Pathogenesis of Lassa Virus Infection in Guinea Pigs

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A rodent model for human Lassa fever was developed which uses inbred (strain 13) and outbred (Hartley) guinea pigs. Strain 13 guinea pigs were uniformly susceptible to lethal infection by 2 or more PFU of Lassa virus strain Josiah. In contrast, no more than 30% of the Hartley guinea pigs died regardless of the virus dose. In lethally infected strain 13 guinea pigs, peak titers of virus occurred in the spleen and lymph nodes at 8 to 9 days, in the salivary glands at 11 days, and in the lung at 14 to 16 days. Virus reached low titers (104 PFU) in the plasma and brain and intermediate titers in the liver, adrenal glands, kidney, pancreas, and heart. In moribund animals, the most consistent and severe histological lesion was an interstitial pneumonia. In contrast, the brain was only minimally involved. The immune response of lethally infected strain 13 guinea pigs, as measured by the indirect fluorescent antibody test, was detectable within 10 days of infection and was similar in timing and intensity to the fluorescent antibody test response of both lethally infected and surviving outbred animals. In contrast to the fluorescent antibody response, neutralizing antibody developed late in convalescence and was thus detected only in surviving outbred guinea pigs.

The availability of a rodent model for human Lassa fever in uniformly susceptible strain 13 guinea pigs should facilitate detailed pathophysiological studies and efficacy testing of antiviral drugs, candidate vaccines, and immunotherapy regimens to develop control methods for this life-threatening disease in humans.

The development of animal models for human Lassa fever would facilitate the development of effective treatment and immunization regimens and might provide insight into the pathogenesis of this life-threatening disease. We recently described Lassa virus infection in rhesus monkeys and the effective treatment of monkeys with the antiviral drug ribavirin (3). Previously, other investigators have described Lassa virus infections in humans (10) and squirrel monkeys (9). We have extended these descriptive studies to include cynomolgus, African green, and capuchin monkeys (P. B. Jahrling, manuscript in preparation). Studies with primates are extremely expensive, however, especially when conducted in maximum containment (P4) laboratories, and the availability of a small rodent model for lethal Lassa virus disease approximating the human disease would be useful. The use of outbred Hartley strain guinea pigs was explored previously (9); the authors concluded that the disease process in outbred guinea pigs was too different from human Lassa fever to warrant extensive investigation. This conclusion deserves reexamination, however, in light of our recently reported observations (4) that inbred strain 13 guinea pigs develop a uniformly lethal infection resembling human Lassa fever after infection with an adapted strain of Pichinde virus, an arenavirus distantly related to Lassa virus (4), whereas outbred guinea pigs are more resistant. These observations prompted us to reexamine Lassa virus infection in guinea pigs, comparing strain 13 and outbred animals to gain insight into the determinants for susceptibility and resistance to Lassa infection and to develop a uniformly susceptible model for testing treatment and immunization regimens. We report here the exquisite sensitivity of strain 13 guinea pigs to lethal Lassa virus infection, the replication of virus in target tissues, the development of histological lesions, and humoral immune responses to Lassa virus infection.

MATERIALS AND METHODS

Preparation of virus stocks and virus assay. Lassa virus strain Josiah was isolated in 1976 from the serum of a 40-year-old man in Sierra Leone, Africa (11). This isolate was passaged four times at low multiplicity (1 PFU per 100 cells) in monolayer cultures of Vero cells (an African green monkey kidney cell line) and grown to confluency in 75-cm² flasks containing 5 x 10⁶ cells. This stock virus suspension (3), stored at -70°C, contained 2.5 x 10⁴ PFU/ml.

All of the infectious Lassa virus assays were performed by counting PFU on Vero cell culture monolayers grown in 10-cm² wells of plastic plates and maintained under medium containing 1% agarose, basal medium (Eagle) with Earle salts, HEPES (N-2-...
hydroxyethylpiperazine-N'2-ethanesulfonic acid) buffer (25 mM), and 2% fetal calf serum as described previously (3). Cells were incubated at 36°C for 5 days in a humidified atmosphere containing 5% CO₂. After the incubation, 2 ml of neutral red diluted in Puck saline A to a final concentration of 1:6000 was added to the cells; plaques were counted at an additional incubation period of 18 to 24 h.

