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Protection of Lassa Virus-Infected Guinea Pigs With Lassa-Immune Plasma of Guinea Pig, Primate, and Human Origin

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Lassa virus-immune plasma has been used to treat human Lassa fever patients; however, criteria for plasma selection were based arbitrarily on available serologic tools and protective efficacy was never directly assessed. To test the validity of plasma therapy for Lassa virus infections in an animal model, and to develop biologically relevant criteria for selection of protective immune plasma, inbred, strain 13 guinea pigs were infected with a lethal dose of Lassa virus and treated with various Lassa-immune plasmas obtained from guinea pigs, primates, and convalescent human patients. Neutralizing antibody titers were determined in a virus dilution, plaque reduction test, and were expressed as a $\log_{10}$ plaque-forming units (PFU) neutralization index (LNI). All guinea pigs treated with immune plasma 6 ml/kg/treatment on days 0, 3, and 6 after virus inoculation were protected, provided the LNI exceeded 2.0. Plasmas obtained from donors in early convalescence (32–45 days) had low titers of N-antibody (LNI < 2) and failed to confer protection, despite high titers of Lassa antibody measured in the indirect fluorescent antibody (IFA) test. Higher doses of marginally titered plasma conferred increased protection. The degree of protection and suppression of viremia was closely associated with LNI and not IFA titers. Administration of low-titered plasma did not result in immune enhancement. A high dose of human plasma from Liberia (12 ml/kg/treatment) was required to confer complete protection to guinea pigs infected with a Lassa virus strain from Sierra Leone (LNI = 1.6), while a lower dose (3 ml/kg/treatment) was sufficient for protection against a Liberian strain (LNI = 2.8), suggesting that a geographic matching of immune plasma and Lassa virus strain origin may increase treatment success. These studies support the concept of plasma therapy for Lassa infection and suggest that the plaque reduction neutralization test is more appropriate than the IFA test for predicting protective efficacy of passively administered plasma.

Key words: Lassa virus, immunotherapy, guinea pigs

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INTRODUCTION

Lassa virus-immune plasma has been used to treat human Lassa fever since the disease was first recognized in 1969 as a new disease entity in Nigeria [Leifer et al, 1970]. The promise of the initial report of treatment success was diminished by subsequent reports that plasma therapy success was equivocal [Keane and Gilles, 1977], and occasionally others have speculated that at times it may even have been deleterious [Clayton, 1977]. The beneficial effects of immune plasma in reducing acute mortality associated with related arenavirus diseases have been clearly demonstrated for Argentine hemorrhagic fever in humans [Maiztegui et al, 1979] and Bolivian hemorrhagic fever in experimentally infected rhesus monkeys [Eddy et al, 1975]. However, in both reports, a disturbing incidence of late neurologic sequelae following treatment was noted, raising concerns related to immunologic enhancement of disease.

For Lassa fever therapy, immune plasma has until now been selected on the basis of complement fixation or fluorescent antibody titers, rather than neutralizing antibody titers, and protective efficacy has never been directly assessed.To evaluate plasma treatment in a more systematic manner, we developed a uniformly responding animal model (strain 13 guinea pigs) and a biologically relevant neutralizing antibody assay for Lassa virus [Jahrling et al, 1982]. In this report, we present data which support the validity of immune plasma therapy for Lassa virus infection, and establish preliminary criteria for the selection of protective Lassa-immune plasma and its effective administration.

MATERIALS AND METHODS

Preparation of Virus Stock and Virus Assay

Lassa virus, strain Josiah, was isolated in 1976 from the serum of a human patient in Sierra Leone [Wulff and Johnson, 1979] and was used in the fourth Vero cell passage. Unless otherwise noted, this virus stock was employed throughout the present study. A second Lassa virus strain (Z-132) was isolated in our laboratory from a serum collected by Dr John Frame in Liberia in 1981, and was used in second Vero cell passage. All infectious Lassa virus assays were performed by counting plaque-forming units (PFU) on Vero cell culture monolayers grown and maintained in 10-cm^2 wells of plastic plates as described previously [Jahrling et al, 1980]. All manipulations of infectious material were conducted within the maximum biologic containment (P4) facilities at the Institute.

