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BOLIVIAN HEMORRHAGIC FEVER IN RHESUS MONKEYS: TREATMENT WITH SPECIFIC, HOMOLOGOUS ANTIBODY

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To introduce this paper let us consider a hypothetical situation that might develop in a modern military hospital. In this scenario a Captain reports to the dispensary with nonspecific clinical signs including fever, malaise, inappetence and vague aches and pains. The condition is diagnosed as a viral illness, the patient receives appropriate symptomatic medication and is instructed to rest at home and come back in three days if not improved. There is an influenza epidemic at the time and the staff is a bit harassed.

For the great majority of patients there would be no further need for treatment and the dispensary would not see him again, but in our hypothetical situation the patient returns in three days. He is more obviously ill with fever, nausea, occasional diarrhea, severe headache, pain in the lumbar region and shaking chills. He is hospitalized, and a workup by the staff reveals nothing very extraordinary. The leukocyte count is low, the pain in the lumbar area is particularly severe, there is occasional epistaxis and the patient's skin is extremely sensitive. He has several episodes of vomiting and soils the bed linens numerous times. It is of interest also that 16 days prior to entering the hospital he returned from a trip to tropical South America to inspect a Civil Affairs unit that was assisting a disaster relief mission.

Our patient becomes more severely ill, but eventually his fever breaks and the blood pressure drops a bit. He makes a slow uneventful recovery. His discharge diagnosis is that of a severe viral disease of undetermined etiology with complications.

Within two weeks of admission of our Captain there are four more instances of a somewhat similar illness at the dispensary. None are initially recognized as unusual nor can they be distinguished from

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the many influenza patients. The only clue to something more serious is that one of the new patients is the wife of our Captain and the other three are hospital staff-members. Eventually all find their way into the hospital, and two of them die. One of the senior medical officers recalls some rather similar cases from his service in Korea and the staff is quickly alerted to the possibility of a nosocomial viral infection.

All persons known to have had contact with either the index case or the known secondary cases are contacted and members of the hospital staff who become ill are questioned carefully about contact with patients. Within six weeks from the first signs of illness of the Captain there are at least 17 secondary and tertiary cases and 7 deaths. Each illness has occurred in either hospital employees or in family members of suspected cases. Rumors begin to spread. The disease is thought to be one of the South American hemorrhagic fevers and the appropriate state, national and international health agencies are notified. Virologists, epidemiologists and other specialists arrive en masse along with members of the local and national press. Statements by high government officials are made before nationwide television cameras.

The major local problem at this point however, is fear, particularly in the hosptial staff. The virus is identified as Machupo virus, which causes Bolivian hemorrhagic fever (BHF), and the first indications of headache, fever and sore throat in an at-risk staff member raise serious problems. The physician who examines him is in a quandary as to whether to put him on the special contagious ward for hemorrhagic fever patients or to send him home to recover from influenza. Many of the hospital staff refuse to enter the special ward and those who do are fearful for themselves and their families. As the number of cases and deaths mount, panic spreads to the surrounding locality. Fear increases when the disease is diagnosed in a nearby civilian hospital. That diagnosis ultimately proves to be incorrect, but in the ensuing days and weeks day to day interpersonal contacts in the community decrease to an absolute minimum. People who are geniunely ill are afraid to go to the military hospital and the civilian hospitals refuse to admit military personnel or their dependents. Eventually the number of new cases diminishes due to the rigorous precautions initiated in the hospital and dispensary. The disease disappears after having affected 31 people and causing 14 deaths.

This hypothetical situation is not a wild delusion. Recently an outbreak of fatal BHF occurred in a hospital in Cochabamba, Bolivia. But, because the clinical signs and symptoms of the disease were somewhat different from the typical BHF symptoms and because the hospital was well outside the BHF endemic area it was not initially diagnosed. Machupo virus had never been known to exist in Cochabamba.

Eventually it was learned that the index patient had traveled in the Department of Beni, where BHF is endemic, and became ill there before returning home to Cochabamba. This knowledge plus the deaths of the index case and three of the four secondary cases caused a near panic in the city (1). Members of the hospital staff were reluctant to care for a sick pathologist who was exposed during a necropsy of one of the secondary cases and who subsequently became ill and died.

Although other instances of human to human transmission of Machupo virus have been reported (2), this was the first nosocomial outbreak. Hospital associated infections have occurred with the serologically related Lassa fever virus in Africa and the reports of the outbreaks there are remarkably similar to that described for the Cochabamba epidemic (3).