**Inoculation of guinea pigs and harvest of tissues.** All manipulations of Lassa virus-infected animals, and unfixed samples obtained from infected animals, were conducted within the maximum biological containment (P4) facilities at the USAMRIID. Inbred male strain 13 guinea pigs weighing 350 to 500 g were obtained from the Institute’s colony; male strain 2 guinea pigs (450 to 600 g) were obtained from Crest Caviary (Covina, Calif.), and outbred male Hartley strain guinea pigs (400 to 500 g) were from Buckberg Farms (Tomkins Cove, N.Y.). All of the guinea pigs were inoculated subcutaneously (s.c.) with virus suspensions diluted in Eagle minimal essential medium with Earle salts plus 10% fetal calf serum. Virus doses were varied as stated below. Plasma samples were incubated for 4-8 min in a humidified atmosphere containing 5% CO₂. After staining, all of the slides were observed under P4 facilities at the USAMRIID. The endpoint was the highest dilution of serum which obtained from ether-anesthetized guinea pigs bled repetitively at 2- to 3-day intervals from the retroorbital sinus into heparinized syringes with 25-gauge needles. Guinea pigs killed to provide infected tissues and large volumes of plasma were anesthetized with chloroform and bled from the heart into heparinized syringes. Tissues were removed aseptically, and a portion of each was homogenized in minimal medium plus 10% fetal calf serum with mortars and pestles and containment conditions. Challenge virus was the Lassa virus strain Josiah preparation described above diluted initially 1:10 in NTM, in which it had a titer of 6.2 × 10⁶ PFU/ml. To test the neutralizing capacity, we diluted the sera (freshly obtained or stored frozen at −70°C until used) 1:10 in NTM and divided the sera into a series of six aliquots (0.9 ml each). Challenge virus was then serially diluted in tenfold increments in the NTM containing test serum, and the reaction mixtures were incubated at 37°C for 1 h. As a control, normal guinea pig serum was substituted for immune guinea pig test serum. After incubation, the reaction mixtures were assayed for residual infectivity (i.e., PFU) on Vero cells as described above. Wells containing 10 to 100 PFU were counted. N-antibody activity was expressed as a log₁₀ neutralization index (LNI), calculated by the formula LNI = log₁₀ (PFU in control) − (PFU in test serum).

**RESULTS**

Lassa virus strain Josiah infected and killed all of the strain 13 guinea pigs inoculated s.c. with 2 PFU or more (Table 1); tenfold less virus (0.2 PFU s.c.) killed 4 of 10. The surviving strain 13 guinea pigs did not seroconvert by the IFA test, nor did they resist backchallenge, suggesting...
TABLE 1. Infectivity and lethality of Lassa virus strain Josiah for guinea pigs inoculated s.c.

<table>
<thead>
<tr>
<th>Guinea pig strain</th>
<th>Inoculum (PFU)</th>
<th>No. dead/no. tested (%)</th>
<th>Mean day of death (range)</th>
<th>Sero-converted*</th>
<th>Resisted rechallenge*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 13</td>
<td>240,000</td>
<td>5/5 (100)</td>
<td>15.5 (11-17)</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>2,400</td>
<td>15/15 (100)</td>
<td>17.4 (16-19)</td>
<td>17.1 (15-19)</td>
<td>18.4 (17-24)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10/10 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>4/10 (40)</td>
<td>20.0 (18-22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1/10 (10)</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain 2</td>
<td>2,400</td>
<td>10/10 (100)</td>
<td>18.1 (15-20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred 1</td>
<td>240,000</td>
<td>9/30 (30)</td>
<td>17.0 (15-19)</td>
<td>21/21</td>
<td>21/21</td>
</tr>
<tr>
<td>Hartley</td>
<td>2,400</td>
<td>6/20 (30)</td>
<td>17.0 (15-19)</td>
<td>14/14</td>
<td>14/14</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>6/19 (32)</td>
<td>16.8 (15-19)</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10/30 (30)</td>
<td>17.6 (16-19)</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>4/25 (16)</td>
<td>18.0 (17-19)</td>
<td>9/21</td>
<td>13/21</td>
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</tbody>
</table>

* Considered positive if IFA titers were ≥10 on days 45 to 50.

