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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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Bethesda, Maryland 20205**

REPORT DATE: April 1, 1993

TYPE OF REPORT: Interim Report

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Development Command, Fort Detrick
Frederick, Maryland 21702-5012**

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 1 April 1993	3. REPORT TYPE AND DATES COVERED Interim Report (3/1/92 - 2/28/93)	
4. TITLE AND SUBTITLE Development of Safe, Effective Vaccines for Dengue Virus Disease by Recombinant Baculivirus			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 20205			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)				
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 Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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In our previous Annual Report, covering the period 3/1/91-2/28/92, we described our finding that cells infected with recombinant baculovirus expressing the 80% N-terminal portion of the dengue virus envelope (E) glycoprotein secreted the 80%E protein into the surrounding medium. This property was unique for recombinant baculoviruses containing the dengue E gene constructed in this laboratory. Viruses containing this E fragment from dengue type 2, 3 or 4 were designated b(DEN2, 80%E), b(DEN3, 80%E) or b(DEN4, 80%E), respectively. Mice inoculated with the secreted DEN4 80%E protein in partially concentrated medium developed a stronger antibody response to DEN4 E, as determined by Western blotting, than did mice inoculated with lysates of cells infected with b(DEN4, 80%E) or with previously constructed recombinants b(DEN4, 93%E), b(DEN4, C-preM-E-NS1-NS2A), or b(DEN4, RSVG-E). Since the dengue virus protein products of the latter two recombinants appeared to partially protect rhesus monkeys against challenge with wild-type DEN4 virus in an earlier experiment, we decided to test b(DEN4, 80%E) in a second monkey experiment, to see if we could obtain a more convincing protective response.

A total of 16 rhesus monkeys were inoculated intramuscularly with baculovirus products. Two control monkeys received a lysate of cells infected with wild-type baculovirus, and two received medium from the same cells. Four monkeys were inoculated with a lysate of cells infected with b(DEN4, 80%E), four with medium from these cells, and four with a lysate of cells infected with b(DEN4, 93%E), which does not secrete its E product. Each monkey was inoculated three times with 2 ml of baculovirus product; boosting was done at 4 weeks and 13 weeks. All monkeys were challenged at 16 weeks by subcutaneous injection of 10^5 pfu of DEN4 strain 341750, provided as a lyophilized preparation from WRAIR (1/86). Monkeys were bled during the course of immunization to evaluate their immune response and after challenge to assess viremia.

The antibody response to immunization, determined by 50% plaque reduction neutralization using pre-challenge sera, was very poor. One of four monkey recipients of b(DEN4, 93%E) lysate had a titer of 1:10, two animals which received b(DEN4, 80%E) lysate had titers of 1:10, and two monkeys immunized with b(DEN4, 80%E) medium had titers of 1:10 and two had titers of 1:20. All other monkeys, including controls, were negative.

Tests for post-challenge viremia, using amplification on C6/36 mosquito cells followed by C6/36 plaquing, failed to demonstrate viremia. Control animals immunized with wild-type baculovirus material were positive on only a few scattered days, and when the amplification and plaquing were repeated for this group, plaques were again obtained on scattered days, but these differed from the first result. The cause of this failure is not known. We can draw no conclusion from this experiment as to the protective efficacy of these baculovirus products in monkeys, but it is evident that they are only weakly immunogenic.

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