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Protection of rhesus monkeys from fatal Lassa fever by vaccination with a recombinant vaccinia virus containing the Lassa virus glycoprotein gene

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ABSTRACT **Lassa fever is an acute febrile disease of West Africa, where there are as many as 300,000 infections a year and an estimated 3000 deaths. As control of the rodent host is impracticable at present, the best immediate prospect is vaccination. We tested as potential vaccines in rhesus monkeys a closely related virus, Mopeia virus (two monkeys), and a recombinant vaccinia virus containing the Lassa virus glycoprotein gene, V-LSGPC (four monkeys). Two monkeys vaccinated with the New York Board of Health strain of vaccinia virus as controls died after challenge with Lassa virus. The two monkeys vaccinated with Mopeia virus developed antibodies measurable by radioimmunoprecipitation prior to challenge, and they survived challenge by Lassa virus with minimal physical or physiologic disturbances. However, both showed a transient, low-titer Lassa viremia. Two of the four animals vaccinated with V-LSGPC had antibodies to both Lassa glycoproteins, as determined by radioimmunoprecipitation. All four animals survived a challenge of Lassa virus but experienced a transient febrile illness and moderate physiologic changes following challenge. Virus was recoverable from each of these animals, but at low titer and only during a brief period, as observed for the Mopeia-protected animals. We conclude that V-LSGPC can protect rhesus monkeys against death from Lassa fever.**

Lassa fever is an acute febrile disease widespread in West Africa, where it has been estimated to cause as many as 300,000 infections per year (1, 2). Although the overall mortality is probably 1–2%, in hospitalized patients it is 16%, and in some hospitals as many as 10–15% of adult medical patients are admitted due to Lassa fever, equaling or surpassing malaria as a cause of hospitalization of adults (3, 4). Lassa fever is commonly seen in children, accounting for 10% of outpatient visits, with a case fatality of 12–15%. The case fatality from Lassa fever virus infection in the third trimester of pregnancy is 30%, with an overall fetal/neonatal loss of >80% (5). Since many of the patients are young adults, the impact of the disease on the community and on the economy is significant.

Most human infections result from contact with the multimammate rat *Mastomys natalensis* or its urine, usually in village housing, which is widely infested with these rodents in Lassa fever-endemic regions of West Africa (1, 2). The rodent is persistently infected for life, excreting large quantities of virus in urine (6). Although person-to-person spread occurs, it appears to be less important. Control of the disease would ideally lie with rodent extermination, or at least prevention of close contact between people and rodents. However, in the context of many West African societies this approach presents some insurmountable practical problems.

The best immediate prospect for control in endemic areas lies with the use of a vaccine. A vaccine prepared from inactivated purified virus affords no protection to rhesus monkeys challenged with a lethal dose of Lassa virus (J.B.M., unpublished data). At present there exists at least one candidate for a live nonpathogenic vaccine. A virus closely related to Lassa virus, Mopeia virus, originally isolated from rodents trapped in Mozambique, is thought not to be pathogenic in non-human primates (7). This virus is immunologically related to Lassa virus and has been used successfully to protect rhesus monkeys from lethal Lassa virus challenge (8). However, since its safety in humans cannot be readily assessed, its potential as a vaccine remains academic.

We recently produced and tested a candidate recombinant vaccinia virus vaccine for Lassa fever (9). A cDNA clone derived from the 5′ half of the small genome RNA of the Josiah strain of Lassa virus and encoding the surface glycoproteins (10) was inserted into the thymidine kinase gene of the New York Board of Health (NYBH) strain of vaccinia virus under control of the $P_{7.5}$ promoter. This recombinant virus, V-LSGPC, was shown to express the Lassa virus glycoprotein precursor, GPC, and its posttranslational cleavage products, GP1 and GP2. Further, guinea pigs vaccinated with this live recombinant virus vaccine were protected from a lethal challenge with Lassa virus. Here we examine the potential of V-LSGPC to protect primates from fatal Lassa infection, which in these animals follows a clinical course virtually identical to severe human Lassa infection (11).