Lived 45 days or longer after s.c. inoculation of 2,400 PFU of Lassa virus strain Josiah.

that inapparent infections did not occur in the strain 13 guinea pigs. Likewise, all of the 10 strain 2 guinea pigs inoculated with a moderate Lassa virus dose (2,400 PFU s.c.) were lethally infected. In contrast, outbred Hartley strain guinea pigs were relatively resistant; approximately 30% of them died at all doses of 2 PFU or more tested (Table 1). Inapparent infections occurred, since all of the surviving guinea pigs inoculated with 2 PFU or more seroconverted and all resisted backchallenge. These results suggested that the strain 13 guinea pig would serve as an excellent model for analyzing the pathogenesis of lethal Lassa virus infection. In contrast, outbred guinea pigs would be useful for producing Lassa immune serum.

To characterize Lassa virus infections of guinea pigs in more detail, we compared virus titers among groups of 15 guinea pigs inoculated with 3.4 log_{10} PFU and bled repetitively until day 21 or until they died (Fig. 1). Viremia in strain 13 guinea pigs developed more rapidly and reached higher titers than it did in either the lethally infected or surviving outbred groups. On days 4 and 6, viremias for strain 13 guinea pigs were significantly (P < 0.05) higher than for either outbred group. Viremia persisted at the highest titer in strain 13 guinea pigs and at somewhat lower titers in lethally infected outbred guinea pigs. In the surviving outbred animals, viremias declined to undetectable titers by day 21. These data suggest that viral replication proceeded more efficiently in strain 13 than it did in the outbred animals and that host response mechanisms did not effectively restrict viral replication in the strain 13 guinea pigs or in outbred guinea pigs with an ultimately fatal infection.

Humoral antibodies to Lassa virus were measured for all three groups. Despite differences in the viremia titer curves, the antibody responses measured by IFA were similar in both timing and magnitude among the groups. IFA and LNI antibody responses in the outbred guinea pigs which survived infection are compared in Fig. 2. IFA titers reached 1:40 or more by day 10 and 1:320 to 1:640 by day 16. In surviving guinea pigs, viremia declined to undetectable titers by day 21. However, N-antibody was not detectable on days 21, 28, or 32. N-antibody titers were first detected on day 45 and increased with time for 180 days, the last time tested.

![Graph](attachment:image.png)
Further descriptive studies of fatal Lassa virus pathogenesis were initiated with strain 13 guinea pigs, which were uniformly killed by Lassa virus infection. Leukopenia was not severe; even in moribund animals, the average total leukocyte count was 6,200 cells per mm\(^3\). However, differential counts revealed absolute lymphopenia which was offset by neutrophilia. Hemoglobin concentrations increased by day 8 but receded to near normal values by day 16 (Fig. 3). SGOT activity increased steadily throughout the disease course but never exceeded 200 IU/liter.

To obtain additional insight into the tissue tropisms and histological lesions produced by Lassa virus, we inoculated guinea pigs with 2,400 PFU s.c. Groups of infected animals were killed at 3- to 4-day intervals to obtain tissues for infectivity titrations; in addition, eight guinea pigs were killed when moribund on days 16 to 18 to correlate the presence of infectious virus with the distribution of viral antigens and histopathological lesions in moribund animals. Infectivity data for tissues obtained in the sequential study (Fig. 4) suggested that the virus replicated in all of the extraneural tissues examined but not in the brain. High titers of virus occurred early in the lymph node, spleen, salivary gland, pancreas, and lung; virus concentrations in other tissues increased more slowly. In the group of eight moribund guinea pigs examined 16 to 18 days after infection, the highest titers of virus were recovered from the lung (Fig. 5). As judged by immunofluorescence, all of the eight lungs examined contained viral antigens. The spleen contained the second highest concentration of virus, followed by the pancreas, lymph node, adrenals, kidney, salivary gland, liver, and heart (Fig. 5). Virus recovered from the brain was of a sufficiently low titer to be attributable to the blood-borne virus. High concentrations of viral antigens were readily detected by immunofluorescence in all lung (Fig. 6A), spleen, pancreas, kidney (Fig. 6B), and salivary gland specimens examined but less frequently detected in liver (five of eight), heart (two of eight), and brain (two of eight) samples.

The distribution and degree of histological damage observed in these tissues was less than would have been predicted on the basis of the infectivity and immunofluorescence data. Lung

![Graph showing development of Lassa viremia (○) IFA responses (○), and N-antibody responses (■) in surviving outbred guinea pigs inoculated s.c. with 3.4 log\(_{10}\) PFU.]

