Protection of monkeys against Machupo virus by the passive administration of Bolivian haemorrhagic fever immunoglobulin (human origin) *

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Bolivian haemorrhagic fever immunoglobulin of human origin, given either prior to or shortly after experimental infection with Machupo virus, protected rhesus and cynomolgus monkeys against initial clinical illness. Some survivors developed severe neurological signs 30–47 days after virus inoculation and died 4–6 days later. Results from one of the experiments suggested that the development of neurological signs was associated more frequently with high doses of immunoglobulin than with intermediate or low doses.

In a joint effort by the Bolivian Ministry of Health, the Pan American Health Organization, the Middle America Research Unit, the Biologic Laboratories of the Commonwealth of Massachusetts, and our Institute, 221 units of plasma were collected by plasmapheresis in approximately equal quantities from 14 persons with prior histories of Bolivian haemorrhagic fever (BHF) and with previously assayed neutralizing antibody titres to Machupo virus, the etiological agent of BHF. The plasma was fractionated, and the resulting immunoglobulin was packaged and lyophilized. This report describes the *in vitro* and *in vivo* testing of the final product.

MATERIALS AND METHODS

BHF immunoglobulin, human origin

Approximately 1600 ml of immunoglobulin were prepared by methanol fractionation from 221 units (50.2 litres) of human plasma at the Biologic Laboratories of the Commonwealth of Massachusetts. After safety and sterility testing, 1400 ml were packaged and lyophilized in vials containing 10 ml per vial. The serum protein content of the final, reconstituted product was 16.7 g/100 ml.

Virus and virus neutralizing antibody assays

Machupo virus, virus plaque assays, and assays for plaque reduction neutralizing antibody were identical with those described by Eddy et al. (1).

Infection and observation of monkeys

Rhesus or cynomolgus monkeys, as described elsewhere (1), were experimentally infected by the subcutaneous inoculation of 1000 plaque-forming units of Machupo virus. Treated monkeys were inoculated intramuscularly with a high (1.0–1.5 ml/kg), intermediate (0.3–0.5 ml/kg) or low (0.1–0.15 ml/kg) dose of the reconstituted BHF human immunoglobulin either 4 h before or 4 h after inoculation of the virus. Virus-inoculated controls received no immunoglobulin.

RESULTS

In vitro testing

Equal volumes of plasma from each of 14 donors were pooled and tested for Machupo virus neutralizing antibody. By repeated testing of serial 2-fold dilutions the titre of the pooled plasma was determined as 1:128 and that of the immunoglobulin as 1 : 2048.

Efficacy of passively administered immunoglobulin

We carried out 2 dose-response experiments to measure the efficacy of immune plasma, one in

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Table 1. Prophylactic dose response of BHF immunoglobulin (human) in rhesus monkeys

Dose [a] (ml/kg)	Initial clinical signs			Days viraemic/ days tested [b]	Late neurological signs		
	Positive/ total	Severity (number)	Deaths (mean day of death)		Positive	Mean day of onset (mean day of death)	Deaths
1.5	0/3	− (3)	0	0/15	3	41 (45)	2
0.5	3/3	± (3)	0	0/15	1	47 (51)	1
0.15	3/3	++ (2) ++++ (1)	1 (19)	1/14	0	–	0
None	3/3	++++ (3)	3 (19)	11/12	–	–	–

[a] Monkeys were inoculated subcutaneously with 1000 PFU of Machupo virus and 4 h later received BHF immunoglobulin at the dosage shown.

[b] Days 7, 10, 14, 17, and 21 of surviving monkeys. Day 0 sera were assayed for virus, found negative, and not included in tabulated data.

rhesus monkeys and a second in cynomolgus monkeys. In both experiments groups of 3 monkeys each received high, intermediate, or low doses of immunoglobulin 4 h after inoculation of virus. The monkeys were observed daily. Serum samples were collected for viraemia assays on days 0, 7, 14, 17, and 21 and for neutralizing antibody assays on days 0, 1 (second experiment only), 4, 7, 10, 14, 17, 21, 28, 35 (second experiment only), 42, and 56. Brain, spleen, inguinal lymph node, kidney, liver, and serum specimens for virus assay were collected in the second experiment from monkeys that died following neurological signs of illness.