METHODS

Immunization of Animals. Eight adult rhesus monkeys (*Macaca mulatta*) were used. Two (Rh1 and Rh3) received 10^9 plaque-forming units (pfu) of the NYBH strain of vaccinia virus, and four (Rh4, -5, -6, and -8) received the same dose of V-LSGPC. Two (Rh2 and Rh7) more were given 10^4 pfu of Mopeia virus 37 days before challenge. All procedures, including the initial immunizations, were conducted in the biosafety level 4 laboratory at the Centers for Disease Control. All animals were vaccinated with 0.2 ml intradermally in each of four separate sites (each forearm and the lateral aspects of the upper legs). Each site was observed for the development of a typical vaccinia skin lesion and for enlargement of local lymph nodes.

Lassa Virus Challenge. All animals except Rh4 and Rh5 were challenged 37 days after vaccination, with 10^4 pfu of Lassa virus, Josiah strain, in 0.5 ml, subcutaneously. Rh4 and Rh5 were challenged 284 days after vaccination, with 10^4 pfu

Abbreviations: NYBH, New York Board of Health; V-LSGPC, recombinant vaccinia virus expressing the Lassa glycoprotein gene; RIP, radioimmunoprecipitation; pfu, plaque-forming unit(s); ATIII, antithrombin III; VIIIrAg, factor VIII-related antigen; GP1 and GP2, Lassa glycoproteins 1 and 2; NP, nucleoprotein.

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of Lassa virus, Josiah strain, in 0.5 ml, intramuscularly. The animals were then monitored daily for general health and food and water intake, and on alternate days temperature and weight were measured and a close physical examination was performed. All animals were bled regularly, as previously described (11), for routine hematologic studies; assays of platelet function, antithrombin III (ATIII) and factor VIII-related antigen (VIIIrAg), coagulation, and viremia; antibody studies; and clinical biochemistry. Tissue samples were obtained by open biopsies of an enlarged axillary lymph node and of liver and spleen from animal Rh6, 119 days after challenge, under general anesthesia.

Virology and Immunology. Virus titers were obtained by plaque titration, and virus-specific total and IgM antibodies were determined by indirect immunofluorescence assay (12). The specificity of response to the proteins of Lassa virus was determined by radioimmunoprecipitation (RIP) and immunoblot methods (13–16). The ability of sera to neutralize Lassa virus was measured by a plaque-reduction assay (17).

Attempts at cocultivation of virus from tissues taken at open biopsy were made by incubating fresh triturated lymph node, liver, and spleen tissue and leukocyte suspensions separated on LSMR Lymphocyte Separation Medium (Organon) with monolayers of Vero E-6 cells. These cultures were passed once and probed with fluorescently labeled anti-Lassa virus monoclonal antibodies.

Hematology and Chemistry. Hemoglobin, leukocyte and erythrocyte counts, and hematocrit were determined with a Hycel 555 cell counter (Boehringer Mannheim). Differential leukocyte counts and platelet counts were determined manually. Blood biochemistry studies were performed with Gilford diagnostics kits and a model 250 spectrophotometer (Gilford).

Platelet Function Studies. Blood (5 ml) was drawn into 0.11 M sodium citrate and centrifuged at 180 × g for 10 min to obtain platelet-rich plasma, which was then adjusted to 200,000 cells per ml with platelet-poor plasma. The response of these platelets to a standard dose (206 μM) of ADP (Sigma) was obtained by using a PAP-4 Platelet Profiler (Bio-DATA, Hatboro, PA). Slopes, percent aggregation, and traces were recorded.

Coagulation Studies. Prothrombin and partial thromboplastin times were recorded with an Electra 750 (Medical Laboratory Automation, Mount Vernon, NY) and reagents from Dade Diagnostics (American Scientific Products, Stone Mountain, GA). ATIII and VIIIrAg were measured by gel immunodiffusion techniques incorporating monoclonal antibody to ATIII and to VIIIrAg (Behring Diagnostics). This test was standardized with human reference serum, normal monkey serum, and commercial standards (Thromboscreen, Pacific Hemostasis, Ventura, CA) and adjusted to obtain mid-range readings for monkey sera (18).

Autopsy. Full postmortem examinations were performed on all animals, and tissues were taken for virus isolation from animals Rh1 and Rh3 at death, 12 and 15 days after challenge, respectively. Rh4 and Rh5 were electively sacrificed 31 days after challenge; Rh2, 207 days after challenge; and Rh6, -7, and -8, 251 days after challenge; liver, spleen, lymph nodes, and adrenals were taken for virus isolation.

