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ARMY PROJECT ORDER NO: 90PP0813

TITLE: DEVELOPMENT OF SAFE, EFFECTIVE VACCINES FOR DENGUE VIRUS DISEASE BY RECOMBINANT BACULOVIRUS

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Department of Health & Human Services
Bethesda, Maryland 20892

REPORT DATE: August 25, 1992

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and
Development Command, Fort Detrick
Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	25 August 1992	Annual Report (1 Mar 91-28 Feb 92)

4. TITLE AND SUBTITLE
Development of Safe, Effective Vaccines for Dengue Virus Disease by Recombinant Baculovirus

5. FUNDING NUMBERS
Army Project Order 90PP0813

61102A
3M161102BS13.AA.045
WUDA335461

6. AUTHOR(S)
Ching Juh Lai, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
National Institutes of Health (NIH)
Department of Health & Human Services
Bethesda, Maryland 20892

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Outbreaks and major epidemics of dengue continue to afflect human populations, especially in the tropical and subtropical regions of the world. Dengue viruses, transmitted predominantly by mosquito species of the Aedes genus, include four serotypes that are distinguishable by plaque reduction--neutralization with type specific monoclonal antibodies. Dengue is characterized by fever, rash, severe headache, and joint pain. Its mortality rate is low. However, over the past few decades, a more severe form of dengue, characterized by hemorrhage and shock has been observed with increasing frequency in children and young adults. This severe form of dengue has a high mortality rate. Despite years of intensive research, an effective vaccine is still not available for prevention of dengue infection. For these reasons, research on dengue viruses and development of safe and effective vaccines have been given a high priority by the World Health Organization.

14. SUBJECT TERMS
Dengue Virus; Genetic Engineering; Recombinant DNA; Vaccines; Biotechnology; Diseases; ID; RA I

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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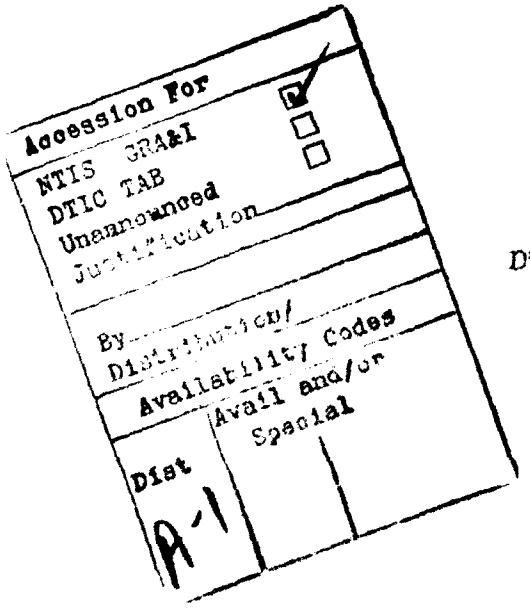
Ching Juh Lai Aug 25, 92

Principal Investigator's Signature Date



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INTRODUCTION

Outbreaks and major epidemics of dengue continue to afflict human populations, especially in the tropical and subtropical regions of the world. Dengue viruses, transmitted predominantly by mosquito species of the Aedes genus, include four serotypes that are distinguishable by plaque reduction--neutralization with type specific monoclonal antibodies (1,2). Dengue is characterized by fever, rash, severe headache, and joint pain. Its mortality rate is low. However, over the past few decades, a more severe form of dengue, characterized by hemorrhage and shock has been observed with increasing frequency in children and young adults. This severe form of dengue has a high mortality rate. Despite years of intensive research, an effective vaccine is still not available for prevention of dengue infection. For these reasons, research on dengue viruses and development of safe and effective vaccines have been given a high priority by the World Health Organization.

Similar to other members of the flavivirus family, dengue virus contains a positive RNA genome and three structural proteins, i.e. capsid protein (C), the small membrane protein (M), and the large membrane or envelope (E) glycoprotein. The RNA genome codes for a long polyprotein that is co- and post-translationally cleaved to produce the three structural proteins and a series of non-structural proteins designated NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 in that order.

One of our research goals has been to develop a safe and effective vaccine for prevention of dengue virus infection.

Earlier, we constructed recombinant vaccinia virus that contained dengue type 4 virus cDNA coding for the C-PreM-E-NS1-NS2A polyprotein. Infection of CV-1 cells with this recombinant virus produced apparently authentic Pre-M, E, and NS1 glycoproteins (3). Mice immunized with this recombinant vaccinia virus were completely protected against lethal dengue encephalitis (4). These animals developed antibodies against the NS1 glycoprotein, but no antibodies against E or pre-M were observed. Subsequent work showed that vaccinia recombinants expressing E, NS1 or pre-M alone also elicited a protective immune response in mice, but only the NS1 recombinant caused a detectable antibody response (4-6). Since the E glycoprotein, the major antigen of the dengue virion, is the target of neutralizing antibodies, an effective dengue vaccine will probably have to induce a strong anti-E response. It thus became an important research goal to express the E glycoprotein in a more immunogenic form. Success in this endeavour is described below.

BACULOVIRUS RESULTS THROUGH 2/28/91 (SUMMARY)

The protective immune response elicited in mice by a vaccinia recombinant expressing the C-preM-E-NS1-NS2a polyprotein prompted us to construct recombinant baculovirus b(C-PreM-E-NS1-NS2A), containing the same cDNA (7). The DNA sequence was first inserted into a baculovirus plasmid vector (kindly provided by Dr. M. Summers, Texas A & M University, College Station, Texas). The cloned DNA was then recombined into the genome of wild-type Autographa californica mononuclear polyhedrosis virus (AcMNPV),

replacing the polyhedrin coding sequence. A recombinant baculovirus was selected and purified by serial dilution and visual selection of plaques lacking polyhedrin. Dengue protein production by infected cells was confirmed by immunofluorescence assay and by radiolabeling, immunoprecipitation and SDS-PAGE. (The same procedure was followed in construction of other recombinants described below.) Immunization of mice with the lysate prepared from recombinant virus-infected insect cells induced near-complete resistance to lethal intracerebral dengue virus challenge (7). Analysis of seroresponse showed that immunized mice developed a high level of antibodies specific for NS1, but antibodies to E or PreM again were not detected.

