lymph nodes and disruption of normal nodal architecture. The discrete HAM56 expression found in normal nodes was replaced by a more diffuse expression. B cell follicles with well-defined germinal centers and mantle zones were absent or remarkably reduced in number and found intermixed throughout the node rather than confined to the cortical regions. CD3-positive cells were found throughout the cortex and medulla, and Ki67 staining revealed marked proliferation of cells (not shown).
In the spleen, immunohistochemical analysis revealed a depletion of CD3-positive lymphocytes, particularly in the periarteriolar sheaths, which were reduced in size and number (Fig. 4). Overall reductions in CD20-positive B cells were also evident, although lymphoid follicles were present in splenic white pulp. As in the lymph node, these changes were accompanied by increased numbers of HAM56-positive macrophages throughout the red pulp and a marked decreased expression of HLA-DR.

**DISCUSSION**

The relatively small size of marmosets, lower caging and feeding costs, and ease of handling in a biosafety environment represent substantial benefits compared with macaques. Experimental infection of common marmosets with Lassa virus resulted in a systemic viral disease with high viremia and high viral RNA load in all tested tissues, elevated liver enzymes, and decreased levels of albumin in plasma, weight loss, and severe morbidity 15-20 days after inoculation. Morphologic features mirrored those described in human cases of fatal Lassa fever, and included hepatic and adrenal necrosis, lymphoid depletion, and interstitial nephritis.

Postmortem histological studies of Lassa fever patients and Lassa virus-infected experimental animals indicate that the liver is one of the most affected organs participating in a systemic breakdown (32, 43, 49). Three categories of morphological changes were found in the liver of patients that have died from Lassa virus hepatitis: active hepatocellular injury, continued damage and early recovery, and regenerative phase with high mitotic activity of hepatocytes. The authors observed a macrophage response to cellular damage but they did not find evidence of lymphocytic infiltration in infected hepatic tissues (32). Despite the substantial liver dysfunction, the degree of hepatic damage was not sufficient
to implicate hepatic failure as the primary cause of death. The relationship between liver
damage and hematological and endothelial dysfunctions leading to shock and death
remains unclear.

Guinea pigs and mice are susceptible to experimental Lassa virus infection (39). In
mice, outcome of the infection depends on MHC background, age of the animal, and route
of inoculation (20, 21). Intracerebral inoculation of Lassa virus into adult mice induces a
fatal convulsive disorder resembling a classical immunopathology cased by LCMV, the
prototype arenavirus (37, 41). Strain 13 guinea pigs are the most sensitive to Lassa virus
and the most commonly used model for elucidating pathogenesis and for developing
effective treatment and protective regimens for Lassa fever (7, 18, 25, 39, 40). However,
evaluation of vaccine candidates in guinea pigs and primates has produced conflicting
results (9, 12).

Common marmosets were successfully used for pathogenesis and protection studies
with Junin virus, a member of the New World Arenaviruses (Tacaribe serogroup) and a
causative agent of Argentine hemorrhagic fever (14, 47, 48). Infection of Callithrix jacchus
with the prototype strain of Junin virus produced a fatal disease with multifocal
hemorrhages and characteristic microscopic lesions such as meningoencephalitis,
interstitial pneumonia, lymphocytic depletion of lymphatic tissue, hepatocytic necrosis, and
a variable decrease in bone marrow cellularity. High virus concentrations correlated with
lesions and with the presence of virus antigen.