RESULTS

The two animals inoculated with the NYBH strain of vaccinia virus and the four given V-LSGPC developed a vesicular lesion typical of vaccinia virus, limited to the site of inoculation except for one satellite lesion each in monkeys Rh6 and Rh8. The lesions resulting from V-LSGPC inoculation were 2–3 mm in size, compared to the 4- to 6-mm lesions resulting from vaccination with the NYBH strain. This is consistent with vaccinia attenuation produced by the thymidine kinase-negative phenotype of the recombinant virus (19). All lesions healed within 17 days. All six animals developed palpable axillary and inguinal nodes proximal to all four sites 2 days after inoculation; these subsided within 17 days. None of the

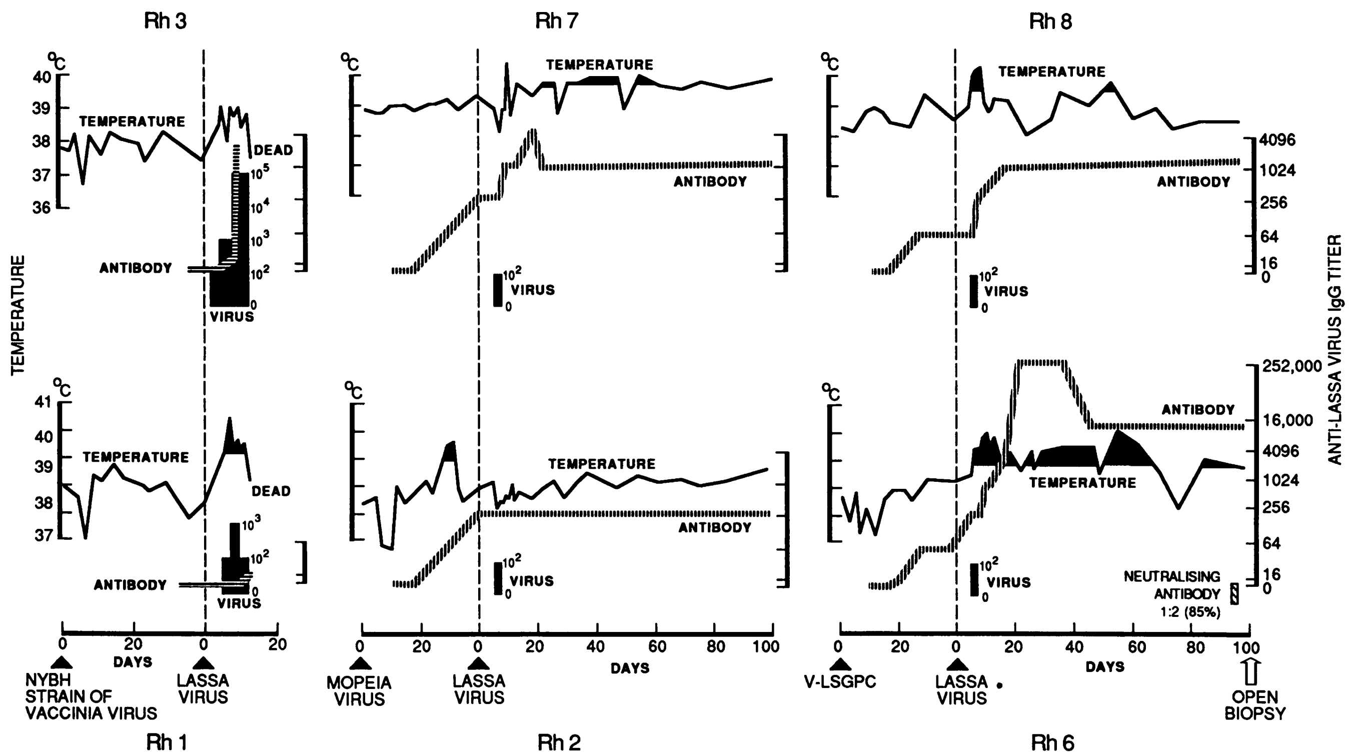

FIG. 1. Temperature, antibody, and viremia in rhesus monkeys following vaccination with the NYBH strain of vaccinia, Mopeia virus, or V-LSGPC and challenge with Lassa virus.

four V-LSGPC-vaccinated animals developed constitutional signs or laboratory abnormalities after receipt of V-LSGPC.