Manipulation of Laboratory Animals

Inbred, strain 13 guinea pigs, 300–350 g males, were obtained from our own colony. Outbred Hartley strain guinea pigs, 500–550 g males were purchased from Buckberg Farms (Tomkins Cove, NY). To produce infection, guinea pigs were inoculated subcutaneously (sc) with 0.5 ml of stock virus suspensions diluted in Eagle's minimal essential medium with Earle's salts (EMEM) to $10^{3.4}$ PFU/0.5 ml. Plasma samples were obtained from ether-anesthetized guinea pigs bled repetitively at 2–3-day intervals from the retro-orbital sinus into heparinized syringes with 25-gauge needles. To obtain large volumes of convalescent guinea pig plasma, surviving outbred guinea pigs were anesthetized with chloroform and bled from the heart into

heparinized syringes. To obtain a Lassa-immune monkey plasma pool, two rhesus monkeys which had survived sc inoculation with $10^{6.1}$ PFU of Lassa virus, strain Josiah, were bled at one-week intervals from the femoral vein into heparinized Vacutainer tubes 180–240 days after infection. All plasma thus obtained was pooled.

Human Lassa-Immune Plasma

Multiple plasma units were obtained from a single human donor who had contracted Lassa fever in Liberia 2–3 years previously, and was plasmapheresed over a period of six months.

Antibody Assays

The IFA test was performed using the procedure of Peters et al [1973] with slight modification [Jahrling, 1980]. In brief, Vero cells infected with Lassa virus or uninfected control cells were dried onto circular areas of Teflon-coated slides (Cel-Line Associates, Minatola, NJ), fixed in acetone, and treated with test serum diluted in phosphate buffered saline (PBS). Each test included two positive control guinea pig sera, one with an anti-Lassa IFA titer of 1:80, the other 1:5,120, to insure standardization of the assay. After incubation for 30 minutes, slides were flooded with fluorescein-conjugated goat antiguinea pig gamma globulin, incubated, washed, mounted, and examined. The titer was expressed as the highest dilution of serum producing definite granular fluorescence of the cytoplasm of infected cells. Since residual infectious virus could routinely be recovered from these slides, even after staining, all slides were observed under P4 containment conditions.

Neutralizing antibody titers were measured in a plaque-reduction test using the constant serum-varying virus format, the rationale for which will be detailed elsewhere [Jahrling, submitted for publication]. In brief, all dilutions were performed in neutralization test medium (NTM) comprised of Hanks' balanced salt solution, HEPES buffer (25 mM), and 10% normal guinea pig serum, either freshly obtained or stored at −70°C, (as a complement source). Challenge virus was the Lassa virus (strain Josiah) preparation described above, diluted initially 1:10 in NTM, in which it titered 6.2×10^6 PFU/ml. To test neutralizing capacity, test sera (freshly obtained or stored frozen at −70°C until used) were diluted 1:10 in NTM and divided 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 reaction mixtures were incubated at 37°C for one hour. As a control, normal serum was substituted for convalescent serum. Following incubation, reaction mixtures were assayed for residual infectivity (ie, PFU) on Vero cells as detailed above. Wells containing ten to 100 PFU were counted. Neutralizing antibody activity was expressed as a $\log_{10}$ neutralization index (LNI), calculated by the following formula: LNI = [Log_{10} (PFU in control) − Log_{10} (PFU in test serum)].

Plasma Protection Studies

Guinea pigs, infected by sc inoculation of Lassa virus as stated in footnotes of the tables were treated with immune plasma or dilutions of immune plasma in normal plasma, inoculated intraperitoneally (ip) in doses ranging from 1 to 12 ml/kg. The first plasma treatment was administered immediately after virus inoculation (day 0); identical second and third treatments were administered ip on days 3 and 6. All treated guinea pigs were bled at 2–4-day intervals until day 21, and were observed

TABLE I. Protective Efficacy of Convalescent Guinea Pig Plasma for Lassa-Virus-Infected* Strain 13 Guinea Pigs (n = 10/Group)