Feeding callitrichids neonatal mice that were infected with LCMV$_{CH}$ caused outbreaks
of callitrichid hepatitis in captive marmosets and tamarins in zoos and animal parks in the
U.S. and in Europe (1, 35, 36, 42). Pathology of both experimental and natural outbreaks
of callitrichid hepatitis in common marmosets and tamarins clearly established the liver as
a principal target for LCMV\textsubscript{CH}. Callitrichid hepatitis was characterized by elevated levels of liver aminotransferases and bilirubin in plasma, random foci of hepatocellular degeneration associated with a mild mononuclear inflammatory cell infiltrate, and a few neutrophils. A characteristic finding was acidophilic structures resembling apoptotic, Councilman-like bodies seen in the sinusoids but also occasionally within Kupffer cells (35). LCMV antigen was observed in hepatic foci as well as in nondegenerating areas in lungs, kidney, urinary bladder, brain, and testes. Interestingly, pygmy marmosets, which died 5-14 days later than the common marmosets and tamarins, had minimal hepatocellular necrosis but an intensive portal mononuclear inflammatory cell infiltration, suggesting involvement of immune-mediated reactions in fatal hepatitis in these animals (35).

In our experiments, Lassa virus infection of common marmosets resulted in a fatal disease with morphologic features similar to those described in patients dying from Lassa disease (32, 49). The most prominent morphological features of Lassa virus-inducible hepatitis in common marmosets were: (i) multifocal hepatic necrosis with mild inflammation presented predominantly by HAM56-positive macrophages; (ii) near absence of CD20-, CD8-, or CD3-positive lymphocytes in necrotic foci; (iv) the complete lack of expression of MHC-II antigen; and (v) hepatocyte proliferation as judged by positive Ki67 staining.

Hepatocyte proliferation was a prominent feature of fatal Lassa virus hepatitis in humans (32) and in LCMV-inducible fatal hepatitis in rhesus macaques (22-24). However, in the LCMV model, the proliferation response was much stronger and it was associated with significant up-regulation of such markers of liver regeneration as Ki67, IL-6, sIL6R, and sTNFR. MHC-II antigens were not described in that model. A striking finding in Lassa virus-infected marmosets was the marked reduction of staining for the MHC-II antigen.
HLA-DP,Q,R and poor T cell responses (Fig. 2). This contrasts with other forms of viral hepatitis in marmosets caused by GB virus B (16) and by SARS-coronavirus (15), in which hepatic inflammation is associated with a marked increase in MHC-II expression. These findings suggest evasion of the normal immune response as a virulence factor in the development of Lassa virus induced hepatitis.

Multifocal interstitial pneumonitis was observed in two animals and was characterized by edema, septal thickening, and inflammatory infiltrates. Pulmonary arterial lesions have previously been recognized in rhesus macaque but not in human tissues (8, 17, 44, 46). In the Lassa virus-infected marmosets, mild to moderate periarterial edema was recognized, but pulmonary arteritis as found in rhesus macaques was not observed.

Adrenal necrosis is a characteristic feature of fatal Lassa virus infection in humans and nonhuman primates (8, 17, 44, 46). Adrenal glands from only two animals were available for evaluation and mild, multifocal adrenal necrosis and adrenalitis was observed. Degenerate cells occasionally contained eosinophilic spherical inclusions.

The significance of the renal lesions remains to be determined. A mild multifocal lymphocytic interstitial nephritis was observed in all animals and is compatible with that described in humans and rhesus macaques.

As described in humans (44), lymphoid depletion was also observed in the spleen and lymph nodes of Lassa virus-infected marmosets. These changes were most pronounced in lymph nodes in which normal architecture was largely effaced through loss of follicles and infiltration by large numbers of histiocytes. Lymphocytic necrosis was occasionally observed, suggesting that this was the primary process and that infiltrates were secondary to cell death.
In addition to liver tissues, a marked reduction in the intensity of HLA-DR staining was also observed in lymph nodes in Lassa virus-infected marmosets (Fig. 4). Immunophenotypic alterations in the spleen included reduction in overall numbers of CD3- and CD20-positive lymphocytes in infected animals compared to controls and disruption of the architecture of lymphoid follicles. Previously, the immunosuppressive phenotype of Lassa virus infection was based on detection of proinflammatory cytokines and immunomodulatory molecules in culture medium of human cells infected in vitro (4, 5, 26, 29), in plasma of experimentally infected animals (22), or in Lassa fever patients (28).