The two animals that had received the NYBH strain died 12 and 15 days after challenge. In previous experiments using the same strain and passage level of Lassa virus in eight unprotected rhesus monkeys, all died after challenge (ref. 11 and J.B.M., unpublished observations). The two animals vaccinated with Mopeia virus and the four that received V-LSGPC prior to challenge survived, though all six showed signs of mild to moderate illness due to Lassa infection, and all six had a brief viremia with Lassa virus.

Fig. 1 shows clinical and virological course in the animals who received the NYBH strain. Monkey Rh1 became febrile 5 days after challenge, and Rh3, 6 days after challenge. Rh1 deteriorated rapidly and died on day 12, and Rh3 survived until day 15, hypotensive with a thready pulse, rapid respiratory rate, and facial edema. Both animals had begun to mount a primary antibody response to Lassa virus before death.

In contrast, the animals who received the Mopeia virus withstood the Lassa challenge with little or no signs of constitutional illness (Fig. 1). One (Rh7) was febrile intermittently between days 9 and 25 postchallenge and continued to have episodes of low-grade fever for the next 3 months. Both animals had antibody to Lassa and Mopeia viruses after vaccination and secondary-type response after challenge. Despite this, Lassa virus was recovered from both animals on one occasion: at low titer on day 9 from monkey Rh2 and on day 7 from Rh7 (Table 1). Virus was not isolated from any other samples. Both animals continued in good health, but Rh2, an elderly female, began to deteriorate 200 days postchallenge and at autopsy 208 days postchallenge was found to have disseminated uterine carcinoma.

The four animals that received V-LSGPC followed an intermediate course. Fig. 1 illustrates this for Rh6 and Rh8. All four animals mounted a brisk secondary antibody response to Lassa virus following challenge, but despite this, Lassa virus was isolated in low titer from all four on at least one occasion between days 7 and 9 postchallenge (Table 1). The antibody response to GP1 and GP2 of Lassa virus was observed by RIP assay for two animals vaccinated with Mopeia and two with V-LSGPC. Although some nonspecific binding comigrated with GP1 and GP2, the V-LSGPC-vaccinated animals had a marked increase in staining in these bands postchallenge. There was a similar but smaller increase for Mopeia-vaccinated monkeys (data not shown).

All four V-LSGPC-vaccinated monkeys became febrile 6–7 days after challenge. Rh6 and Rh8 had mild anorexia but Rh4 and Rh5 continued to eat well. Three of these animals became afebrile by day 10 after challenge, at which time virus could no longer be recovered from any of the four animals. However, Rh6 continued to have intermittent low fever for 3 months. All attempts to recover virus over the next 3 months failed. Rh4 and Rh5 were in good health, aviremic and afebrile, when sacrificed on day 31, as were Rh6 and Rh8 when sacrificed 320 days after challenge. We were unable to recover virus from liver, spleen, adrenal, and kidney from biopsy or autopsy material from any of the animals.

Table 1. Lassa virus titers on days ≥7 postchallenge

		Titer,* log(pfu/ml)										
Vaccine	Monkey	7	8	9	10	11	12	13	15	17	18	>21
NYBH	Rh1	2		3		2	†					
	Rh3	2		3		3	4	5	†			
Mopeia	Rh2	0		2		0	0	0			0	0
	Rh7	2		0		0	0	0			0	0
V-LSGPC	Rh6	2		0		0	0	0			0	0
	Rh8	2		0		0	0	0			0	0
	Rh4	3	0	2	0	0	0		0	0		0
	Rh5	0	2	0	0	0	0		0	0		0

*Values <0.301 are given as 0.
†Monkey died.

Animals immunized with Mopeia and V-LSGPC developed antibodies to Lassa Josiah GP1 and GP2 before challenge, at which time only the Mopeia animals, as would be expected, had antibodies to the viral nucleoprotein (NP). The animals vaccinated with V-LSGPC exhibited an increase in antibodies by RIP specific for GP1 and GP2 of Lassa virus after challenge, but the animals vaccinated with Mopeia and NYBH viruses did not. At this time antibody to NP became detectable in the V-LSGPC-vaccinated animals.

