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Protection against Yellow Fever in Monkeys by Immunization with Yellow Fever Virus Nonstructural Protein NS1

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Immunization of monkeys with yellow fever virus-specified nonstructural protein NS1 resulted in protection against fatal hepatitis as well as marked reduction in the magnitude of virema after subcutaneous challenge with yellow fever virus. The results may be relevant to the design of possible subunit or recombinant flavivirus vaccines.

The virus-specified nonstructural protein gp48, currently designated NS1 (7), is highly conserved among flaviviruses. It is expressed on the surfaces of infected cells and is probably the complement-fixing (CF) antigen (1, 10). We and others have recently shown that monoclonal antibodies to NS1 of the 17D vaccine strain of yellow fever virus (17D YF) confer passive protection in mice against lethal intracerebral challenge with 17D YF (6, 9). The protective capacity of monoclonal antibodies to NS1 correlated with their CF activity and with their ability to lyse virus-infected cells (9). It was further shown that active immunization with NS1 conferred solid protection in the absence of detectable neutralizing or other anti-virion antibodies. Because these findings may be relevant to the design of possible subunit or recombinant flavivirus vaccines, we extended our studies to immunization of monkeys against subcutaneous challenge with wild YF. Protection in monkeys against the wild strain of YF has previously been accomplished only by immunization with live-virus vaccines.

Domestic rhesus monkeys (6 to 10 kg) were prescreened for the absence of anti-flavivirus antibody by hemagglutination inhibition and complement fixation testing against dengue (serotypes 1 through 4), St. Louis encephalitis, Japanese encephalitis viruses, and YF and by neutralization assay against YF. Immunoaffinity-purified NS1 was prepared from lysates of 17D YF-infected Vero cells as previously described (9). Five rhesus monkeys were given combined intradermal and subcutaneous injections of NS1 adsorbed to an alum adjuvant (Alhydrogel; Accurate Chemical and Scientific Corp., Westbury, N.Y.). Four monkeys injected in parallel with ovalbumin in alum served as controls. Animals were injected initially with 100 μg and given booster injections of 50 μg of NS1 or ovalbumin at 3 and at 7 weeks; they were challenged 1 to 2 weeks after the second booster injection by subcutaneous inoculation of a lethal dose (375 to 700 PFU) of African strain Dakar 1279 YF. Subcutaneous inoculation of 50 to 200 PFU of this virus is uniformly lethal for rhesus monkeys. Serum anti-NS1 antibody responses were measured by an enzyme-linked immunoassay (9) with a goat anti-monkey immunoglobulin G detector and by a standard complement fixation assay (2). Anti-virion antibody was measured by plaque reduction neutralization assay of Dakar 1279 YF in Vero cells in the presence of fresh frozen human plasma (3, 5) and by conventional hemagglutination inhibition assay (4). The viral protein specificity of immune serum was determined by radioimmunoprecipitation and sodium dodecyl sulphate-polyacrylamide gel electrophoresis by using previously described methods (8).

There were no apparent ill effects of hyperimmunization with NS1. High-titer (1:1,000 to 1:10,000) anti-NS1 antibody was detected by enzyme-linked immunoassay within 2 weeks of the initial injection and reached sustained high levels (1:40,000 to 1:300,000) after the first booster injection in all NS1-immunized monkeys, with marginal or no increase after the second booster injection. Sera from control monkeys were uniformly negative (<1:10) by enzyme-linked immunoassay. Changes in anti-NS1 antibody titer were paralleled by changes in CF antibody levels. CF and neutralizing antibody titers are shown (Table 1). Serum from control monkey X673 was slightly anti-complementary. Of the five NS1-immunized monkeys, three had no detectable neutralizing antibody (no plaque reduction in Vero cells at 1:2 dilution), but neutralization (90% plaque reduction) was observed at a 1:32 dilution of the prechallenge serum of monkeys 15 and 503C. No hemagglutination inhibition activity (<1:10) was detected in any of the prechallenge sera. Sera taken immediately prechallenge with YF gave strong precipitin bands with NS1 as determined by radioimmunoprecipitation and polyacrylamide gel electrophoresis (Fig. 1). The fainter precipitin bands with E protein that were observed are similar to those previously noted with monoclonal antibodies to NS1 (8) and with the 8G4 antibody to NS1 on the same gel.