Immune plasma pool infused[a]						
Pool	Days convalescent	Dilution	IFA	LNI	% survival	MTD[b] (range)
1	32	0	2560	0.3	0	18 (15–21)
		1:3	640	0.1	0	17 (14–19)
		1:9	160	0.1	0	17 (14–21)
2	45	0	2560	0.9	0	21 (19–24)
		1:3	320	0.6	0	24 (16–22)
		1.9	160	0.3	0	21 (19–22)
4	90	0	2560	3.4	100	
		1:3	640	3.1	100	
		1:9	160	2.0	90	19
		1:27	40	1.4	50	17 (16–17)
5	180	0	2560	3.8	100	
		1:3	640	2.8	100	
		1:9	320	2.2	80	25 (24–26)
		1:27	40	1.5	30	21 (12–32)
		1:81	10	0.8	0	23 (19–27)
Normal plasma					0	17 (15–19)

*3.4 $\log_{10}$ PFU Lassa virus, strain Josiah, SC.
[a]6 ml/kg of convalescent guinea pig plasma diluted in whole, fresh guinea pig plasma, inoculated IP into recipient guinea pigs on days 0, 3, and 6 after virus inoculation.
[b]Mean day of death.

for late deaths until day 60 when survivors were bled to determine seroconversion rates.

RESULTS

Neutralizing antibody activities in plasma of Lassa virus-inoculated guinea pigs evolved late, relative to Lassa-reactive antibodies measured by the IFA test. Neutralizing antibody titers (as determined by an LNI), were measured in four plasma pools obtained from surviving outbred guinea pigs at 32, 45, 90, and 180 days after virus inoculation (Table I). The LNI titers were 0.3, 0.9, 3.4, and 3.8, respectively, while IFA titers remained constant at 1:1,280–1:2,560. LNI titers were rapidly diminished by dilution of immune plasma, while IFA titers diminished proportionately with dilution (Table I).

The ability of these convalescent plasmas to protect Lassa virus-inoculated strain 13 guinea pigs was also tested (Table I). The availability of plasma pools with various IFA-LNI ratios facilitated the correlation of protective efficacy with either the LNI or IFA titers. Plasma treatment was initiated day 0 (day of infection) and repeated on days 3 and 6, at a dose of 6 ml/kg. Immune plasma obtained in early convalescence (days 32 and 45) conferred no protection, even when administered without dilution despite IFA titers of 1:2,560. In contrast, late plasma obtained at 90 and 180 days protected guinea pigs even when diluted 1:9; however, the 1:27 dilutions (where LNI were < 2), protected only some of each group, and the 1:81 dilution of plasma 5