Repeated attempts failed to detect neutralizing antibodies by a constant serum, varying virus dilution, log(neutralization index) technique (17), at a serum dilution of more than 1:2 until 46 days postchallenge (Table 2). A plaque reduction of 80% at a serum dilution of 1:2, which is the minimum required for demonstration of neutralizing activity, was observed in three surviving animals. No serum from any of the animals after vaccination and prior to challenge had any neutralizing activity.

Hematologic and clinical chemistry findings in the monkeys that died of Lassa fever (Rh1 and Rh3) were consistent with those previously reported for Lassa fever in rhesus monkeys and in severely ill human patients (11). Fig. 2 shows that after early lymphopenia, neutrophilia supervened, most markedly in the animal surviving to day 15 (Rh3). The two Mopeia-immunized monkeys had little or no change in baseline hematology, though both briefly had atypical lymphocytosis, and one (Rh7) had persistent lymph node en-

Table 2. Plaque-reduction assays of monkey sera for neutralizing antibodies to Lassa virus

			% reduction†	
Vaccine	Monkey	Time,* days	1:2	1:20
NYBH	Rh1 or Rh3	0 and 29 PV	–	–
		21 PC	–	–
Mopeia	Rh2	0 and 29 PV	–	–
		21 PC	–	–
		98 PC	50	–
	Rh7	0 and 29 PV	–	–
		21 and 46 PC	–	–
		98 PC	50	50
V-LSGPC	Rh6	0 and 29 PV	–	–
		21 PC	–	–
		25 PC	50	–
		46 PC	50	50
		98 PC	80	50
	Rh8	0 and 29 PV	–	–
		21 PC	–	–
		98 PC	50	–
	Rh4	0, 68, 115, and 282 PV	–	–
		7 PC	–	–
		21 PC	80	50
	Rh5	0, 68, 115, and 282 PV	–	–
		7 PC	–	–
		21 PC	80	80

*PV, postvaccination; PC, postchallenge; day 0 PV, prevaccination.
†Sera were assayed at dilutions of 1:2 and 1:20. See ref. 17 for details of assay; –, <50% plaque reduction; 50%, not significant; 80%, significant.

largement up to 3 months after challenge. The changes in the V-LSGPC-immunized animals were the most pronounced of the surviving monkeys (Fig. 2). These had early lymphopenia consistent with Lassa virus infection, and monkey Rh6 then developed a moderate neutrophilia and a marked lymphocytosis with atypical lymphocytes. This animal then went on to have a persistent lymphocytosis concurrent with palpably and visibly enlarged axillary lymph nodes. Liver, spleen, and lymph node biopsy material from this animal on day 96 postchallenge was negative for virus isolation.

As previously observed (11), platelet counts remained within normal limits in the unprotected animals, but the *in vitro* response to ADP was depressed (Fig. 3). We detected no abnormalities of platelet function in the Mopeia-immunized monkeys, whereas the V-LSGPC-immunized monkeys demonstrated transient mild depression of aggregation responses at the time of viremia. Prothrombin times remained normal in all animals, but partial thromboplastin times were prolonged in the unprotected animals and normal in four surviving animals (Fig. 3). ATIII levels fell in all animals. There was a sharp rise in VIIIrAg in six animals following challenge.

DISCUSSION

We have shown that a recombinant vaccinia virus vaccine expressing the glycoprotein gene of Lassa virus can protect rhesus monkeys against death from a lethal challenge of Lassa virus. Recombinant vaccines have been shown to protect smaller animals used as disease models for respiratory syncytial virus, influenza, and rabies (19–21); however, our results demonstrate protection of primates by a live recombinant vaccine from an otherwise uniformly fatal disease. These data extend the success of this recombinant vaccine in guinea pigs (9) and contrast with the failure of a killed vaccine to provide any protection, despite eliciting measurable antibodies, as determined by immunoblot and RIP, to all the viral proteins prior to challenge. The marked similarity of human and primate Lassa fever, and a mortality approaching 100% in rhesus monkeys infected with this dose and strain of Lassa virus, makes the protection highly significant (11, 17, 18).

We were also able to establish, as expected, that V-LSGPC was not pathogenic in the rhesus monkey, and this virus is now handled in biosafety level 2 animal facilities. Despite the low pathogenicity, Mopeia virus remains classified as a biosafety level 4 agent.