![Graph showing hemoglobin concentrations in blood and SGOT activity in the sera of strain 13 guinea pigs inoculated s.c. with 3.4 log\(_{10}\) PFU of Lassa virus. Points are arithmetic means (± standard error) of 10 guinea pigs.]
FIG. 4. Infectious Lassa virus concentrations recovered from serum and tissues of strain 13 guinea pigs inoculated s.c. with $3.4 \log_{10}$ PFU. Points are geometric means (± standard error) of five guinea pigs. The viremia curve (-----) is superimposed on each tissue curve (●●●) to facilitate comparison.

tissue from all eight guinea pigs revealed areas of interstitial pneumonia, which varied in degree from mild to moderate (Fig. 6C). Four of eight kidney sections displayed acute necrotizing nephritis, a lesion complicated in two of the four instances by the presence of bacterial colonies (Fig. 6D). Likewise, five of eight spleen sections contained necrotizing lesions of varying severity.
dose-independent pattern of resistance among early in the disease course suggests early repli-
cation of the virus in these tissues and is consist-
ent with the reticuloendothelial tropism reported
involving bacteria. Mild myocarditis was ob-
served in four of eight guinea pigs, and minimal
hepatitis accompanied by diffuse fatty change
was found in five. Although the virus clearly
replicated in the adrenal and salivary glands and
pancreas, no remarkable lesions were seen in
these tissues. Brain tissues from two guinea pigs
were examined but contained no lesions.

**DISCUSSION**

The studies reported here suggest that guinea
pigs are a potentially useful model for elucidat-
ing the pathogenesis of Lassa virus infection and
for developing effective treatment and protec-
tion regimens. Strain 13 guinea pigs were uni-
formly killed by Lassa virus infection, whereas
outbred Hartley strain animals were relatively
resistant. Thus, strain 13 guinea pigs are prefera-
able to outbred animals for studying the patho-
genesis of acutely lethal Lassa virus infection
and for assessing vaccine and treatment regi-
mens. Conversely, outbred guinea pigs offer an
opportunity to generate convalescent antisera.

The recovery of infectious Lassa virus from
all of the extraneural tissues examined, in con-
centrations which suggest viral replication, was
similar to the tissue distributions reported by us
for Lassa virus in rhesus monkeys (3) and Pi-
chinde virus in guinea pigs (4), and by others for
Lassa virus in humans (10) and guinea pigs (9).
The presence of high concentrations of infec-
tious Lassa virus in the spleen and lymph nodes
early in the disease course suggests early repli-
cation of the virus in these tissues and is consist-
ent with the reticuloendothelial tropism reported
for other arenaviruses in animal models (5, 6). In

<table>
<thead>
<tr>
<th>Tissue</th>
<th>LOG$_{10}$PFU/g</th>
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<tbody>
<tr>
<td>SERUM</td>
<td></td>
</tr>
<tr>
<td>LUNG</td>
<td></td>
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<tr>
<td>SPLEEN</td>
<td></td>
</tr>
<tr>
<td>PANCREAS</td>
<td></td>
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<tr>
<td>LYMPH NODE</td>
<td></td>
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<tr>
<td>ADRENAL</td>
<td></td>
</tr>
<tr>
<td>KIDNEY</td>
<td></td>
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<tr>
<td>SALIVARY GLAND</td>
<td></td>
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<tr>
<td>LIVER</td>
<td></td>
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<tr>
<td>HEART</td>
<td></td>
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<tr>
<td>BRAIN</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 5.** Infectious Lassa virus concentrations re-
covered from sera and tissues of moribund strain 13
guinea pigs obtained 16 to 18 days after s.c. inocula-
tion with 3.4 log$_{10}$ PFU. Titer are geometric means
(± standard error) of eight moribund guinea pigs.
These are the same animals whose tissues were exam-
ined by immunofluorescence for viral antigens and by
light microscopy for histological lesions.

**TABLE 1.** Intranasal and intraperitoneal and for assessing vaccine and treatment regi-
mens. Conversely, outbred guinea pigs should facilitate adoptive cell transfer stud-
ies to approach these problems. Differences in
the susceptibility of guinea pig strains may also
depend on genetically determined differences in
cellular susceptibility to productive viral infec-
tion or perhaps to defective interfering particle
generation. The existence of increased numbers
of target cells in susceptible animals may also be
a factor. In an analogous study (11), the increased
susceptibility of MHA strain hamsters as com-
pared with LVH hamsters was correlated with
increased numbers of natural killer cells in the
splens of MHA hamsters; natural killer cells
appeared to be critical targets for Pichinde virus
replication.