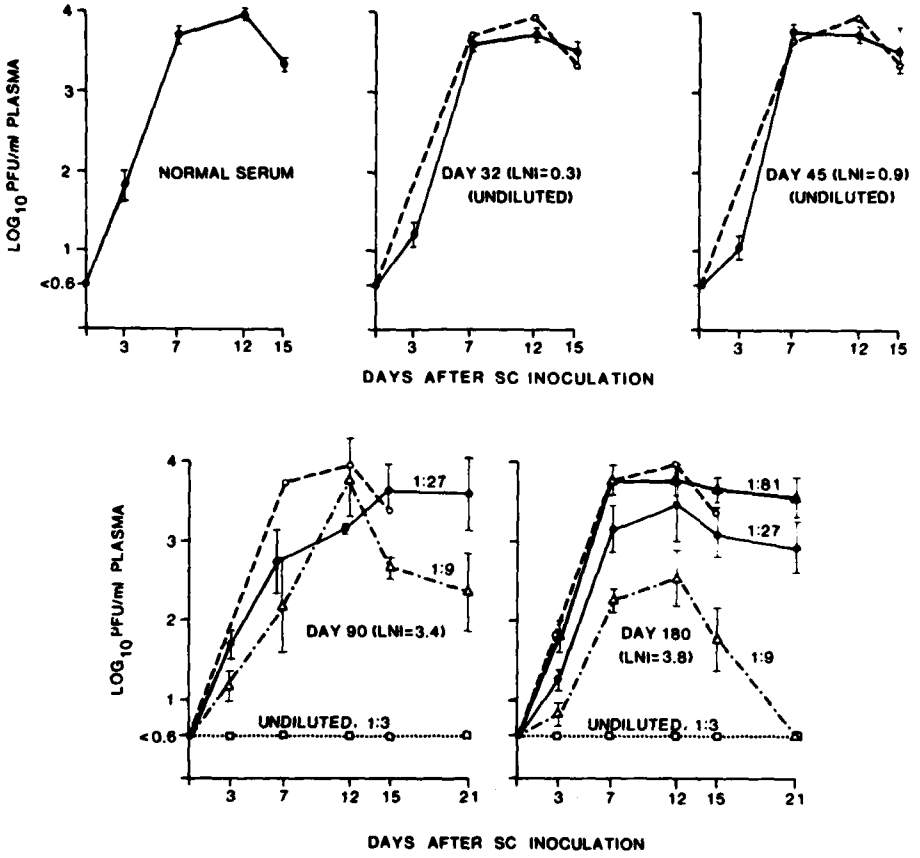

Fig. 1. Effect of immune plasma treatment of Lassa viremia in guinea pigs. Strain 13 guinea pigs (n = 10) inoculated with 3.4 $\log_{10}$ PFU Lassa virus, strain Josiah, were treated ip on days 0, 3, and 6, with 6 ml/kg of immune plasma or plasma dilutions obtained from Lassa convalescent guinea pigs on days 32, 45, 90, or 180, following virus inoculation. Viremias in normal serum-treated guinea pigs (first panel) are superimposed (0-----0) on subsequent panels to facilitate comparison. LNI pertains to undiluted plasma obtained on the stated day of convalescence, and the dilution of plasma tested is stated next to the appropriate viremia curve.

(LNI = 0.8) protected none of the guinea pigs. Protective efficacy of Lassa-immune plasma clearly correlated with LNI more closely than with IFA titers.

Viremia titers were measured in all groups of immune plasma-treated and control guinea pigs (Fig. 1). Guinea pigs treated with late convalescent (days 90 and 180) plasmas in low dilution, failed to develop any detectable viremia, although they all eventually seroconverted, developing IFA titers of 1:1,280–1:2,560. Guinea pigs treated with higher dilutions of late plasma (containing lower LNI) were less adequately protected, and viremias increased, approaching control (normal plasma-treated) viremias. Viremias following treatment with plasma obtained early (days 32 and 45) also approximated controls (Fig. 1). There was no suggestion that inadequate plasma treatment enhanced viremia.

TABLE II. Protection of Lassa-Virus-Infected* Guinea Pigs as a Function of Convalescent Guinea Pig Plasma Dose

Immune plasma pool infused					
Pool	Days convalescent	Dilution	LNI	ml/kg[a]	% Survival (n = 10)
3	60	0	2.2	1	0
				3	50
				6	100
				12	100
5	180	1:12	2.0	1	10
				3	60
				6	100
				12	100
None					0

*3.4 $\log_{10}$ PFU Lassa virus, strain Josiah, SC.
[a]Plasma or plasma dilution inoculated IP on days 0, 3, and 6, after virus inoculation.

TABLE III. Protection of Strain 13 Guinea Pigs* by Convalescent Rhesus Monkey Plasma†

Plasma dilution	LNI	Dead/5 (% survival)	MTD[a] (range)	Viremia – $\log_{10}$ PFU/ml by days (SEM[b])				
				6	9	12	15	19
0	3.6	0	—	<[c]	<	<	<	<
		(100)		(–)	(–)	(–)	(–)	(–)
3	2.5	0	—	<	<	<	<	<
		(100)		(–)	(–)	(–)	(–)	(–)
9	1.2	5	19	1.46	2.98	3.42	3.02	3.00
		(0)	(13–36)	(0.16)	(0.21)	(0.29)	(0.36)	(1.00)
27	0.5	5	19	2.68	3.76	4.02	3.23	3.30
		(0)	(19–21)	(0.26)	(0.11)	(0.10)	(0.13)	(–)
Control	—	5	18	3.72	3.85	4.10	3.65	4.80
		(0)	(16–20)	(0.31)	(0.19)	(0.14)	(0.25)	(–)

*4.0 $\log_{10}$ PFU Lassa virus, strain Josiah, SC.
†6 ml/kg of convalescent rhesus monkey plasma diluted in whole fresh monkey plasma, inoculated IP on days 0, 3, 6.
[a]MTD = Mean day of death.
[b]< = Undetectable, less than 0.7 $\log_{10}$ PFU/0.2 ml.