Baculovirus recombinants expressing NS1 alone were also studied. It was previously shown that recombinant vaccinia virus expressed apparently authentic NS1 either when the complete NS1-NS2A genes were present in tandem (8), or when only the NS1 gene was inserted. Expression of NS1 was 2-3 fold higher in the latter case (Hori and Lai, unpublished observation). Equivalent baculovirus recombinants b(DEN4, NS1-NS2A) and b(DEN 4, NS1) were constructed, and were shown to produce apparently authentic NS1 glycoproteins. However, although cells infected with vaccinia recombinants expressing NS1 secrete a small amount of NS1 into the medium, no secretion from baculovirus-infected cells was observed.

In order to clarify the role of E in inducing resistance to dengue virus challenge, recombinant baculovirus b(DEN4, RSVG-E) expressing only E was constructed utilizing the amino terminal 71

amino acid sequence of respiratory syncytial virus (RSV) G glycoprotein as a signal for proper glycosylation of E. Mice immunized with a lysate of insect sf9 cells infected with this recombinant virus failed to develop a detectable seroresponse to E, but were completely resistant to dengue virus challenge (8). We next constructed two dengue 4 E recombinants, both of which encode the native N-terminal hydrophobic signal sequence. The first, b(DEN4, 93%E), encodes the N-terminal 93% of the E protein, deleting the C-terminal hydrophobic sequence. The second encodes the entire E gene, plus, for reasons incidental to the cloning procedure, the first three amino acids of NS1. An equivalent 100% E constructs was produced for dengue type 2, while for type 3 a precise 100% E, without the short NS1 sequence, was constructed by means of PCR. Recombinants b(DEN4, 93%E) and b(DEN3, 100%E) were readily shown to produce an apparently authentic E glycoprotein of expected size. However, although cells infected with b(DEN4, 100%E) or b(DEN2, 100%E) were strongly positive by immunofluorescence assay, the products of these recombinants were not detectable by radio-immunoprecipitation and SDS-PAGE. It is thought that this may be the result of strong binding of these proteins to lipid membranes of their host cells, mediated by the hydrophobic C-terminus of E plus the additional amino acids of NS1, which may be acting as a membrane anchor. The immunogenicity and protective efficacy of these recombinants will be discussed below.

The protective efficacy of two of the above recombinant baculoviruses has been evaluated in rhesus monkeys. One of six

monkeys immunized with dengue virus proteins expressed by recombinant b(DEN4, C-PreM-E-NS1-NS2A) was protected against dengue virus challenge, as was one of three monkeys immunized with dengue E alone expressed by b(DEN4, RSVG-E) (10). Although monkeys inoculated with the first recombinant developed antibodies to NS1, neither elicited a response to E. Three monkeys immunized with vaccinia recombinant v(DEN4, C-preM-E-NS1-NS2A) developed only a very low antibody response to NS1, none to E, and were not protected.

CONSTRUCTION OF RECOMBINANT BACULOVIRUS EXPRESSING HIGHLY IMMUNOGENIC DENGUE ENVELOPE (E) GLYCOPROTEIN

Recent results obtained from our laboratory showed that the C-terminal truncation of E increased its immunogenicity in mice (11). A series of C-terminal deletions was engineered and introduced into recombinant vaccinia virus, resulting in a library of recombinants expressing from 9% to 100% of the N-terminal portion of dengue 4 E. It was observed that proteins constituting approximately 80% or more of E appeared to retain a native tertiary structure, in that they could be immunoprecipitated by hyperimmune mouse ascitic fluid (HMAF) raised against authentic E, while smaller E proteins reacted weakly with HMAF, but were immunoprecipitated more efficiently using antibodies raised against linear peptide sequences. This implied that these proteins had lost the conformational epitopes of the native E protein. It was also noted that proteins 80% in size were detected both on the

surface and in the medium of infected cells, while no cell surface expression or secretion of larger E proteins was observed. Immunization of mice with a recombinant vaccinia virus expressing the 80% E protein, which retained a native conformation and was both expressed on the cell surface and secreted from the infected cell, was found to elicit a strong anti-E response . These mice were fully protected against dengue virus challenge.

During the past year we have constructed recombinant baculoviruses which express 80% E of dengue type 4, dengue type 2 and dengue type 3, as presented below. This report presents the results of experiments to analyze quantitatively the E product expressed by each recombinant, its level of secretion, and the results of immunization experiments in mice, in terms of serologic response and protection against homotypic dengue virus challenge.

Baculovirus 80% E constructs: protein production and immunogenicity

a. Dengue 4 80% E

Cells infected with baculovirus recombinants expressing dengue 4 E products were strongly reactive by immunofluorescence assay using anti-dengue 4 HMAF. Radio-immunoprecipitation and SDS-PAGE revealed glycoproteins of expected size in lysates of cells infected with b(DEN4, 93%E) and b(DEN4, 80%E), but, as mentioned above, the product of b(DEN4, 100%E) was not detected (not shown).Synthesis and secretion of the 80% E protein was analyzed in a pulse-chase experiment, as described in the legend of Figure 1. After a two-hour label and a six-hour chase, the concentration

of the 46-50kD 80% E protein in the medium reached approximately 15% of the concentration within the cells. Examination of the medium without immunoprecipitation (Figure 1, lane "6" next to molecular weight markers) revealed a band co-migrating with the labeled E protein, but the majority of this material failed to immunoprecipitate with HMAF, and proved to be the baculovirus storage protein. The secreted E protein would have to be purified to remove this and other viral or insect-cell proteins before it could be used for human immunization. However, for animal immunization studies, the secreted E protein can readily be concentrated to any desired extent by high-speed centrifugation of the medium through a Centricon (protein-retaining) filter.