The results of testing in rhesus monkeys (Table 1) showed that the high and intermediate doses of immunoglobulin protected against the development of initial signs of illness. Three of the monkeys that received the intermediate dose exhibited slight conjunctivitis and a pallid complexion, signs not unequivocally attributable to infection. All monkeys in the low dosage group became ill. One severely ill monkey died, but 2 moderately ill monkeys recovered uneventfully. Viraemia was not detected in the 2 higher dosage groups and was detected on only one day in one monkey in the low dosage group. That monkey died.

By day 30 all survivors appeared normal. Between days 30 and 47 all monkeys from the high dosage group and one from the intermediate group developed severe neurological signs, including intention tremors, ataxia, depression, and coma resulting in death in 3 of the 4 affected monkeys. The disease course was brief. Clinical signs were predominantly neurological and entirely different from those seen during illness in control monkeys.

A similar study was done in cynomolgus monkeys with a slightly lower dosage of immunoglobulin (Table 2). This study yielded similar results except that all monkeys in the low dosage group were viraemic on 9 of 13 days tested and all died during the early phase. Late neurological signs developed in only one monkey in each of the 2 highest dosage groups. As in the previous experiment, the monkeys in the two highest dosage groups exhibited little or no illness during the first 4 weeks and the late neurological phase of disease was severe, brief, and without detectable viraemia. Tissue and serum specimens from the 2 monkeys that died following late neurological signs of illness were negative for virus.

Antibody titres in passively protected monkeys inoculated with Machupo virus

A third experiment was initiated to determine the effect of administration of immunoglobulin 4 h prior to virus inoculation. In this experiment only a high dose (1.2 ml/kg) of the product was used in 3 rhesus monkeys. None of the 3 monkeys experienced initial clinical illness whereas control monkeys died on days 14 and 16. The rate of decline in passive antibody was slower in the monkeys pretreated with immunoglobulin than in the monkeys given immunoglobulin 4 h after virus inoculation. The decline in antibody

Table 2. Prophylactic dose response of BHF immunoglobulin (human) in cynomolgus monkeys

Dose[a] (ml/kg)	Initial clinical signs			Days viraemic/ days tested[b]	Late neurological signs		
	Positive/ total	Severity	Deaths (mean day of death)		Positive	Mean day of onset (mean day of death)	Deaths
1.0	0/3	-	0	0/15	1	30 (33)	1
0.3	1/3	±	0	2/15	1	34 (39)	1
0.1	3/3	++++	3 (24)	9/13	-	-	-
0	2/2	++++	2 (23)	6/7	-	-	-

[a] Monkeys were inoculated subcutaneously with 1000 PFU of Machupo virus and 4 h later received BHF immunoglobulin at the dosage shown.

[b] Days 7, 10, 14, 17, and 21 of surviving monkeys. Day 0 sera were assayed for virus, found negative, and not included in tabulated data.

titres for the 3 experiments is summarized in Fig. 1. The geometric mean antibody titres of the 6 monkeys inoculated with virus prior to immunoglobulin (3 with 1.5 ml/kg and 3 with 1.0 ml/kg, Tables 1 and 2, respectively) are shown in comparison to the group of 3 monkeys that received virus 4 h after administration of immunoglobulin. Antibody was detectable at a titre of 1 : 4 in the latter group on day 28 whereas the antibody in the former group of monkeys became undetectable between days 14 and 21. The slopes of the linear regression lines for the two treatment categories were significantly different ($P < 0.01$).

The antibody titres of 6 monkeys receiving the intermediate dose of immunoglobulin 4 h after virus inoculation ranged from 1 : 4 to 1 : 8 between days 1 and 4 and then became undetectable between days 7 and 14.