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**TABLE 2.** Infectious Lassa virus concentrations re-
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**FIG. 5.** Infectious Lassa virus concentrations re-
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(± standard error) of eight moribund guinea pigs.
These are the same animals whose tissues were exam-
inished by immunofluorescence for viral antigens and by
light microscopy for histological lesions.
FIG. 6. (A) Lassa viral antigens in thickened alveolar septae of lung (direct fluorescent antibody test). (B) Lassa viral antigens associated with renal tubules (direct fluorescent antibody test). Glomeruli contained no viral antigens. (C) Interstitial pneumonia, moderately severe, accompanied by edema and hemorrhage (hematoxylin and eosin stain). (D) Acute necrotizing nephritis with associated bacterial colonies; same kidney as shown in Fig. 6C (hematoxylin and eosin stain). (x440)

In the present study, central nervous system involvement appeared to be minimal; immunofluorescent staining of brain sections revealed infection of occasional, isolated cells in association with small blood vessels, which could be of pathological significance especially in animals which survive the acute, viscerotropic infection. Lassa virus concentrations in guinea pig tissues were slightly lower than those we reported for rhesus monkey tissues (2); for most tissues, this difference was only about 1 log_{10} PFU/g. However, of the guinea pig tissues tested, the lungs contained the highest concentration of virus, 6.9 log_{10} PFU/g, which was slightly higher than that reported for monkey lung (3). Conversely, the liver did not appear to be a major site of Lassa virus replication in guinea pigs as compared with monkeys, in which the liver contained the highest concentration of virus (7.6 log_{10} PFU/g) of all of the tissues tested. Virus titers in the tissues obtained in the sequential study (Fig. 5), the relatively modest SGOT elevations (Fig. 3), and the immunofluorescence study all confirmed that the liver is not a major target of Lassa virus replication in guinea pigs. Thus, with respect to hepatotropism the Pi-
Increased hemoglobin concentration observed in various animal models. The cardiovascular functions and capillary permeability changes in blood, hepatic, and hematological changes in blood, hepatic, and renal tissues can be observed. Effective treatments may be studied in more detail. Insight critical to the physiology of arenavirus disease remains to be determined. Terminal bacteremia has been a significant factor in the pathogenesis of this disease. Bacteremia may be a frequent occurrence in infected individuals, which contain high concentrations of Lassa anti-hemorrhagic fever virus antibodies.

Histological lesions were generally mild. Interpretation of the lesions was complicated by the presence of bacterial colonies, which may have been a significant factor in the pathogenesis of this disease. Terminal bacteremia has occasionally been documented for human Lassa fever patients (J. B. Frame, personal communication) and more frequently in cynomolgus, rhesus, and African green monkeys experimentally infected with Lassa virus (P. B. Jahrling, unpublished data). Bacteremia may be a frequent rather than incidental consequence of Lassa virus infection of the lymphoreticular organs, leading to reticulooendothelial cell dysfunction and possibly to a generalized immunosuppression. However, in guinea pigs the severe histological lesions, including necrotizing hepatitis and interstitial pneumonia, usually associated with Lassa virus infection in primates (2; R. T. Callis, P. B. Jahrling, and A. DePaoli, submitted for publication) occurred infrequently or were of minimal severity.

The replication of Lassa virus in tissues showing little or no histological damage or inflammation is a recurrent theme (3, 9). Disease may result as a consequence of functional impairment of infected cells, which appear histologically normal but are inhibited from performing the speciality functions of differentiated cells (7). For example, the infection of adrenocortical cells, which contain high concentrations of Lassa antigens as visualized by immunofluorescence, may result in an imbalance in corticosteroid metabolism which could contribute to death. The pathophysiology of arenavirus disease remains to be studied in more detail. Insight critical to the effective treatment of Lassa virus infections may be obtained by studying the biochemical and hematological changes in blood, hepatic, and cardiovascular functions and capillary permeability changes in various animal models. The increased hemoglobin concentration observed (Fig. 3) is presently unexplained but is consistent with the hemococoncentration associated with other human hemorrhagic fever virus infections. Our initial pathophysiological studies will focus on Pichinde in guinea pigs (4), since this virus poses a reduced biohazard as compared with Lassa virus. However, critical pathophysiological studies will be repeated with Lassa virus in guinea pigs. In the interim, we anticipate that strain 13 guinea pigs will be useful for testing antiviral drugs, candidate vaccines, and immunotherapy regimens.

**LITERATURE CITED**