In the course of work with these baculovirus products, it was noted that the amount of detectable E protein in a sample of cell lysate sometimes diminished, in a manner consistent with temperature lability. This was tested by exposing samples of lysate and supernatant to room temperature and removing aliquots at time points during the course of one hour (Figure 2). The amount of E protein detectable by immunoprecipitation with HMAF in the two lysates fell by 50%, while the secreted protein remained stable. The data suggest either the presence of protease activity in the cell lysate or spontaneous loss of native conformation of the E products in the lysate environment. In light of this finding, material used for mouse immunogenicity studies described below was always kept on ice until immediately prior to injection. The stability of secreted 80%E and the purity of the protein in the

supernatant are potentially highly advantageous features of this baculovirus product.

The relative immunogenicity of the products of b(DEN 4, 93%E) and b(DEN4, 80%E) were determined by inoculating identical quantities into mice and assessing the antibody response (Figure 3). As noted in the legend to that figure, the concentration of the products of b(DEN4, 93%E) in a cell lysate and of b(DEN4, 80%E) in a lysate and in a concentrated volume of medium was first determined by Western blotting and probing with antiserum and ^{125}I-labeled staph A protein. Inocula were adjusted so that mice received identical quantities of antigen. Recombinant b(DEN4, 100%E) was not used in this experiment because of the difficulty of detecting and quantitating its protein product, as described above. All mice inoculated with secreted 80% E (lanes 8-14) and one of three mice given a lysate of b(93%E)-infected cells (lane 4) developed antibodies to the E protein, while other mice inoculated with a lysate of cells infected with b(93% E), with b(80% E), or with wild-type baculovirus showed no antibody response, as determined by radio-immunoprecipitation. Screening for neutralizing antibodies has not yet been performed.

The quantitated products were also used in two experiments to determine their protective efficacy in mice, as described in the legend of Table 1. Although both experiments were marked by less than 50% mortality of challenged mice in the negative control groups immunized with a lysate of cells infected with wild-type baculovirus, the combined mortality of those groups was still

significantly higher than the combined mortality of groups inoculated with the product of b(93%E) or with the intracellular or secreted product of b(80% E) ($p<.025$). Scored for morbidity, all mice immunized with the wild-type product developed signs of encephalitis following challenge, while none immunized with the secreted 80% E product showed signs of disease. Approximately half of all mice given the 93% E product, and nearly all given the 80% E product as a cell lysate, developed encephalitis.

b. Dengue 2 80% E

Analysis of the production and secretion of the product of b(DEN 2, 80%E) showed it to be similar to that of its dengue 4 counterpart. Since the product of b(DEN2, 100%) could not be directly quantitated, as described above, comparative analysis of the immunogenicity of a lysate of cells infected with b(DEN2, 100%E) and the intra- and extracellular forms of b(DEN2, 80%E) was performed by an indirect method. The E proteins in the supernatant and lysate of cells infected with b(DEN2, 80%E) were quantitated as described, and a lysate of cells infected with b(DEN2, 100%E) at the same M.O.I., and for the same length of time, as b(DEN2, 80%E), was diluted to the same extent. The immunogenicity of these products was assessed as described in the legend of Figure 4. All mice inoculated with the secreted 80% E protein developed a strong antibody response to native dengue 2 E, while the antibody response of mice given the intracellular 80% E product was weaker and more variable. Mice given the 100% E product or wild-type-infected cell lysate did not develop antibodies to E. The antibody response to

secreted 80% E was comparable to that seen in mice immunized with a recombinant vaccinia virus expressing dengue 2 80% E.

The protective efficacy of these dengue 2 E products was assessed as described in the legend of Table 2. The secreted 80% E product gave a statistically significant level of protection, in comparison to the mortality of negative control mice ($p<.025$). The mortality of mice immunized with lysates of cells infected with b(DEN2, 100%E) or b(DEN2, 80%E) did not differ from that of the control group.

c. Dengue 3 80% E

As shown in Figure 5, cells infected with recombinants b(DEN3, 100%E) and b(DEN3, 80%E) produced E proteins of expected size. Although the medium of cells infected with b(DEN3, 100%E) shows what appears to be 100% E protein, this probably represents leakage of labeled protein from cell lysis, as most gels do not show 100% E in the medium. The level of secreted 80% E is somewhat lower, relative to the intracellular protein, than was observed for the dengue 4 recombinant in Figure 1. In this experiment, immunoprecipitation with either anti-dengue 3 or anti-dengue 2 HMAF was performed, and cross-reactivity is evident. Figure 6 shows a Western blot of lysates of cells infected with six individual plaques of b(DEN3, 80%E) or with two plaques of b(DEN3, 100%E). All show an E band not present in the wild-type lysate; in contrast to the 100% E product of types 4 or 2, that of dengue 3 is readily detected.

Mice were inoculated with lysates of cells infected with b(DEN3, 80%E), b(DEN3, 100%E), or wild-type baculovirus, or with concentrated supernatant from cells infected with b(DEN3, 80%E), as described in the legend of Figure 7. As that figure demonstrates, only mice inoculated with the secreted 80% E product developed detectable antibodies against the native E protein. Sera from immunized mice were further tested for the presence of protective antibodies in a passive transfer experiment. Since dengue 3 virus has not been successfully adapted to growth in mouse brain to the point that it will cause fatal encephalitis in adult mice, but will only kill suckling mice, the efficacy of immunization can only be assessed through serum transfer. Suckling mice are inoculated intra-peritoneally with the immune serum, followed by intra-cerebral challenge with partially mouse-brain-adapted dengue 3 virus.