Here we have presented the first experimental evidence that replication of Lassa virus in target tissues of a nonhuman primate, the common marmoset, is associated with alterations that would be expected to impair adaptive immunity.

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Table 1. Microscopic Findings in Lassa-Infected Marmosets

<table>
<thead>
<tr>
<th>Tissue/Pathology</th>
<th>Animal Number and Dose</th>
<th>10&lt;sup&gt;3&lt;/sup&gt; PFU</th>
<th>10&lt;sup&gt;6&lt;/sup&gt; PFU</th>
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<tbody>
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<td></td>
<td></td>
<td>17102</td>
<td>18164</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Hepatitis</td>
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<tr>
<td>Eosinophilic inclusions</td>
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<tr>
<td>Hepatic necrosis</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Portal infiltrates</td>
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<td>-</td>
<td>+</td>
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<td>Lymphoid and hematopoietic</td>
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<tr>
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<tr>
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<td>+</td>
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<tr>
<td>Bone marrow</td>
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<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Interstitial nephritis</td>
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<td>Interstitial pneumonitis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mesothelial hypertrophy</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Other tissues</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis in adrenal gland</td>
<td>NE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>NE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Skin</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NSF&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup>NE, not examined.

<sup>b</sup>NSF, no significant findings.
FIGURE LEGENDS

FIG. 1. Lassa virus infection in common marmosets. ALT (A) and albumin (ALB) (B) levels in plasma of four monkeys infected with Lassa virus. C, Gross pathology: enlarged liver with pale foci in animal 17280, which was infected with $1 \times 10^6$ PFU of Lassa virus. D, Viral load in tissues of four infected monkeys was measured by qRT-PCR as described in Materials and Methods and expressed as mean+SD.

FIG. 2. Hepatic pathology in Lassa-inoculated common marmosets. Multifocal random hepatic necrosis accompanied by a mixed inflammatory cell infiltrate consisting of macrophages and lymphocytes (A) Degenerate hepatocytes contained well circumscribed eosinophilic to amphophilic cytoplasmic inclusions and individual hepatocyte necrosis was apparent (insert: Councilman body). (B) Cellular aggregates in regions of hepatocellular necrosis were largely composed of HAM56 macrophages and were devoid of CD3- (C) and CD20-positive cells (D). A marked reduction in the intensity of HLA-DP,Q,R staining (E) was observed compared with normal control tissue (insert). Increased numbers of cells positive for the proliferation marker Ki67/MIB1 were observed (F).

FIG. 3. Pulmonary pathology in common marmosets infected with Lassa virus. A multifocal interstitial pneumonitis was observed in 2 of 4 animals (Table 1) evidenced by septal thickening, alveolar edema, and increased numbers of inflammatory cells (A). In these animals multifocal periarterial edema (B) and multifocal hypertrophy of pleural mesothelial cells (C) was observed.
FIG. 4. Immunophenotypic alterations in lymph node during experimental Lassa infection. A marked reduction in the intensity of HLA-DR staining was observed in lymph nodes obtained from Lassa-infected animals (B) compared to controls (A). Increased numbers of HAM56-positive cells were noted in the infected animals (D) compared with controls (C) and the discrete staining observed in normal lymph node was largely absent.

FIG. 5. Immunophenotypic alterations in spleen of marmosets infected with Lassa virus. Overall numbers of CD20-positive B lymphocytes were reduced in Lassa-infected animals (B) compared with controls (A) and lymphoid follicles lacked their normal architecture. Similarly, CD3-positive T cells within the periarteriolar sheath were reduced in number or absent from the infected animals (D) compared to controls (C).
A  ALT in plasma

B  ALB in plasma

C  Gross Pathology

D  Viral Load in Tissues

- Spleen
- LN
- Lung
- Liver
- Kidney
- Brain
- Adrenals

Log10PFU equivalents per 100 mg