Pathology of Chronic Bolivian Hemorrhagic Fever in the Rhesus Monkey

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Gross and microscopic lesions of Bolivian hemorrhagic fever (BHF) are described in 10 rhesus monkeys that survived from 30 to 75 days after subcutaneous inoculation with a dose of 10^6 plaque-forming units (PFU) of Machupo virus, a dose which produces a severe and generally fatal disease. Six of the monkeys had been given low doses of homologous immune globulin when initial signs of infection appeared. Monkeys exhibited clinical signs in two phases. The initial signs of acute infection which began to appear about 1 week following inoculation included: diarrhea, depression, anorexia, dehydration, and skin rash. The survivors of this early phase of the illness usually showed improvement before relapsing into the second (or chronic) phase, which was characterized clinically by central nervous system disturbances including incoordination, tremors, convulsions, paresis, and muscle atrophy. Microscopic lesions were similar in both immune globulin-treated and untreated animals. These included widespread lymphoreticular infiltrates in the walls and adventitia of blood vessels of the brain, spinal cord, pancreas, intestine, liver, kidney, adrenal, parathyroid, heart, and skeletal muscle. Diffuse lymphocytic infiltrates not confined to the vascular or perivascular tissues were present in a variable degree in many of these and other organs. Several monkeys exhibited lymphocytic inflammation of the choroid, meninges, peripheral nerves, and ganglia. (Am J Pathol 84:211-224, 1976)

The pathology of Bolivian hemorrhagic fever (BHF) (Macaca mulatta) was previously studied and reported by Terrell et al.\(^1\) The acute form of BHF was described as a highly fatal disease characterized by severe epithelial necrosis of the skin, gastrointestinal tract, liver, and adrenals. Monkeys that died late in the course of the infection had central nervous system (CNS) lesions consisting of mild lymphocytic vasculitis, perivascular cuffing, and gliosis.\(^1\)

This paper represents a retrospective study of a chronic fatal neurovascular disease which developed in 10 BHF-infected rhesus monkeys that survived the acute form of the disease. Six of these monkeys had received low doses of homologous immune globulin after the first signs of illness appeared. Four monkeys received no supportive therapy. All mon-
keys in this study survived for 30 days or more following inoculation and developed similar clinical signs of CNS disease.

Bolivian hemorrhagic fever is highly fatal in rhesus monkeys, so it was necessary to use monkeys from immune globulin therapy experiments in order to obtain a sufficient number of chronically infected animals for study. It should be emphasized that BHF in rhesus monkeys can be successfully treated when immune globulin is properly administered.2

Materials and Methods

Inoculation

A suckling hamster brain pool of the Carvallo strain of Machupo virus was used. The virus titer in the stock solution was $2 \times 10^4$ plaque-forming units (PFU) of virus per milliliter. The virus was diluted to contain $10^3$ PFU 0.5 ml and each monkey was inoculated subcutaneously with 0.5 ml of the diluted virus. Virus plaque assay in Vero cell culture at the time of infection indicated that the inoculum contained $10^3$ PFU of virus.

Animals

All monkeys were confined in individual primate cages during the period of infection. Four monkeys received no treatment. Six monkeys received various dosages of homologous immune globulin at the onset of clinical illness. Days 5 or 10 of infection (Table 1).

Histology

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 to 8 $\mu$, and stained with hematoxylin and eosin for histopathologic examination.

Results

Clinical Observations

All monkeys showed the initial signs of disease as described by Terrell et al.3 including diarrhea, depression, dehydration, anorexia, and necrosis of the skin. These signs varied somewhat in onset and severity among the monkeys that were treated with the various dosages of immune globulin. Clinical improvement was seen in several of the treated monkeys, then all monkeys began to show signs of neurologic involvement from 16 to 32 days after inoculation (Table 1). These signs included incoordination, tremors, paresis and convulsions, and in 1 animal lateral curvature of the spine. Mucopurulent nasal discharge, alopecia, diarrhea, emaciation, and muscle atrophy were commonly observed during the late course of the disease. Terminally, monkeys were often unable to feed themselves due to weakness, tremors, and incoordination. Clinical signs were difficult to evaluate in these debilitated animals. Necropsies were performed immediately after death or euthanasia of moribund animals.
Table 1—Therapy, Day of Death, and Onset of Central Nervous System (CNS) Signs of BHF-Infected Rhesus Monkeys