The design of experiments in which the protective efficacy of baculovirus dengue 3 E products was assessed is given in the legend of Table 3. Both experiments failed to show protection by experimental immune serum, though anti-dengue 3 serum was protective in the second experiment. Since serum from mice immunized with secreted dengue 3 80% E contained readily detectable antibodies to E, the reason for the failure to produce any protective effect is not known.

CONCLUSIONS

The research goals of this study are to; (1) produce authentically processed dengue subunit protective antigens using

the high yielding baculovirus-insect cell expression system; and (2) design recombinant DNA constructs that code for highly immunogenic protein products, and achieve a high level expression of these products. In work prior to this reporting year, recombinant baculoviruses expressing the dengue type 4 C-preM-E-NS1-NS2A polyprotein, NS1 alone or E alone were shown to produce apparently authentic proteins. These recombinants elicited complete or nearly complete protective immunity in mice, and the polyprotein and an E recombinant were partially protective in a primate study. However, none of these recombinants elicited a strong seroresponse to the E glycoprotein, which we believe is probably an essential quality of an immunogen for effective immunization against dengue.

The discovery of the high immunogenicity of C-terminally truncated 80% E may have solved the problem of the lack of an antibody response to E. As described in this report, work during the past year has resulted in the construction of recombinant baculoviruses expressing the 80% E protein of dengue type 4, 2 or 3. Cells infected with these viruses secrete a relatively large fraction of the E protein into the surrounding medium, and the protein can be concentrated through a simple centrifugation procedure. In contrast to intracellular E proteins in cell lysate preparations, the secreted protein is stable at room temperature. Secreted E elicits a readily detectable anti-E seroresponse. However, sera have not yet been screened to determine neutralizing

antibody titers, so it is not known how much of the antibody response is truly protective.

Immunization studies have shown that the secreted products of b(DEN4, 80%E) and b(DEN2, 80%E) protect mice against homotypic dengue virus challenge. The data for protection against dengue 4 would be more convincing were the mortality of negative-control mice higher; this factor appears to vary with the source of the mice used (D. Dubois, personal communication). Further testing will be carried out using mice obtained from Jackson Laboratories, which traditionally have been more susceptible to dengue 4 challenge. Protection against dengue 2 challenge was incomplete, despite the strong antibody response elicited by the 80%E product. Since the challenge virus failed to kill all negative controls in this experiment, incomplete protection cannot be attributed to a high degree of virulence of the challenge virus; the cause of this experimental outcome is unknown. Similarly, even though sera from mice immunized with secreted dengue 3 80% E protein contained readily demonstrable antibodies against the native E protein, the pooled serum was not protective in a passive transfer experiment. This may be related to any of the several variables involved in this experiment: age and source of mouse pups, serum volume and antibody titer, and challenge virus titer. Further work remains to be done to assess these promising recombinant baculoviruses.

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TABLE 1.

Response to dengue 4 virus i.c. challenge of mice immunized with recombinant baculovirus-expressed dengue 4 envelope glycoprotein products

Product expressed by recombinant baculovirus	Experiment 1 Mortality	Experiment 2 Mortality	Combined Morbidity
DEN4 93% E lysate	0/10	0/10	40-50%
DEN4 80% E lysate	0/10	1/10	90%
DEN4 80% E supernatant	0/10	0/10	0
WT baculo lysate	3/10	4/9	100%

Female BALB/c mice were inoculated i.m. at age 3 weeks with a total of 0.1 ml lysate or supernatant preparation, boosted 2 weeks later, and challenged at age 6 weeks by intra-cranial inoculation of 100 LD_{50} of mouse-brain-adapted H241 strain dengue 4 virus. The mice were observed for 28 days for signs of encephalitis and for death.

TABLE 2.

Response to dengue 2 virus i.c. challenge of mice immunized with recombinant baculovirus-expressed dengue 2 envelope glycoprotein products

Product expressed by recombinant baculovirus	Morbidity	Mortality
DEN2 100% E lysate	12/12	9/12
DEN2 80% E lysate	12/12	5/12
DEN2 80% E supernatant	5/12	3/12
WT baculo lysate	14/14	10/14

Female BALB/c mice were inoculated i.m. at age 3 weeks with a total of 0.1 ml lysate or supernatant preparation, boosted 2 weeks later, and challenged at age 6 weeks by intra-cranial inoculation of 100 LD_{50} of mouse-brain-adapted New Guinea C strain dengue 2 virus. The mice were observed for 28 days for signs of encephalitis and for death.

TABLE 3.

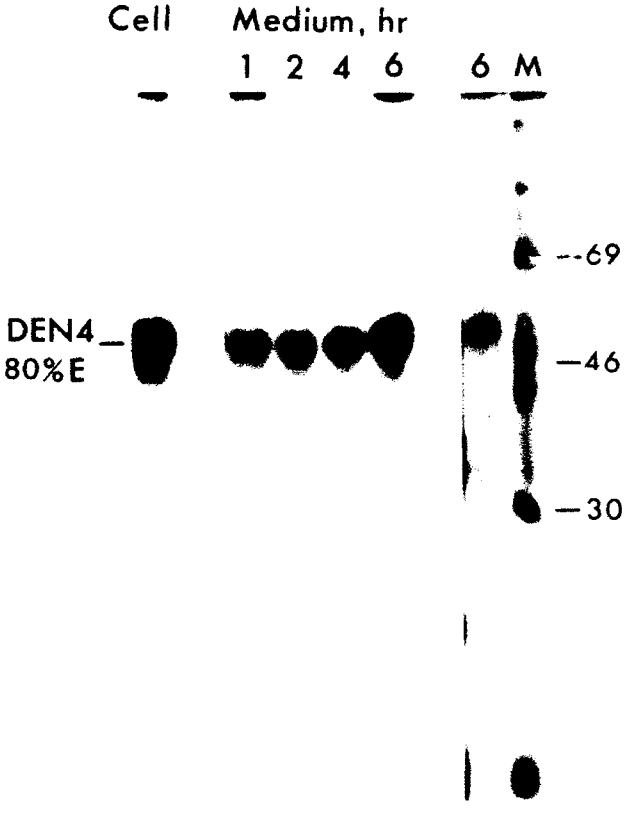
Response to dengue 3 virus challenge of suckling mice after inoculation with serum from mice immunized with recombinant baculovirus-expressed dengue 3 envelope glycoprotein products