Hematologic and pathophysiologic observations in the two animals that died following the Lassa challenge were in accord with the abnormalities previously reported in unvaccinated primates infected with Lassa virus (12). In all eight animals the levels of the acute-phase reactant VIIIrAg rose and the levels of ATIII fell. Both the Mopeia virus-vaccinated and the V-LSGPC-vaccinated animals developed a mild fever and a brief and low-titer viremia. All four V-LSGPC-vaccinated animals made a complete recovery, but they did develop "mild" Lassa fever, previously undescribed in monkeys, in whom infection is usually fatal within 20 days of challenge. They demonstrated mild but nevertheless measurable abnormalities of acute Lassa virus infection: increased serum glutamic-oxaloacetic transaminase (data not shown), depressed platelet function (Fig. 3), and lymphopenia and neutrophilia (Fig. 2). These changes were absent in the Mopeia-immunized monkeys, suggesting that despite the brief viremia, they were spared the physiological processes of Lassa fever and thus, by definition, illness. Epitopes of NP and GP2 of Mopeia virus are shared by Lassa virus (J.B.M., unpublished observations); however, despite its closely related antigenicity, Mopeia virus appears to lack epitopic determinants responsible for eliciting a fully protective response to Lassa virus. It is not possible to draw conclusions from this experiment on the relative contribution of the separate products of the *S* gene (NP, GP1, and GP2).

Serum antibodies to Lassa virus do not usually neutralize Lassa virus *in vitro* in a classical replication-inhibition, serum dilution neutralization assay (18). We were able to demon-

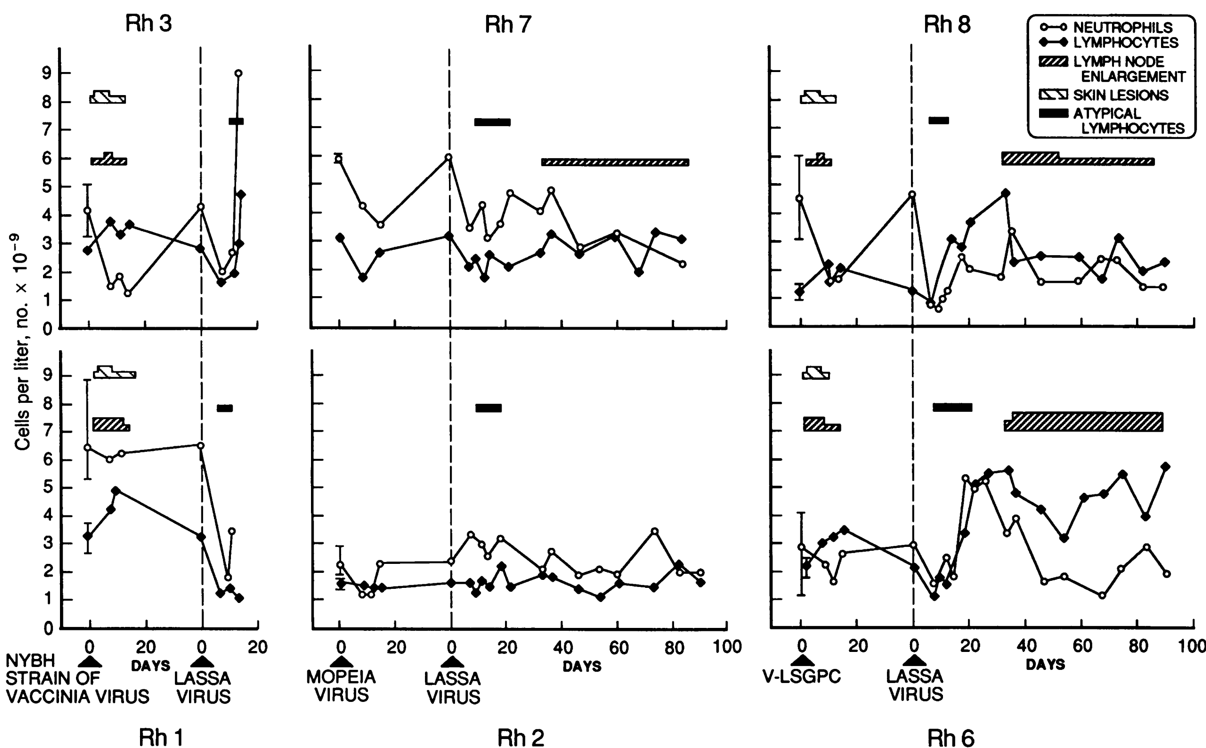

FIG. 2. Differential leukocyte responses and lymph node enlargement in rhesus monkeys following vaccination with the NYBH strain of vaccinia, Mopeia virus, or V-LSGPC and challenge with Lassa virus. Error bars indicate the preimmunization range.