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Immune serum (ml/kg)</th>
<th>Day of onset of CNS signs</th>
<th>Day of death</th>
<th>CNS signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>27</td>
<td>30</td>
<td>Tremors, paresis</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>32</td>
<td>35</td>
<td>Tremors, muscle atrophy</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>22</td>
<td>39</td>
<td>Tremors</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>20</td>
<td>43</td>
<td>Paresis, tremors, muscle atrophy</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>16</td>
<td>30</td>
<td>Paresis, tremors</td>
</tr>
<tr>
<td>6</td>
<td>0.4</td>
<td>20</td>
<td>31</td>
<td>Tremors</td>
</tr>
<tr>
<td>7</td>
<td>0.04</td>
<td>17</td>
<td>35</td>
<td>Paresis, muscle atrophy, spinal curvature</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>21</td>
<td>35</td>
<td>Tremors, paralysis</td>
</tr>
<tr>
<td>9</td>
<td>1.2</td>
<td>19</td>
<td>44</td>
<td>Tremors</td>
</tr>
<tr>
<td>10</td>
<td>0.8</td>
<td>22</td>
<td>78</td>
<td>Tremors, muscle atrophy</td>
</tr>
</tbody>
</table>

**Gross Pathology**

Gross lesions were usually minimal. A dry scaly dermatitis of the face and abdomen was seen in 6 monkeys. Four monkeys had contracture and muscle atrophy, most severe in the legs. Nine of the monkeys were moderately to severely emaciated. Thymic atrophy was a common finding.

**Microscopic Pathology**

The location and severity of microscopic lesions are summarized in Table 2. The principle histopathologic lesions of chronic BHF infection were widespread lymphoreticular infiltrates in the walls and adventitia of small arteries and veins in the brain, spinal cord, pancreas, intestine, liver, kidney, adrenal, parathyroid, heart, and skeletal muscle. A diffuse lymphocytic inflammation of practically all organs and tissues was present to a variable degree.

**Blood Vessels**

The lymphoreticular vascular inflammation was present in small arteries and veins and was segmental and proliferative, often involving perivascular tissues. Lymphocytes and macrophages predominated in the inflammatory reaction. Plasma cells were occasionally seen. Vascular endothelium was often swollen. Vascular and perivascular inflammation was essentially the same regardless of the organ system involved.

**Nervous System**

The most striking lesions were seen in the brain and spinal cord. All monkeys had microscopic evidence of lymphocytic inflammation and
Table 2—Microscopic Lesions in Chronic Bolivian Hemorrhagic Fever Infection of the Rhesus Monkey

<table>
<thead>
<tr>
<th>Lesion</th>
<th>No treatment</th>
<th>Immune serum therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monkey 1 Day 30</td>
<td>Monkey 2 Day 35</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Neuritis, ganglioneuritis</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Enteritis</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Adrenalitis</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Nephritis</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Lymphoid necrosis or depletion</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

+ = minimal, ++ = mild, +++ = moderate, ++++ = severe, - = none.

* Day of death.
vasculitis in the CNS. Lesions were present throughout the brain and spinal cord but were most severe in the midbrain, medulla, and cerebellum. Lesions varied considerably in severity. Some monkeys had only a lymphocytic perivascular cuffing (Figure 1) with mild gliosis. Others had severe vascular and perivascular inflammation which appeared to obliterate small arteries (Figures 2 and 3). The choroid and meninges were occasionally infiltrated with lymphocytes (Figure 4). Neuronophagia was rare, and there was no evidence of demyelination. Ganglia and peripheral nerves were diffusely infiltrated with lymphocytes. Ganglia of the stomach, small intestine, and adrenal were often severely affected (Figure 5).

**Liver**

The liver was occasionally the site of mild lymphoreticular vasculitis. Hepatic lesions consisted mainly of a minimal to moderate chronic perportal hepatitis. Hepatic changes were generally mild, especially when compared to the necrotic process present in this organ in acute BHF infection.

**Pancreas**

Nine of the 10 monkeys had microscopic evidence of chronic pancreatitis characterized by infiltration of the lobules and interlobular connective tissue with lymphocytes and monocytes and by mild acinar cell ectasia. Loss and atrophy of acinar cells was a common finding (Figure 6).

**Kidney**

Mild to moderate lymphocytic interstitial nephritis, occasionally with vasculitis, was a common finding. Glomeruli appeared normal.

**Gastrointestinal Tract**

Enteric lesions were present in all monkeys. Chronic enteritis was consistently seen in the small and large intestine. Necrotic debris was often present in dilated crypts as well as in macrophages of the lamina propria. Vasculitis and perivasculitis of small submucosal or mesenteric vessels were a frequent finding (Figure 7).

**Adrenals**

Six monkeys had lymphoreticular infiltrates in the cortex and medulla of the adrenal glands (Figure 7). Small foci of necrosis were seen in the adrenal cortex of 2 monkeys.
Lymphoid Organs

Lymphoid depletion of lymph nodes, spleen, and thymus was commonly seen. Lymphoid necrosis with mild hemorrhage was present in some cases.