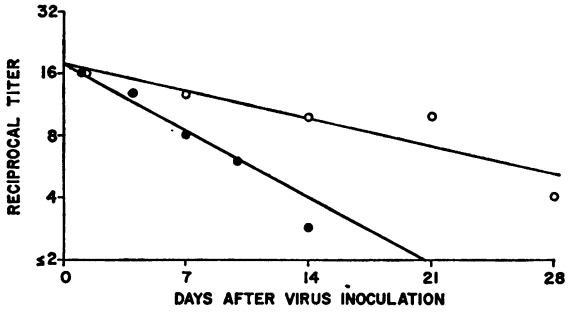

Fig. 1. Geometric mean antibody titres of monkeys receiving a high dose (1.0–1.5 ml/kg) of BHF immunoglobulin either 4 h before (O) or 4 h after inoculation of virus (●). Upper and lower lines represent respective linear regression lines plotted for the two sets of titres.

Active immunity and response to rechallenge

All survivors were tested for antibody between days 42 and 56. All but 2 of the monkeys receiving virus before immunoglobulin developed active antibody by day 56. The exceptions were 2 surviving rhesus monkeys from the intermediate treatment group (Table 1) that failed to develop active antibody. Regardless of antibody status, however, all the surviving monkeys from the first 2 experiments were immune to virus rechallenge at 56 days.

The 3 monkeys receiving a high dose of immunoglobulin before virus inoculation apparently developed a brief surge of active antibody. All had titres of 1 : 4 on day 28 (Fig. 1) and the titres rose to a mean of 1 : 16 (1 : 8, 1 : 16, 1 : 32) on day 35. On days 42 and 56 the 3 monkeys had no detectable neutralizing antibody titres at 1 : 4 serum dilutions. Challenge of these monkeys on day 56 produced no immediate clinical response. One of the rechallenged monkeys became ataxic 25 days later. Within another 10 days it exhibited convulsions and developed an apparent blindness without observable corneal opacity. The other 2 monkeys exhibited no clinical response to rechallenge.

All monkeys without detectable antibody on day 56 exhibited a relatively brisk antibody response

following rechallenge. Antibody was invariably detectable by day 14 to day 21.

DISCUSSION

We are unable to offer an explanation for the development of late encephalitis in monkeys inoculated with Machupo virus. The occurrence of late encephalitis in the rhesus monkeys given immunoglobulin after virus inoculation suggested an association with a high dose of globulin more frequently than with the low or intermediate dose. No relationship was apparent in cynomolgus monkeys. When we inoculated immunoglobulin before virus none of the monkeys developed early clinical illness and late encephalitis occurred in only one monkey and then only after rechallenge.

Late encephalitis as seen in these studies was very acute, with an abrupt onset usually leading to death within 4–6 days. Except for the abrupt onset, it did not differ clinically from the late encephalitis described in our report on the pathogenesis of Machupo virus infection in primates (1). The major immunological difference between late encephalitis after prophylactic immunoglobulin compared to that occurring spontaneously was that at the time of onset of neurological signs only 1 of the 7 recipients of immunoglobulin had detectable neutralizing antibody. The other monkeys had circulating neutralizing antibody to the virus at titres usually in excess of 1 : 128.

With respect to the concept of postexposure prophylaxis with immunoglobulin, the data at present available seem to indicate that a dosage adequate to provide a neutralizing antibody titre of 1 : 4 to 1 : 8 will protect against severe initial clinical signs of illness. Whether the complicating late encephalitis described herein will present an obstacle to effective therapy in man remains to be determined.

RÉSUMÉ

PROTECTION DES SINGES CONTRE L'ARÉNAVIRUS DE MACHUPO PAR L'ADMINISTRATION PASSIVE D'IMMUNOGLOBULINE ANTI-FIÈVRE HÉMORRAGIQUE DE BOLIVIE (D'ORIGINE HUMAINE)

Des essais *in vitro* et *in vivo* sur l'immunoglobuline anti-fièvre hémorragique de Bolivie, d'origine humaine, ont montré que le pool de plasma primitif et le produit résultant contiennent des anticorps neutralisants contre l'arénavirus de Machupo (titres 1 : 64 et 1 : 2448 respectivement) et que des doses élevées (1,0-1,5 ml/kg), et intermédiaires (0,3-0,5 ml/kg) d'immunoglobuline protègent les singes contre la maladie clinique à ses débuts. Plusieurs des singes à qui l'on avait administré de l'immunoglobuline après inoculation du virus ont été atteints d'encéphalite 30 à 47 jours plus tard. Les résultats d'une des expériences semblent indiquer que l'apparition tardive d'une encéphalite est associée plus fréquemment à des doses élevées d'immunoglobuline qu'à des doses faibles.