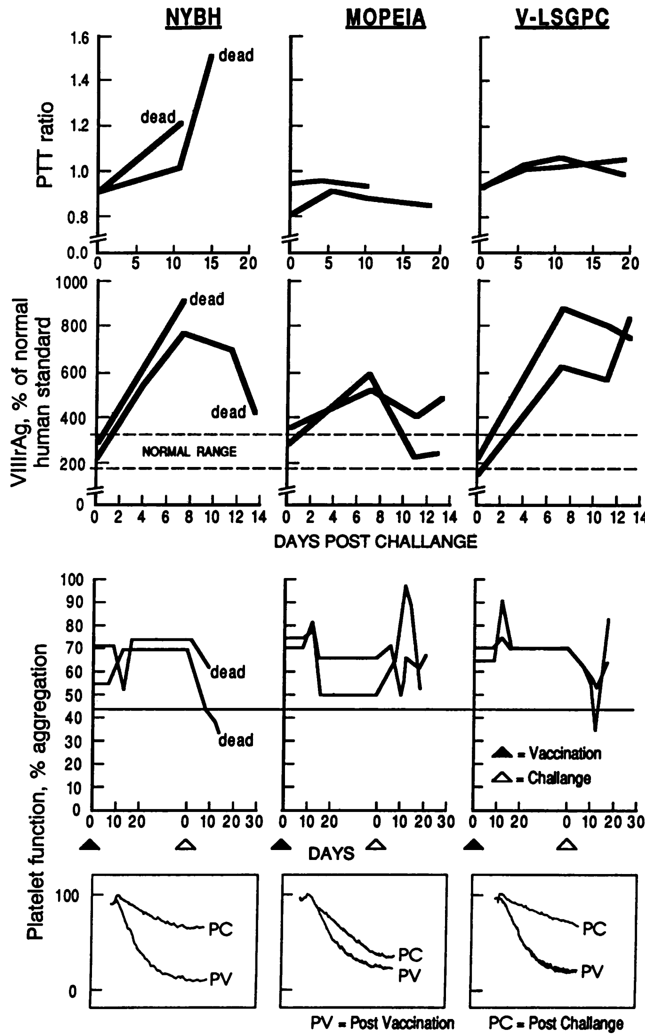

FIG. 3. Partial thromboplastin time (PTT) ratios, VIIIrAg, and platelet function following challenge with Lassa virus in rhesus monkeys vaccinated with the NYBH strain of vaccinia, Mopeia virus, or V-LSGPC.

strate neutralizing antibody by a fixed serum, varying virus dilution technique in only a few samples between 21 and 97 days postchallenge, at a time when the antibody titer by indirect immunofluorescence assay was as much as 1: 250,000, and when all animals had long since cleared virus from their serum. None of the prechallenge specimens had any neutralizing activity. We conclude that neutralizing antibody does not play any role in clearance of virus from serum in acute infection and that it is unlikely to play a major role in protection from challenge. It is possible, however, that were we able to induce neutralizing antibodies by immunization, full protection from challenge might have been achieved.

Lassa virus infects the neonatal mastomys, in which it establishes persistent infection for life with profuse excretion of virus in urine and immunologic tolerance in the face of virus-specific antibody. In humans a brisk primary antibody response of both the IgG and the IgM class is invariably observed. However, virus persists in serum and occasionally in urine for up to 3 months. The persistence of fever and lymphadenopathy, particularly in Rh6, led us to perform open biopsy to obtain tissue for virus isolation. We have not detected persistence of Lassa virus in these animals.