Product expressed by recombinant baculovirus	Experiment 1 Mortality	Experiment Mortality
DEN3 100% E lysate	9/9	9/11
DEN3 80% E lysate	9/9	9/11
DEN3 80% E supernatant	10/10	9/11
WT baculo lysate	12/12	8/11
POSITIVE CONTROLS:		
anti-DEN3 immune serum (HMAF) 1:10 dilution		0/7
"" 1:100	6/6	11/11

Serum donors: female BALB/c mice were inoculated i.m. at age 6 weeks with a total of 0.2 ml lysate or supernatant preparation and boosted 2 weeks later. Beginning one week after boosting the donors were bled 4 times, at 5-7 day intervals, and the serum from each group was pooled. Positive control HMAF was provided by WRAIR. Suckling BALB/c mice (4 days old in Experiment 1, 6 days old in Experiment 2) were inoculated intra-peritoneally with .050 ml (Experiment 1) or .10 ml (Experiment 2) of serum, and challenged the following day by intra-cranial inoculation of 100 LD_{50} of mouse-brain-adapted H87 strain dengue 3 virus. The mice were observed for 23 days for signs of encephalitis and for death.

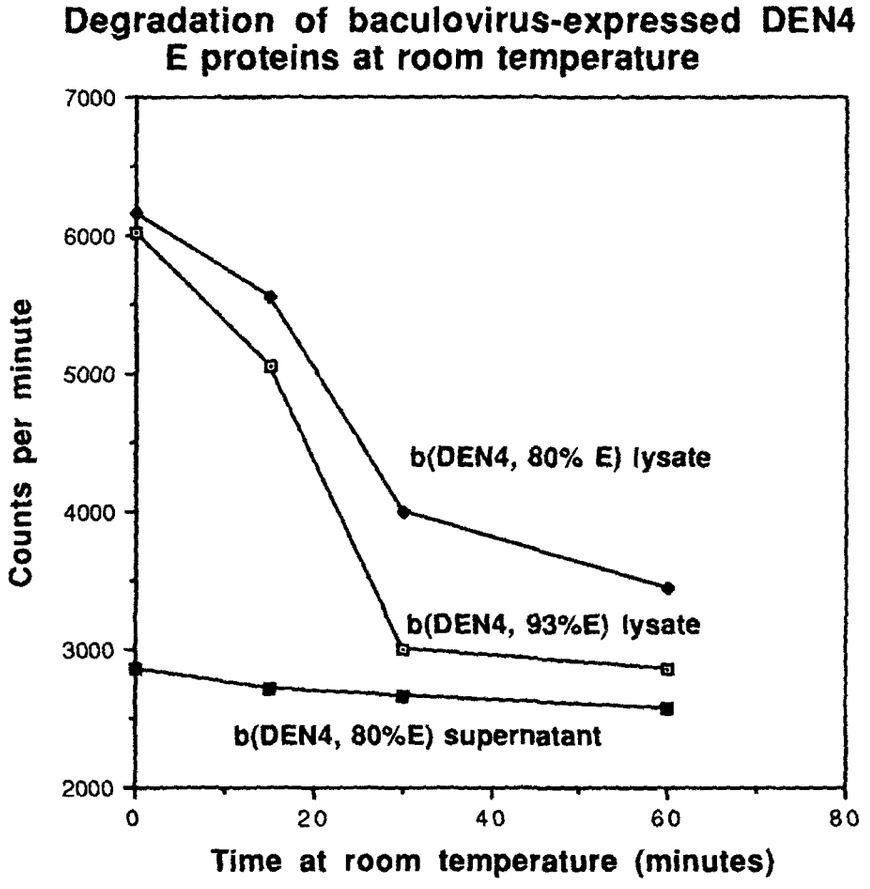
FIGURE 1.

Secretion of dengue type 4 virus 80% envelope (E) protein from Sf9 cells infected with recombinant baculovirus



Confluent S. frugiperda Sf9 cells infected with recombinant baculovirus were radio-labeled for 2 hours with ^{35}S-methionine, and the labeling medium was replaced with serum-free Grace's medium. Aliquots of medium were collected at the indicated times. At 6 hours the medium was removed and the cells lysed. Equivalent aliquots of medium and cell lysate were immunoprecipitated with anti-dengue 4 HMAF, followed by SDS-12%PAGE, to detect extracellular and intracellular 80% E. The 6-hour medium sample was also analyzed without immunoprecipitation (column at right).
M: molecular weight markers in kilodaltons.