A consistent pattern of high-grade viremia and evolution of clinical signs followed infection of the control monkeys. Fever appeared by postchallenge day 3 followed by listlessness, progressive jaundice, hemorrhage, striking elevations of serum glutamic pyruvic transaminase levels (2,170 to 7,960 U/ml; normal range, 5 to 45 U/ml), and prolonged prothrombin times (26 to 60 s; normal, 11 to 16 s). All four control monkeys died, as expected, whereas four of the five immunized monkeys survived ($P < 0.04$, Fisher's exact test). Death occurred on day 5 or 6, and massive liver necrosis was found at autopsy. Of the surviving monkeys, monkey 15 had no detectable viremia and apart from a transient slight increase in serum glutamic pyruvic transaminase level (62 U/ml) did not exhibit any clinically apparent disease. Similar degrees of mild illness with fever, poor feeding, and evidence of hepatitis as determined by slight elevations of serum glutamic pyruvic transaminase

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TABLE 1. Protection against yellow fever in rhesus monkeys by immunization with NS1

Monkey no.	Prechallenge serum titer (reciprocal) by:		Viremia[a] on postchallenge day:									Outcome
	CF test	PRNT[b]	1	2	3	4	5	6	7	8	9	
NS1 immunized												
V268	256	<2	0[c]	0	0	4.3	4.3	0	0	0	ND[d]	Alive
15	64	32	0	0	0	0	0	0	0	0	ND	Alive
11	32	<2	0	0	0	3.5	5.2	4.8	2.8	0	0	Alive
503C	128	32	0	0	0	3.3	4.8	0	0	0	0	Alive
957	64	<2	0	0	4.3	6.8	8.3	10.0[e]				Dead
Ovalbumin immunized												
503	<8	<2	0	0	3.0	4.0	8.0	3.0[e]				Dead
X673	16	<2	0	0	3.3	6.0	7.7	8.8[e]				Dead
W272	<8	<2	0	0	4.4	7.4	9.5[e]					Dead
12	<8	<2	0	0	5.1	7.9	9.3[e]					Dead

[a] Log_{10} PFU per ml of serum.
[b] PRNT, Plaque reduction neutralization test against Dakar 1279 YF.
[c] No plaques detected in undiluted serum.
[d] ND, Not done.
[e] Death.

levels (138 to 223 U/ml) but normal prothrombin times were observed in the other three surviving NS1-immunized monkeys. Viremia in these three monkeys was delayed by 1 day in onset, and peak titers were uniformly lower than those in control monkeys, averaging 10,000-fold less (Table 1). Surviving monkeys developed neutralizing antibody at high titers (1:1,024 1:20,480), confirming replication of the challenge virus. There was no correlation between pre- and postchallenge neutralizing antibody titers.

These experiments provide evidence that protection in monkeys against lethal YF infection was achieved by immunization with a purified nonstructural, virus-specified protein. The presence of low-titer neutralizing antibody to Dakar 1279 YF in the prechallenge serum of two of the five immunized monkeys raises the possibility of contamination with small amounts of YF envelope protein (E) in the NS1 preparation used to immunize or of antigenic cross-reactivity between E and NS1, or both. We have been unable to detect E in our NS1 preparations by polyacrylamide gel electrophoresis or by radioimmunoprecipitation with polyclonal mouse antibody to 17D YF or by monoclonal antibodies to E, both of which precipitate E from 17D YF-infected cell lysates (8, 9). We were also unable to detect E protein in the NS1 preparations by enzyme-linked immunoassay with monoclonal antibodies to E. The possibility of trace amounts of E in the NS1 preparations cannot be ruled out with certainty by these methods. We think it likely, however, that protection was mediated predominantly by CF antibody to NS1 rather than by biologically active antibody to E alone, since survival in two of four monkeys could not be attributed to neutralizing antibody and CF antibody was present in all monkeys. It is uncertain whether the low-titer neutralizing activity present in two NS1-immunized monkeys provided a protective level of immunity. The results are concordant with our previous findings in mice in which protection was conferred by active immunization with NS1 in the absence of any detectable anti-virion antibody and also by passive immunization with monoclonal antibodies to NS1 (9). In the latter instance, protective capacity correlated with complement fixation titers and with the ability of antibodies to lyse 17D YF-infected cells. Together, the evidence suggests that protection is probably mediated by CF antibodies to NS1 that effect lysis of YF-infected cells bearing surface NS1. The possibility that other immune mechanisms are involved in the protective response to NS1 vaccination remains to be explored.