The degree of protection conferred by immune plasma with LNI ≙ 2 was dose-dependent (Table II). Guinea pigs were treated on days 0, 3, and 6 with either an early convalescent plasma (LNI = 2.2) or a late plasma diluted 1:12 to artificially produce an LNI = 2.0; both immune plasma preparations were tested at various doses (1, 3, 6, and 12 ml/kg). All guinea pigs treated with 6 or 12 ml/kg of either plasma survived. In contrast, only about half of each group treated with 3 ml/kg survived, and almost all guinea pigs treated with 1 ml/kg died. Viremias (not presented) confirmed the expectation that animals receiving higher serum doses sustained lower viremias than animals receiving less plasma.

TABLE IV. Protection of Strain 13 Guinea Pigs by Convalescent Human Plasma*

Lassa[a] virus strain (origin)	Human plasma LNI[b]	Human plasma ml/kg[c]	% Survival (n = 10)	MTD (range)
Josiah	1.6	3	0	17.4 (15–20)
(Sierra Leone)		6	50	26.0 (22–30)
		12	100	—
	—	None	0	18 (15–20)
Z-132	2.8	3	80	24 (22–26)
(Liberia)		6	100	—
		12	100	—
	—	None	0	17.2 (15–19)

*Human plasma (JM) obtained from a convalescent human patient infected with Lassa virus in Liberia.
[a]4.0 $\log_{10}$ PFU Lassa, strain Josiah, or 4.3 $\log_{10}$ PFU Lassa, strain Z-132, inoculated S.C.
[b]LNI versus challenge Lassa virus strain.
[c]ml/kg of undilute plasma inoculated day 0, and repeated days 3 and 6.

To determine if guinea pigs could be used to assess protective efficacy of rhesus monkey Lassa-immune plasma, a plasma dilution experiment analogous to the plan in Table I was performed using a high-titered, late convalescent rhesus plasma whose protective efficacy had also been determined in monkeys [Jahrling, submitted for publication]. Guinea pigs inoculated with undiluted or 1:3 dilutions of rhesus plasma (LNI > 3.6 and 2.5 respectively), 6 ml/kg days 0, 3, and 6, were fully protected, and failed to become viremic (Table III). In contrast, those treated with 1:9 and 1:27 dilutions (LNI = 1.2 and 0.5, respectively) died. Viremias were slightly delayed in the LNI = 1.2 group, and essentially unaffected in the LNI = 0.5 group. These data are very similar to those generated for this same plasma in monkeys, and suggest that guinea pigs may reasonably be substituted for monkeys in efficacy testing of primate plasmas.

Finally, a human plasma was tested for protective efficacy in guinea pigs infected with either of two virulent Lassa virus strains (Table IV). This plasma, obtained from a convalescent patient who was infected with Lassa virus in Liberia, neutralized 1.6 $\log_{10}$ PFU of strain Josiah (the prototype strain from Sierra Leone), and 2.8 $\log_{10}$ PFU of strain Z-132 (a Liberian strain). As predicted by the LNI values, this plasma protected both groups of guinea pigs, but lesser quantities were required to effectively treat Z-132 strain infection.

DISCUSSION

The concept of passive serum transfer for Lassa virus prophylaxis was strongly supported in the present study. The degree of protection conferred to Lassa virus-infected guinea pigs varied with the quantity of neutralizing antibody administered, providing, for the first time, a rational criterion for optimizing therapeutic regimens. Immune plasma obtained from guinea pig donors in early convalescence contained low titers of N antibody (despite high IFA titers), and conferred no protection. Late

convalescent plasma had IFA titers similar to early plasma, but also contained high N antibody titers. Late convalescent plasma protected even when diluted. Plasma or plasma dilutions containing an LNI $\geqslant 2$ were protective, when administered on days 0, 3, and 6, at a dose of 6 ml/kg/treatment. Six ml/kg is approximately the equivalent of 2 units (250 ml) of plasma administered to a 70-kg human patient. Higher-titered plasma or increased volumes of plasma would probably be required to effectively treat Lassa virus infection if initial treatment were delayed after day 0; this is a testable hypothesis.

Immune monkey plasma also conferred protection to guinea pigs; approximately the same minimal titers of N antibody as established for homologous guinea pig plasma were required. In addition, protective efficacy studies of this monkey plasma in monkeys suggest that the minimal protective LNI is approximately 2 [Jahrling, *submitted for publication*]. This observation increases confidence in the LNI as a useful predictive index of protective efficacy, and further suggests that guinea pigs could be used in place of primates for in vivo efficacy testing of primate plasma, including human material.