The viruses that caused these illnesses are both members of the arenavirus group which also includes lymphocytic choriomeningitis virus and Junin virus (the cause of Argentine hemorrhagic fever) in addition to a number of avirulent viruses. Some of the more unusual characteristics of infections with the virulent members of the arenavirus group are incubation periods of two weeks or more and prolonged periods of virus shedding (4,5).

Thus, in this era of frequent, rapid international travel the possibility of an outbreak of an arenavirus disease in a large modern hospital becomes a distinct possibility. Our hypothetical situation may never occur, but a realistic assessment suggests that it could. As the remote tropical regions are developed agriculturally and as alterations take place in their ecology, presently known viruses and new viral entities would seem to be candidates for nosocomial outbreaks.

This report describes studies carried out at the United States Army Medical Research Institute of Infectious Diseases during the last 18 months wherein we have defined a rhesus monkey model for studying Machupo virus infections and have successfully treated BHF in monkeys by devising therapy with specific, homologous antibody.

BHF IN RHESUS MONKEYS

In order to work in reasonable safety with Machupo virus our studies were carried out in airtight hoods or in pressurized suits(6). After developing virus plaque assays in Vero cell culture and *in vitro* tests for virus neutralizing antibody, we assessed the rhesus monkey as a model for this infection by observing clinical signs and by measuring leukocyte counts, viremias, and antibody responses. Complete necropsies were performed on monkeys that died.

Figure 1 shows the observed development of clinical signs in monkeys each inoculated with approximately 1,000 plaque forming units

(PFU) of virus. These signs were similar to those reported in humans (7) except that the onset was earlier in monkeys and the disease course more severe. The earliest monkey deaths occurred about day 15, and 80 to 90% of the monkeys died by day 30, the mean day of death was about day 20.

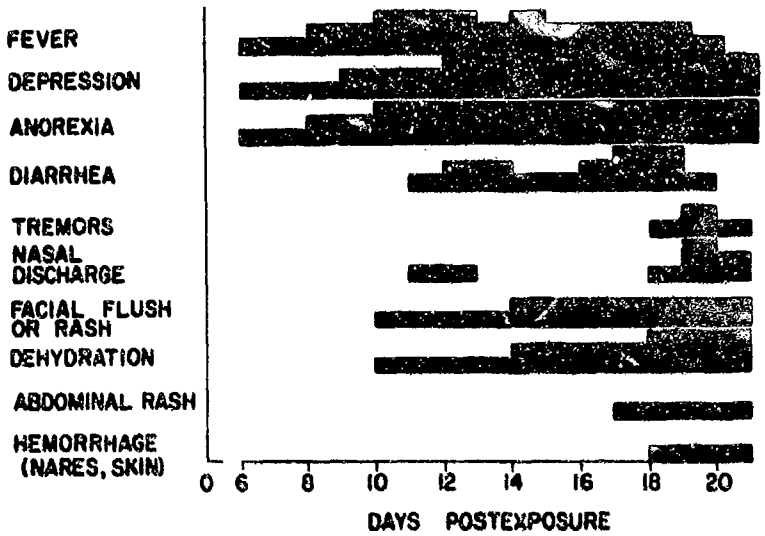

Figure 1. Clinical Signs of BHF in Rhesus Monkeys

Like the reported human cases hemorrhage was not pronounced in the monkeys. There was occasional epistaxis and many monkeys showed some bleeding around the gums. The rash on the face and abdomen did not occur at all in some monkeys but was extremely severe in others.

The neutrophil count dropped precipitously after postexposure day 5 and reached a low on day 13 to 14; other blood parameters that declined included lymphocytes and packed red cell volume. All began to rise on day 13 to 14. Defervescence began at this time although some monkeys had a second fever peak on day 15 to 16.

Histopathology did not reveal consistent hemorrhagic manifestations although hemorrhages were observed in the skin, heart, brain and nerves of some monkeys. Generally, the histopathologic lesions were consistent. There was hepatic necrosis, adrenal cortical necrosis, necrotizing enteritis and epithelial necrosis. Monkeys which died after postexposure day 18 also had nonsuppurative meningoencephalitis and lymphoid necrosis. Most of the histopathologic lesions reported in human autopsies were reproduced in the monkey, however the necrosis of the skin, oral mucosa,

gastrointestinal mucosa and adrenal cortex have not been described in man. Despite these latter discrepancies the rhesus monkey was a satisfactory histopathologic model for the study of BHF.

Ten to 20% of the untreated monkeys in our several experiments survived the initial effects of the virus and began to show some improvement by day 30. They regained their appetite, became more active and were no longer dehydrated, but approximately half of them then developed evidence of central nervous system (CNS) disease. They exhibited paresis, depression, occasional convulsions and incoordination. These clinical signs appeared from day 30 to as late as day 90 and about half of the monkeys died. The histopathologic lesions in the monkeys that died with these signs was primarily vasculitis. Typically it affected all the major organs of the body including the brain.