REFERENCES

1. EDDY, G. A. ET AL. Pathogenesis of Machupo virus infection in primates. *Bull. World Health Organ.*, **52**: 517-522 (1975)
2. STINEBAUGH, B. J. ET AL. Bolivian hemorrhagic fever. A report of four cases. *Amer. J. Med.*, **40**: 217-230 (1966)
3. JOHNSON, K. M. ET AL. Hemorrhagic fever of Southeast Asia and South America: a comparative appraisal. *Progr. med. Virol.*, **9**: 105-158 (1967)

DISCUSSION

K. JOHNSON: Does anyone know whether or not there is reason to suspect that there might be differences in the way antibodies function when contained in unalterated plasma as opposed to when they are concentrated and purified in the form of immunoglobulin? I think it unlikely that the results you have described are due to that process because you get the same neurological disease in a fraction of the monkeys that survive initial

infection, but I should like to know whether there is any evidence of such differences in the biological activities of antibodies.

EDDY: Of course, one thing that is lost from methanol-fractionated immunoglobulin is IgM; we have some very tenuous data suggesting that IgM will lyse cells infected with Machupo virus, whereas late convalescent serum will not.

RAWLS: The ability to suppress the acute manifestations of Machupo virus infection by immunoglobulin without altering the late neurological disease suggests that the inoculum may contain two viruses: one virus responsible for Machupo-like illness and another antigenically distinct virus responsible for the encephalitis. This possibility is further suggested by the inability to recover virus from the brains of the deceased animals. What evidence is there that the late neurological disease is in fact due to Machupo virus?

EDDY: We cannot entirely rule out the possibility of a second virus. We have done some cloning of Machupo virus, but all this work was done with a single pool of the third or fourth suckling hamster brain passage of the prototype isolate, Carvallo strain.

ISAÄCSON: The late Dr Karl F. Meyer and others have shown in experimental work with live attenuated plague vaccines in *Cercopithecus aethiops* and other primate species that the pathogenic effects may vary considerably. The vaccine, relatively harmless to man, was lethal in a high proportion of vervet monkeys. It is advisable to be cautious when interpreting results obtained in animal models.

EDDY: I quite agree. The monkey is far more susceptible to Machupo virus than humans; they have a far higher viraemia.

NATHANSON: The late encephalitis in the passively immunized animals develops about the time when their antibody titres have disappeared. What happens if you give them repeated doses of globulin? In other words, does the late disease develop because their passive immunity is wearing off and they are not developing much of an active immune response?

EDDY: We have not tried giving repeated doses of globulin. I am inclined to suspect that there is some kind of immunological imbalance, but I have not any idea what it is. If there is too much antibody in the host at the time he is exposed to the virus, this would have profound implications in regard to the possible use of a live or an attenuated vaccine. And I would point out that whether they developed clinical signs of late encephalitis or not, all of the monkeys ended up with vasculitis. We have never had a monkey infected with Machupo virus that did not end up with vascular cuffs.

MAIZTEGUI: In relation to the beneficial effects obtained in the monkeys inoculated with BHF immunoglobulin, your observations are in complete agreement with those of clinicians treating AHF patients with convalescent plasma. However, I have never seen a patient with AHF develop the late neurological syndrome you find in monkeys. If this were related to the passive immune treatment, I would expect the disorder to have become apparent in the several thousand AHF patients treated. I wonder if the neurological disease could be related to the use of purified globulin instead of whole plasma.