The protection of guinea pigs with human, Lassa-immune plasma (Table IV) provided direct evidence that human plasma could confer protection. Less plasma was required to effectively treat guinea pigs inoculated with Liberian Lassa strain (Z-132) than with a Sierra Leone strain (Josiah). Higher efficacy against strain Z-132 may well be related to the higher LNI of this human plasma (of Liberian origin) against the homologous Z-132 strain than against the serologically distinct Josiah strain. More definitive proof will require the reciprocal experiment in which we would anticipate that human immune plasma (of Sierra Leonean origin) would protect against Josiah strain infection more effectively than against strain Z-132. Preliminary cross-neutralization data from this laboratory using single-injection guinea pig sera suggest that Lassa strains of different geographic origins are serologically distinct. Lassa strains isolated from Sierra Leone, Liberia, Nigeria, Guinea, Mozambique, Central African Republic, and Rhodesia can all be differentiated from each other on the basis of cross-neutralization [Jahrling, submitted for publication]. In the absence of LNI data, treatment success could probably be enhanced by matching the geographic origin of immune plasma with the origin of the infecting Lassa virus strain. The possibility of antigenic diversity among Lassa virus strains and its impact on cross protection and passive immunization were suggested previously [*Monath et al*, 1974], but further investigation required development of a neutralization test. However, more critical to treatment success than the geographic match of virus and plasma origins may be the selection of plasma with the highest LNI against the infecting strain. Thus the Liberian human immune plasma tested in Table IV, with LNI = 1.6 versus Sierra Leonean Lassa, was more effective in treating this infection than two other plasmas obtained from Sierra Leone (with LNI = 0.8 and 1.1), which protected no guinea pigs inoculated with the Josiah strain [Jahrling, unpublished data].

Low LNI titers to Lassa are typical for human immune plasma samples. Of 130 sera obtained from Lassa-convalescent patients in Liberia (in collaboration with Dr John Frame), only 18 contained LNI > 2 against a Liberian strain, and none contained LNI > 3 [Jahrling, manuscript in preparation]. The generally low LNI encountered in human convalescent plasma may compound the problem of collecting and administering adequate quantities of protective antibody, if LNI > 2 is actually required to treat human patients effectively.

An alternative to the use of marginally titered plasma might be the fractionation and concentration of IgG from whole plasma, using established procedures [Condie, 1979a]. Human IgG prepared in this way has been successfully used to treat life-threatening cytomegalovirus infections in human patients [Condie, 1979b]. We intend to investigate this approach, but recognize that while concentration of immune globulin may circumvent this problem of low N antibody titers, the cost is measured in terms of a total supply reduced by the approximate concentration factor.

Another alternative to collection of large quantities of human plasma is the generation of high-titered antibody in a large laboratory animal, followed by despeciation using techniques presently applied to production of botulism antitoxin in horses [Layton et al, 1972]. Eventually, monoclonal antibodies suitable for human use may be developed with sufficient neutralizing antibody activity to confer protection to human Lassa fever patients.

The guinea pig protection studies reported herein contribute to the development of guidelines for the collection and testing of immune plasma for serotherapy of human Lassa fever. The N antibody titer predicted efficacy more accurately than the IFA test. Early convalescent antisera, in addition to having low LNI and low protective efficacy, frequently contain both IFA antibodies and infectious virus, as reported for guinea pigs [Jahrling et al, 1982], monkeys [Jahrling et al, 1980], and humans [Wulff and Lange, 1975]. Human immune plasma, selected without the benefit of an LNI determination, should be obtained at least three months or more after infection to reduce the hazard of transferring infectious virus and to increase the possibility of obtaining an adequate LNI. Since increased protection was obtained by infusing high doses of marginal sera in guinea pigs, infusion of higher doses of marginally titered human plasma might be considered in the treatment of human Lassa fever.

The guinea pig protection studies clearly demonstrate that plasma therapy can favorably affect the disease course in a model Lassa virus infection. Thus it seems appropriate to continue collecting human immune plasma for controlled therapy trials, screening for infectious virus and testing for N antibody, while searching for alternative practical means to obtain immune globulin with enhanced N antibody titers suitable for use in human patients.

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The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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