No neutralizing or complement fixing (CF) antibody was detected in monkeys that died prior to day 21. After that time however, the survivors developed CF titers that gradually rose to 1:512 by day 60. The first neutralizing antibody was detectable by day 28 and reached titers of 1:3200 or more by day 60. These titers are substantially higher than those reported for humans (8) and provided a source of neutralizing antibody for additional studies to define a method of therapy.

The patterns of viremia which developed in monkeys are shown in Table 1. These data represent mean values for several monkeys from three different studies. This relatively prolonged, high viremia is unusual. In most virus infections viremia persists for only 3 to 4 days, and the more severe clinical signs often occur while the viremia is either waning or after it has disappeared. Arenavirus diseases are less acute however, and the prolonged viremia suggests that the pathogenesis might be successfully interrupted with specific antibody therapy. We therefore undertook studies to determine whether convalescent immune serum would protect monkeys if it were administered either at about the time of exposure or after the onset of clinical illness.

PROPHYLAXIS AND TREATMENT OF BHF IN MONKEYS WITH PASSIVE ANTIBODY

Table 2 shows the effect of homologous immune serum given to monkeys that were inoculated 2 hours later with approximately 1,000 PFU of Machupo virus. The quantity of serum given each monkey is shown in the column on the left. The titers on day 4 represent passive antibody and those on day 40 the active antibody titers. The data show that monkeys with low passive antibody titers of 1:4 by CF and 1:8 to 1:32 by neutralization

titers were protected from severe disease. Monkeys with higher passive antibody levels were completely protected from illness but not, apparently, from infection. When the survivors were challenged with Machupo virus on day 60 none became ill. Some monkeys were apparently fully immune despite the absence of detectable neutralizing antibody thereby suggesting a role for cell mediated immunity in this virus infection.

Table 1. Mean viremia titers in Machupo virus infected rhesus monkeys.

Exper. No.	Inoculum Dose	Days Postinoculation (Number Viremic/total)					
		6	10	12	14	17	21
1	3.0*	-	2.7* (5/5)	3.6 (5/5)	4.6 (5/5)	3.7 (3/5)	1.0 (0/1)
2	5.0	2.6 (2/2)	3.4 (2/2)	1.8 (2/2)	-	-	-
3	3.0	-	3.2 (2/2)	2.8 (2/2)	4.8 (2/2)	4.5 (2/2)	2.2 (1/1)

*Log_{10} PFU of virus

Table 2. Immune serum protection of rhesus monkeys against Machupo virus infection

Serum Dose ml	Reciprocal Day 4 Antibody Titer				Severity of Illness	Deaths/2	Reciprocal Day 40 Antibody Titer			
	Neut.		CF				Neut.		CF	
5.0	32	128	16	16	0	0	<8	<8	<4	<4
1.5	32	32	8	8	0	0	8	32	<4	4
0.5	8	32	4	4	++	0	128	512	64	64
0.15	<8	<8	<4	<4	++++	1	2048		512	
None	<	<8	<4	<4	++++	2	-	-	-	-

Nevertheless, the prophylactic efficacy of antibody was obvious. We therefore assessed the effects of immune serum given to monkeys at various times postinfection. Table 3 shows the results of our efforts to treat monkeys on days 5, 7, 9 or 11 postexposure by giving each monkey 10 ml of immune serum. Bearing in mind that the monkeys became symptomatic on about day 6, it is apparent that the typically fatal disease course was favorably modified after the onset of clinical signs.

Table 3. Treatment of Machupo virus-infected monkeys with homologous immune serum

Day Treated	Severity of Illness	Time to Recovery	Death/2	Reciprocal CF Titer Day 44
5	Trace	1-2 days	0	32 64
7	+	6-8 days	0	64 256
9	+++	2-3 Weeks	0	512 512
11	++++	2 months	1	512 -
None	++++	-	2	- -

In general we were sble to reproduce the foregoing results by using ethanol fractionated gamma globulin rather than whole serum. However, treatment after the onset of clinical signs appeared to be less effective with intramuscular injections of gamma globulin than with the equivalent amount of serum given subcuteneously. We reasoned that this may be due to the route of administration and that the deep intramuscular injection was less efficacious than other possible routes.

Table 4 sh s the results of giving immune gamma globulin by two routes on day 8 postexposure. The two monkeys treated intravenously suffered a mild clinical disease whereas three of the four monkeys given intramuscular gamma globulin experienced a more severe illness.

