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The World Reference Center for Arboviruses Characterized Sabia and Guanarito viruses, two new arenaviruses causing hemorrhagic fever in Brazil and Venezuela, respectively. A new genotype of Japanese encephalitis virus was discovered from Indonesia. Classifications of Venezuelan encephalitis and arenaviruses were revised. Outbreaks of arenavirus hemorrhagic fever in Venezuela and Brazil were investigated. Military watchdogs had antibody to Japenese encephalitis virus. Rapid PCR-based diagnostic techniques for encephalitides were developed. Neutralization tests of sera of army volunteers supported the licensing of the Japanese encephalitis inactivated vaccine. Recombinant vaccinia viruses expressing the prM/E genes were developed as candidate vaccines. Reagents were distributed to laboratories in 24 countries.

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FOREWORD

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RSS For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

RSS In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Robert E. Shope June 7, 1994

PI Signature Date

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INTRODUCTION

The World Reference Center for Arboviruses was established at the Yale Arbovirus Research Unit in 1965 as an outgrowth of The Rockefeller Foundation program on arboviruses which was moved in 1965 to Yale University from New York City. The U.S. Army has supported this program since 1972, initially through joint Navy-Army funding, then through separate contracts and grants, and during the past 3 1/2 years by this grant. The progress of this period is included in this report; it covers the work for the entire project which received support from the World Health Organization and NIH in addition to that of this grant.

BODY OF REPORT

1. Virus identification. Viruses were identified from Japan, Sudan, Venezuela, Brazil, United States, Indonesia, Thailand, Mexico, Egypt, Peru, Colombia, China, Malaysia, India, Italy, Senegal, Central African Republic, France, and Angola. Among these were:

a. TOGAVIRUSES. A variety 1E Venezuelan encephalitis virus was identified from a horse during an epizootic in Mexico. This was the first demonstration of this variety implicated in epizootic equine disease.

b. FLAVIVIRUSES. A new genotype of Japanese encephalitis virus was identified in Indonesia from among viruses isolated by the U.S. Naval Medical Research Unit Detachment in Jakarta. This genotype was recognized by sequencing 240 base pairs of the prM gene. The new genotype was represented in Bali, Flores, and Java.

c. BUNYAVIRUSES. Batai virus was identified for the first time from sera of human fever cases in Sudan. The virus was referred to the Center by the U.S. Naval Medical Research Unit #3 in Cairo.

d. ARENAVIRUSES. Sabia virus was a newly recognized arenavirus isolated from a fatal human case of hemorrhagic fever in Sao Paulo State, Brazil. The virus was identified in Brazil using reference reagents supplied from the Yale Center.

Guanarito virus was a newly recognized arenavirus isolated in Venezuela from a fatal human case and identified at Yale. This virus was shown to be the cause of Venezuelan hemorrhagic fever.

2. Classification of arboviruses and arenaviruses. The classification of the Venezuelan encephalitis virus complex was revised. During a study of the 1F variety from Sao Paulo Brazil, neutralization tests revealed that subtype 2 (Everglades) virus was more closely related to variety 1AB than previously described. It was proposed that subtype 2 be reclassified as a variety of subtype 1.

The classification of arenaviruses was revised to include the new members, Sabia and Guanarito from the New World. Complement fixation test results indicated that there are 2 sets of New World arenaviruses; one set contained Guanarito, Junin, Tacaribe, and Amapari; the other set contained Latino, Parana, Tamiami, Flexal, and Pichinde viruses.

3. Diagnosis of disease. An outbreak of hemorrhagic fever was investigated in Portugesa State, Venezuela. Fifteen cases were confirmed by virus isolation and the clinical syndrome was described. Nine of the 15 cases were fatal. This was apparently a new disease for this region with a clinical picture very similar to that of Argentine and Bolivian hemorrhagic fevers.

A single fatal case of hemorrhagic fever in Sao Paulo State, Brazil was shown to be caused by a new arenavirus, Sabia virus. The exact site of exposure of the patient was not determined and is still not known.