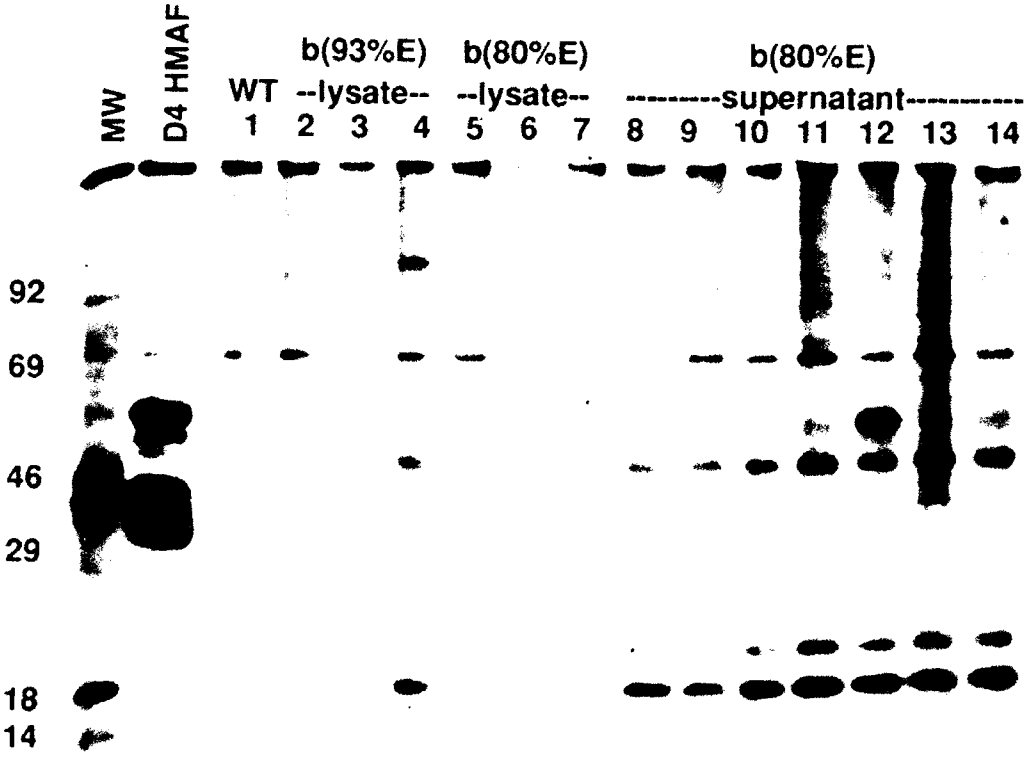
FIGURE 2.



Samples of b(DEN4, 93%E)-infected Sf9 cell lysate and b(DEN4, 80%E)-infected Sf9 cell lysate and supernatant were frozen and thawed, vortexed and kept at room temperature. At the indicated times, aliquots were removed, mixed with sample buffer and placed on ice. The samples were then subjected to SDS-12%PAGE and transferred to a nitrocellulose filter, which was probed with anti-dengue 4 antiserum and ^{125}I-labeled staph protein A. The E bands were cut out and counted in a liquid scintillation counter.

FIGURE 3.

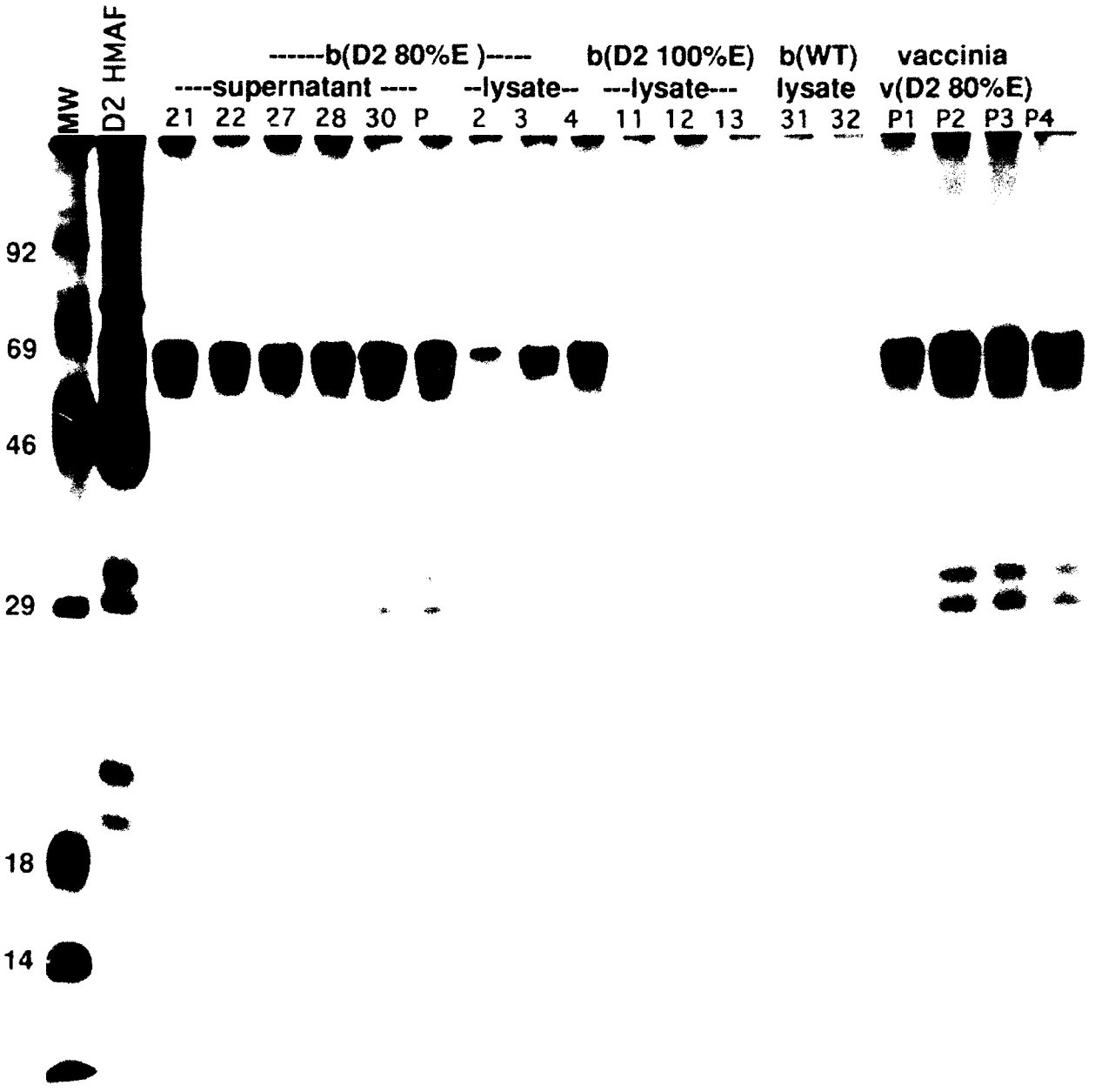
Seroresponse of mice to dengue type 4 envelope (E) glycoproteins produced by cells infected with recombinant baculovirus