Yellow fever in monkeys induced by systemic challenge with the virus should provide a more suitable model than intracerebral inoculation of mice for evaluation of potential subunit YF vaccines in humans. That protection was incomplete may reflect the limits of effectiveness of immunization with NS1 or it may reflect the extreme susceptibility of monkeys to fulminant hepatitis when infected with this virus. It is possible that more effective immunization might be achieved with a homologous Dakar YF NS1 preparation. The structural and antigenic relatedness among flaviviruses makes it likely that information gained from these studies can be exploited for the development of similar vaccines against other flaviviruses.

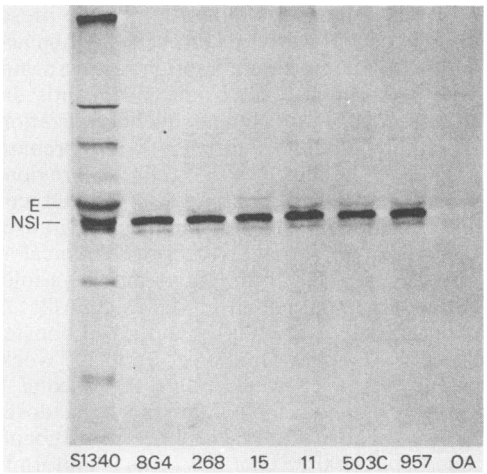

FIG. 1. Immunoprecipitation of [^{35}S]methionine-labeled 17D YF-specific proteins by prechallenge sera from NS1-immunized and ovalbumin-immunized control rhesus monkeys. Proteins were electrophoresed on an 11% linear sodium dodecyl sulfate-polyacrylamide gel. Labeled 17D YF-infected Vero cell lysates precipitated with: pooled sera from five monkeys immunized with standard 17D YF vaccine (S1340); anti-NS1 monoclonal antibody 8G4; prechallenge serum from NS1-immunized monkeys 268, 15, 11, 503C, 957; pooled prechallenge serum from ovalbumin-immunized control monkeys (OA).

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LITERATURE CITED

1. **Cardiff, R. D., and J. K. Lund.** 1976. Distribution of dengue-2 antigens by electron immunocytochemistry. Infect. Immun. **13**:1699–1709.
2. **Casey, H. L.** 1965. Standardized diagnostic complement-fixation method and adaptation to micro test. Public Health monograph no. 74, U.S. Public Health Service publication no. 1228. U.S. Public Health Service, Washington, D.C.
3. **Chappell, W. A., D. R. Sasso, R. F. Toole, and T. P. Monath.** 1971. Labile serum factor and its effect on arbovirus neutralization. Appl. Microbiol. **21**:79–83.
4. **Clarke, D. H., and J. Casals.** 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. **7**:561–573.
5. **Earley, E., P. H. Peralta, and K. M. Johnson.** 1967. A plaque neutralization method for arboviruses. Proc. Soc. Exp. Biol. Med. **125**:741–747.
6. **Gould, E. A., A. Buckley, A. D. T. Barrett, and N. Cammack.** 1986. Neutralizing (54K) and non-neutralizing (54K and 48K) monoclonal antibodies against structural and non-structural yellow fever virus proteins confer immunity in mice. J. Gen. Virol. **67**:591–595.
7. **Rice, C. M., E. M. Lenches, S. R. Eddy, S. J. Shin, R. L. Sheets, and J. H. Strauss.** 1985. Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. Science **229**:726–733.
8. **Schlesinger, J. J., M. W. Brandriss, and T. P. Monath.** 1983. Monoclonal antibodies distinguish between wild and vaccine strains of yellow fever virus by neutralization, hemagglutination inhibition, and immune precipitation of the virus envelope protein. Virology **125**:8–17.
9. **Schlesinger, J. J., M. W. Brandriss, and E. E. Walsh.** 1985. Protection against 17D yellow fever encephalitis in mice by passive transfer of monoclonal antibodies to the nonstructural glycoprotein gp48 and by active immunization with gp48. J. Immunol. **135**:2805–2809.
10. **Smith, G. W., and P. J. Wright.** 1985. Synthesis of proteins and glycoproteins in dengue type 2 virus-infected Vero and *Aedes albopictus* cells. J. Gen. Virol. **66**:559–571.