It may be that in this experiment the high infectious dose of Lassa virus used in the challenge was great enough to overcome complete protection by any immunization schedule. (The challenge was selected empirically when the primate fatal Lassa fever model was established, and the shortage and expense of these animals has not permitted establishment of outcome with lower challenge doses.) Alternatively, it may be that primary infection with Lassa virus itself, with its capacity to establish latent infections and immunotolerance, does not result in solid protection from reinfection, though it does seem likely from previous observations in humans that protection from significant clinical disease during reinfection follows recovery from a primary infection (J.B.M., unpublished observations). These observations are consistent with the idea that the cell-mediated immune response is responsible both for virus clearance and for protection against subsequent reinfection, and any vaccine candidate must be able to induce this type of response. Reliance on the cell-mediated immune response for protection depends on expansion of Lassa virus-specific T-cell clones following infection, and the time required for recruitment of sufficient cells may not allow abolition of the primary viremia. If so, then it may be that complete protection, in the sense of preventing any virus replication, is neither possible nor desirable for such a vaccine, which depends on the cellular immune response for protecting the vaccinee.

Our results suggest that V-LSGPC should be considered as a candidate recombinant vaccinia virus vaccine for human use in Lassa-endemic areas where there are new vaccinia-susceptible populations since the discontinuation of the smallpox eradication program. In the rural areas of West Africa where Lassa is a major cause of morbidity and mortality, the potential hazards of the vaccine may be outweighed by the enormous benefits of prevention to people in poor living conditions with little access to medical care, and where such measures as large-scale rodent control are currently unrealistic.

1. McCormick, J. B., Webb, P. A., Krebs, J. W., Johnson, K. M. & Smith, E. S. (1987) *J. Infect. Dis.* **155,** 437–444.
2. McCormick, J. B. (1987) *Curr. Top. Microbiol. Immunol.* **134,** 69–78.
3. McCormick, J. B., King, I. J., Webb, P. A., Johnson, K. M., O'Sullivan, R., Smith, E. S., Trippel, S. & Tong, T. C. (1987) *J. Infect. Dis.* **155,** 445–455.
4. McCormick, J. B., King, I. J., Webb, P. A., Scribner, C. L., Craven, R. B., Johnson, K. M., Elliott, L. H. & Williams, B. (1986) *N. Engl. J. Med.* **314,** 20–26.
5. Price, M. J., Fisher-Hoch, S. P., McCormick, J. B. & Craven, R. B. (1988) *Br. Med. J.* **297,** 584–587.
6. Monath, T. P., Newhouse, V. F., Kemp, G. E., Setzer, H. W. & Cacciopouti, A. (1974) *Science* **185,** 263–265.
7. Wulff, H., McIntocsh, B. M., Hammer, D. B. & Johnson, K. M. (1977) *Bull. W.H.O.* **55,** 441–447.
8. Kiley, M. P., Lange, J. V. & Johnson, K. M. (1979) *Lancet* **ii,** 738.
9. Auperin, D. D., Esposito, J. J., Lange, J. V., Bauer, S. P., Knight, J., Sasso, D. R. & McCormick, J. B. (1988) *Virus Res.* **9,** 233–248.
10. Auperin, D. D., Sasso, D. R. & McCormick, J. B. (1986) *Virology* **153,** 155–157.
11. Fisher-Hoch, S. P., Mitchell, S. W., Sasso, D. R., Lange, J. V., Ramsey, R. & McCormick, J. B. (1987) *J. Infect. Dis.* **155,** 465–474.
12. McCormick, J. B. (1988) *Manual of Clinical Microbiology*, ed. Fenner, X. (Springer, New York), in press.
13. Kessler, S. W. (1975) *J. Immunol.* **115,** 1617–1624.
14. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
15. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
16. Tsang, V. C. W., Peralta, J. M. & Simons, A. F. (1983) *Methods Enzymol.* **92,** 377–391.
17. Jahrling, P. B., Peters, C. J. & Stephens, E. L. (1984) *J. Infect. Dis.* **149,** 420–427.
18. Walker, D. H., Johnson, K. M., Lange, J. V. & Murphy, F. A. (1975) *Bull. W.H.O.* **52,** 523–534.
19. Olmstead, R. A., Narayanasamy, E., Prince, G. A., Murphy, B. R., Johnson, P. R., Moss, B., Chanock, R. M. & Collins, P. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7462–7466.
20. Smith, G. L., Murphy, B. R. & Moss, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7155–7159.
21. Wiktor, T. J., Macfarlan, R. I., Reagan, K. J., Dietzschold, B., Curtis, P. J., Wunner, W. H., Kieny, M.-P., Lathe, R., Lecocq, J.-P., Mackett, M., Moss, B. & Koprowski, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7194–7198.