Other Observations

Mild to moderate acute epithelial necrosis was observed in 7 monkeys. The epithelium of the skin, lips, nasopharynx, tongue, esophagus, and intestine was consistently involved. Necrosis was occasionally seen in hepatic and pancreatic ductal epithelium and in the epithelium of the renal pelvis. Formalin-fixed epithelium from the pharynx of 1 monkey (No. 2) was examined by electron microscopy and found to contain cytoplasmic virions characteristic of the arenavirus group (Figure 8).

Multifocal and perivascular lymphocytic infiltration was noted in the parathyroid glands of 2 monkeys (Figure 9).

Mild lymphocytic infiltration of the pars nervosa of the pituitary was seen in 2 monkeys.

Four monkeys had mild chronic pneumonitis with interstitial mononuclear infiltrates.

One monkey had multiple acute thrombosis of small vessels in the lung, kidney, liver, adrenals, lymph nodes, and meninges. Thrombosis and hemorrhage were seen in the CNS of a second monkey.

Discussion

In the initial phases of this study, it was considered that the vascular and CNS lesions observed in monkeys dying late in the course of infection were related to the immune serum therapy. Findings of identical lesions in the 4 untreated monkeys in this report have eliminated this possibility. Further evidence that this is not the case was provided in later studies, when a chronic form of BHF developed in a cynomolgus monkey (Macaca fascicularis) and in an African Green monkey (Cercopithecus aethiops) that had received no immune serum therapy.

Machupo virus, the etiologic agent of BHF, produces a chronic virus carrier state in Calomys callosus, a rodent found in endemic areas of infection in Bolivia. The carrier state seen in this animal is similar to that observed in rodents infected with lymphocytic choriomeningitis (LCM), which is also an arenavirus. The LCM carrier state is said to be related to viral lymphotropism, and a similar mechanism has been suggested in the case of Machupo virus infection in C. callosus.

Limited observations on several clinically normal monkeys that have survived BHF infection after immune serum therapy suggest that lymphoreticular inflammation of the CNS may be present to a variable degree
in all surviving monkeys. Examples of this were seen in 4 immune globulin-treated rhesus monkeys that survived the acute phase of BHF infection and had no recognizable clinical evidence of CNS disease. These 4 monkeys were sacrificed 57 days after BHF inoculation and found to have microscopic lesions in the CNS that were similar to, but generally not as severe as, those seen in monkeys with severe neurologic signs. These cases were not included in this study because they were clinically normal and it is possible that they were sacrificed before CNS signs developed.

The epithelial necrosis and possible replication of virus in epithelium may be a continuous process in some monkeys, although this concept awaits confirmation by virus culture and further electron microscopic studies, which are presently being conducted.

It is interesting, and perhaps important in understanding the pathogenesis of BHF, that the lymphocytic inflammation of the CNS seen in chronic BHF infection resembles in some ways the lesions of LCM in mice that are infected intracerebrally as adults. It differs developmentally, however, in that affected monkeys have high levels of serum neutralizing antibody at the time the late neurologic phase of illness develops. Further study is needed to determine if lesions of chronic BHF are mediated by humoral or cellular mechanisms or both.

Extensively studied fatal human cases of BHF have mostly been acute infections in which death occurred less than 2 weeks after onset of illness. Tremors, convulsions, and other signs of CNS inflammation have been observed in human cases of acute BHF, but a chronic form of the disease has not been reported. Although it is not likely that chronic BHF occurs in humans, it would seem worthwhile for clinicians attending convalescent human BHF patients to be aware of the chronic disease as it is seen in monkeys.

References

2. Eddy GA: Unpublished observations

Acknowledgments

The authors express appreciation to Dr. John White for the electron photomicrographs and to George Fry for assistance in performing animal necropsies.
[Illustrations follow]
Figure 1—Moderate lymphocytic perivascular cuffing of a small vessel in cerebellum. Endothelial cells are swollen. (H&E, × 125)

Figure 2—Vasculitis and gliosis (H&E, × 250).
Figure 3—Severe vasculitis and cuffing (H&E, × 900).

Figure 4—Moderate lymphocytic infiltration of choroid (H&E, × 250).
Figure 5—Adrenal ganglion diffusely infiltrated with lymphocytes (H&E, × 250).

Figure 6—Pancreas with moderate acinar cell atrophy and ectasia; diffuse lymphocytic infiltration (H&E, × 250).
Figure 7—Lymphoreticular inflammation around a vein in mesentery. Adjacent artery is not affected. (H&E, × 250)

Figure 8—Electron micrograph of epithelial cell from pharynx. Arrow points to vacuoles which contain pleomorphic granular structures. These granules are the same size as ribosomes (R). Note desmosomes (D). (× 35,000)
Figure 9—Lymphocytes around a vein in parathyroid gland (H&E, × 250).