Three-week-old female BALB/c mice were immunized i.m. with a total of 0.1 ml of material containing an identical amount of dengue 4 E product, as determined by Western blotting and probing with antiserum and ^{125}I-labeled staph protein A, or with a diluted lysate of cells infected with wild-type baculovirus. The mice were boosted two weeks later, then bled one week after boosting. Sera were used to immunoprecipitate a lysate of radio-labeled dengue 4 -infected cells, followed by SDS-12%PAGE.

FIGURE 4.

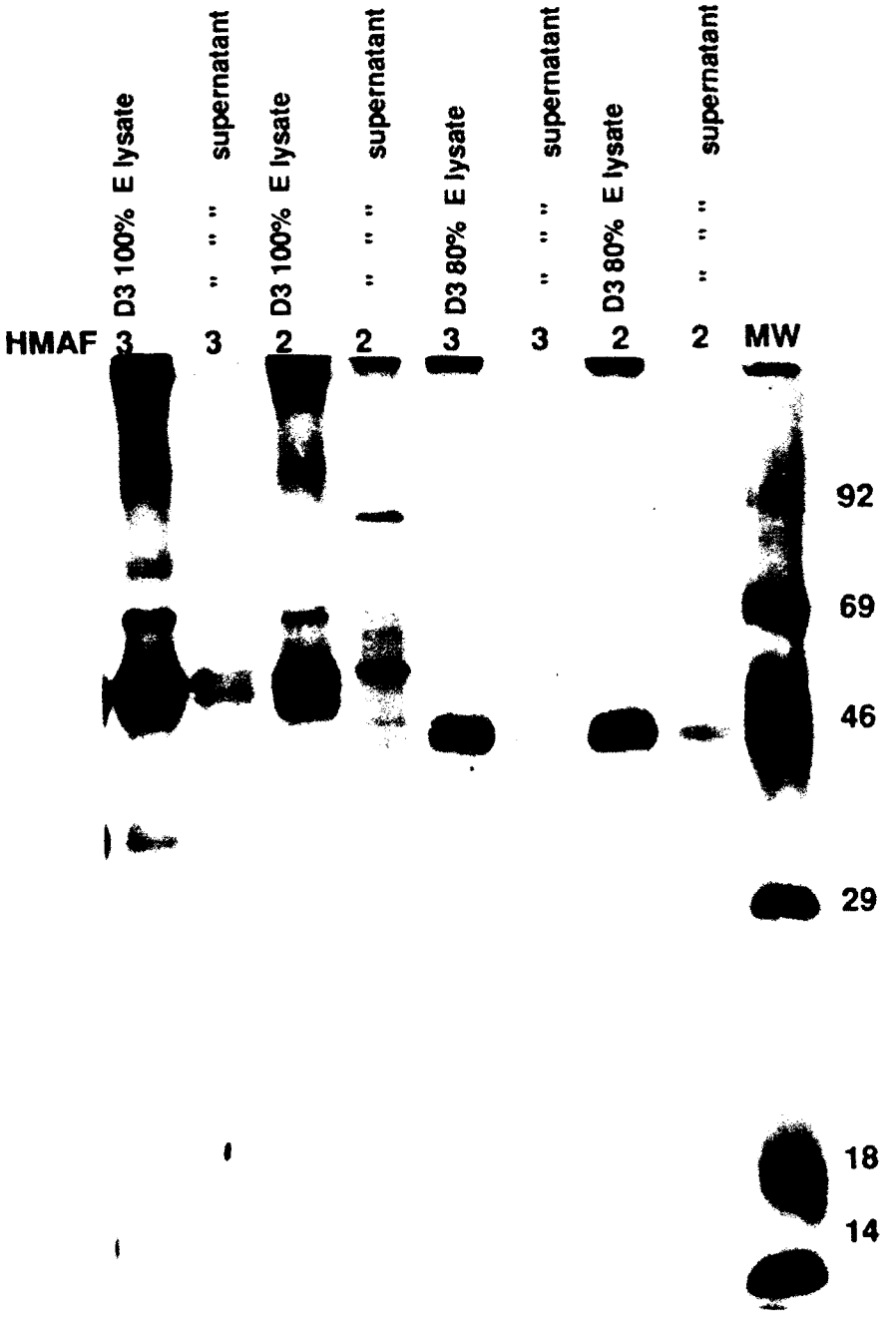
Seroresponse of mice immunized with dengue type 2 100% or 80% envelope (E) proteins produced by recombinant baculovirus or with 80% E produced by recombinant vaccinia virus



A. Immunization with baculovirus-expressed products: Three-week-old female BALB/c mice were immunized i.m. with 0.1 ml of material containing an identical amount of D2 80% E , either in an Sf9 cell lysate or concentrated cell supernatant, as determined by Western blotting and probing with dengue 3-specific antiserum and ^{125}I-labeled staph A protein, or with a lysate of cells infected with b(DEN2, 100%E) at the same M.O.I., for the same length of time, as for b(DEN2, 80%E), and diluted to the same extent, or with a lysate of cells infected with wild-type baculovirus at the same M.O.I., diluted to the same extent. They were boosted 2 weeks later, and bled at age 6 weeks.
B. Immunization with recombinant vaccinia virus expressing dengue type 2 80% E The same schedule was followed with vaccinia immunization; each mouse was inoculated i.p. with 3×10^6 p.f.u. of virus; the mice were bled four times, beginning at age 6 weeks, and the serum pooled.