Table 4. Effect of route of gamma globulin treatment on clinical illness in Machupo virus infected rhesus monkeys

Route	Severity of Illness (Number affected)	Days to Recovery	Deaths/Total
Intravenous	+(2)	10-15	0/2
Intramuscular	+(1)+++(3)	10-15	0/4
None	++++(3)	-	3/3

To quantitate the amount of serum required to treat monkeys early after the onset of clinical signs. Table 5 was prepared as a composite of three studies. It shows the minimum amount of serum or gamma globulin equivalent that resulted in a successful treatment and the maximum amount that did not. In all cases this was given on day 8 and is calculated as the equivalent amount of serum with a neutralizing antibody titer of approximately 1:3000. Thus the amount of potent antiserum required for successful treatment is approximately 3 ml/kg. The human dosage calculated in the column on the right represents the number of 240 ml units of human plasma that would be required to yield similar levels of passive neutralizing antibody in a 70 kg human recipient if the human plasma pool had a neutralizing antibody titer of about 1:100. This represents a substantial amount of plasma, but it could be given as gamma globulin if the need arose.

Table 5. Antibody dosage for early treatment of Machupo virus infected monkeys

Serum Dose (ml/kg)	Severity Illness	Death/total	Equivalent Human Dosage* (Plasma units)
3.0	mild	0/4	26
1.5	severe	1/3	13
None	severe	0/3	-

* See text.

One of the difficulties with antibody therapy later in the clinical course is the rapid turnover that occurs. We observed that monkeys receiving 10 ml of immune serum on postexposure day 10 had barely detectable antibody titers two days later, whereas the data in Table 2 show that monkeys which received 5 ml of serum on day 0 had passive titers of as much as 1:128 on day 4. These data suggested that a single dose of antibody given late in the disease may be removed so rapidly, due to reaction with antigen and to the more rapid turnover of serum protein during severe illness, that successful treatment might require the continuous administration of specific homologous antibody. In a separate experiment virus infected monkeys were treated about 4 days after the onset of illness (day 10 postexposure) with the gamma globulin equivalent of 2 ml/kg of serum intravenously followed by intramuscular doses of one-half that amount daily for the next 10 to 12 days. Although two of the three treated monkeys died, the clinical course and the times to death suggested that such therapy was useful and further studies are now underway.

DISCUSSION

Our data from Machupo virus infected rhesus monkeys showed that species to be a useful model for BHF in humans. The clinical signs, hematologic data and histopathologic lesions were generally similar to those seen in humans except that the disease course was more severe and perhaps somewhat briefer in the monkey. Using the rhesus model we then explored prophylaxis and therapy with homologous antibody. Our data suggest that the use of convalescent plasma might favorably alter an outbreak such as the hypothetical one described in the introduction.

Our data showed that: 1) relatively low prophylactic doses of specific antibody given at about the time of exposure to BHF prevented the development of clinical signs of disease; 2) BHF, unlike most viral diseases, can be successfully treated, in monkeys at least, if antibody therapy is initiated early after the onset of clinical signs; and 3) The maintenance of detectable levels of passive antibody with daily injections may permit successful treatment as much as four days after the clincial signs have become apparent.

On the basis of preliminary data from our BHF studies, the United States Army Medical Research and Development Command funded a project by the Pan American Health Organization (PAHO) and The Middle America Research Unit to collect plasma from BHF immune persons in Bolivia. A number of people in the endemic areas were screened and 16 were selected for plasmapheresis which was carried out by Medical Officers from PAHO and the Bolivian Ministry of Health. A total of 223 units of plasma

was collected and have been sent to the Massachusetts State Laboratory for fractionation of the gamma globulin. The immune gamma globulin will be studied in our laboratory and used in Bolivia.

Although this relatively small amount of antibody will not be adequate for treating large numbers of people, it will provide a means for prophylaxis or therapy of selected medical personnel. There are no BHF immune medical personnel in Bolivia at the present time and it is difficult to persuade susceptible individuals to go into epidemic areas to provide patient care. With a supply of gamma globulin of known potency for prophylaxis and treatment however, the situation of near panic described in Cochabamba may be avoided and medical personnel will be more sanguine about serving in epidemic areas.

In addition to the use of specific human gamma globulin we are exploring the possible use of hyperimmunized monkey serum which many be as much as 100 to 200 times more effective than the available human plasma. This possibility could indeed provide a source of antibody for large scale human therapy if it proves to be safe and effective.

Thus unlike virtually all other viral diseases, arenavirus infections may be unique in that the typical pathogenesis is amenable to specific therapy. Our data suggest that by developing an adequate source, of specific immune serum we may reduce the threat of these rodent-borne viruses.

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