FIGURE 5.

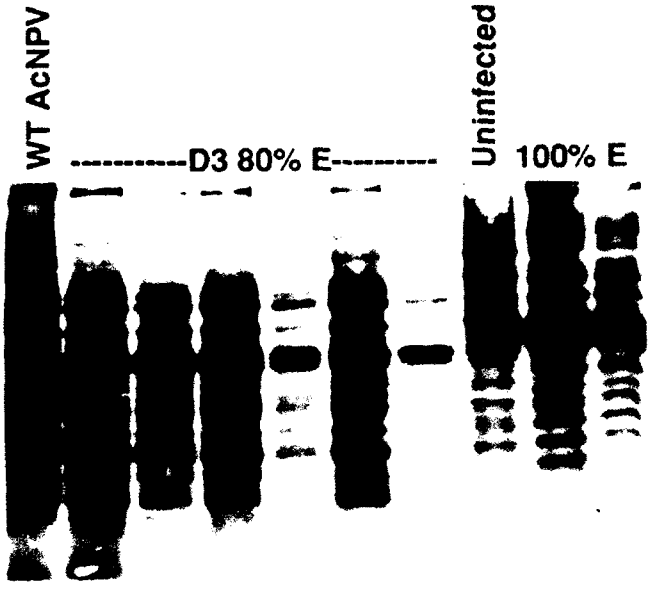
SDS-PAGE analysis of radiolabeled lysates and supernatants of Sf9 cells infected with recombinant baculoviruses expressing 100% or the N-terminal 80% of the dengue type 3 envelope (E) glycoprotein



Confluent S. frugiperda Sf9 cells were infected with recombinant DEN 3 100% E or 80% E baculovirus, at an M.O.I. of 10. After 48 hours of infection, the cells were labeled with ^{35}S-methionine for 4 hours, the supernatants were collected, and the cells were lysed with RIPA buffer. Both supernatants and lysates were immunoprecipitated with anti-dengue 3 or anti-dengue 2 HMAF, as indicated at the tops of lanes. MW: molecular weight in kilodaltons.

FIGURE 6.

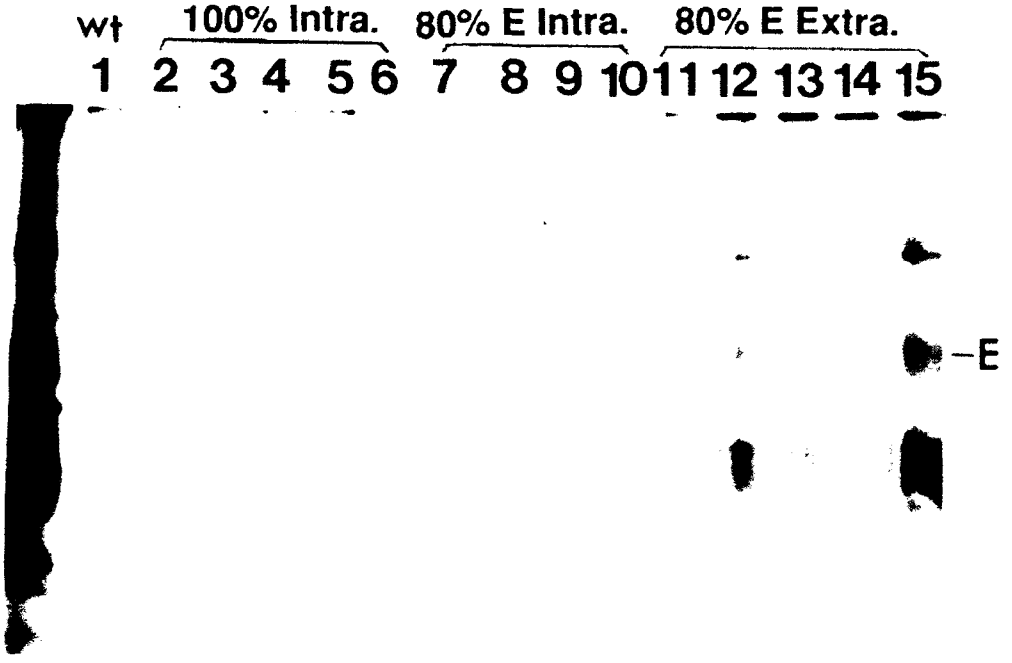
Analysis by Western blotting of products of cells infected with recombinant baculovirus expressing 100% or N-terminal 80% E of dengue type 3 virus



Confluent S. frugiperda Sf9 cells were infected with stocks derived from six individual plaques of recombinant baculovirus expressing dengue 3 virus 80% E, or two plaques of virus expressing100% E, or with wild-type (WT) AcNPV, at an M.O.I. of 1. At 3 days post-infection, the cellswere lysed with RIPA buffer and subjected to 12% SDS-PAGE.After electrophoresis, the protein bands in the gel were transferred to nitrocellulose , and the blot was reacted with anti-DEN 3 HMAF, then probed with ^{125}I-labeled staph protein A.

FIGURE 7.

Seroresponse of Mice to Dengue Type 3 E Proteins Produced by Recombinant Baculovirus



Six-week-old female BALB/c mice were immunized i.m. with a total of 0.2 ml of material containing an identical amount of dengue 3 80% E or 100% E product, as determined by Western blotting and probing with antiserum and ^{125}I- labeled staph protein A, , or with a lysate of cells infected with wild-type baculovirus at the same M.O.I., diluted to the same xtent. The mice were boosted two weeks later, then bled four times from age 9-11 weeks. Serum from the last bleed was used to immunoprecipitate a lysate of radio-labeled dengue 3-infected cells for this gel. Sera from the four bleeds were pooled for passive transfer to suckling mice.