Rift Valley fever returned to Egypt during 1993 after a 13 year absence. Sera of 3 cases of optic retinitis were submitted by the U.S. Naval Medical Research Unit #3. These were confirmed as Rift Valley fever by IgM capture ELISA.

Outbreaks of equine encephalitis in Colombia and Mexico were shown to be caused by Venezuelan encephalitis virus. These were the first epizootics of Venezuelan encephalitis recognized for two decades.

4. Serological surveys. A serosurvey was carried out of Egyptian human residents of a Nile Delta village that was gradually inundated over the past 10 years. High prevalence of West Nile and Sicilian sandfly fever virus antibodies is consistent with the hypothesis that the flooding conditions have created favorable breeding sites for arthropods that in turn increased the transmission of arboviruses.

Sera of 104 dogs, mostly patrol dogs, were referred by the U.S. Army Medical Research Unit, Korea. Sera of 60 dogs were positive in Korea by Japanese encephalitis neutralization test. Tests at Yale with other flaviviruses confirmed the probable specificity of the Japanese encephalitis tests and ruled out Zika, tick-borne flaviviruses, and West Nile (probably). There were some monotypic reactions to Tembusu and Pnom Penh bat viruses, indicating that some dogs were exposed also to other flaviviruses in addition to Japanese encephalitis.

Sera from Sao Paulo State, Brazil from patients suspected of leptospirosis were surveyed for antibody to Hantaan virus by immunofluorescence. One serum from Sao Paulo City was strongly positive.

5. Development of new techniques. A sensitive and rapid technique was developed for detection of St. Louis encephalitis and eastern encephalitis viruses using polymerase chain reaction and gel electrophoresis of amplified cDNA fragments.

The sensitivity and specificity of Vero cell lysate antigens of flaviviruses was examined and compared to those of antibody-captured mouse brain antigens for detection of IgG in yellow fever 17D vaccinees. While the classic mouse brain antigen was slightly more sensitive, the cell lysate method was cheaper, quicker, and of acceptable sensitivity.

Limited primer extension sequencing, approximately 240 base pairs, of dengue-1, dengue-2, and Japanese encephalitis viruses showed multiple genotypes for each virus and geographic clustering. This technique was applied to determine the molecular epidemiology (genotype distribution) of each of these flaviviruses.

6. Flavivirus vaccine development.

a. EVALUATION OF JAPANESE ENCEPHALITIS PRE- AND POST-VACCINATION PLAQUE REDUCTION NEUTRALIZATION IN MILITARY SUBJECTS. Serological studies supported the trial in U.S. Army personnel in Hawaii of the Biken inactivated mouse brain vaccine for Japanese encephalitis. Sera of 532 adult volunteers were tested by plaque reduction neutralization test. Two different dosing schedules produced substantial titers of neutralizing antibody at 60 and 180 days post-vaccination. Tests done at Yale correlated almost completely with tests done by different techniques at Biken and CDC, Fort Collins. The prevaccination sera were also tested at Yale by ELISA for yellow fever antibody (presumably secondary to vaccination). Those subjects with pre-existing yellow fever antibody had slightly better responses to Japanese encephalitis vaccine, but the difference was not statistically significant. The Japanese encephalitis test results were submitted to the Food and Drug Administration to support the request for licensure. The vaccine was subsequently approved and licensed for use in the U.S.

b, DEVELOPMENT OF RECOMBINANT VACCINIA VIRUSES AS FLAVIVIRUS CANDIDATE VACCINES. Japanese encephalitis, yellow fever, dengue-1, dengue-2, dengue-3, and dengue-4 recombinant vaccinia viruses were developed. Plasmids containing the prM/E genes were constructed and transfected into tissue culture cells infected with a rescuing vaccinia virus which then expressed the proteins. Candidate Japanese encephalitis vaccines were made in NYVAC attenuated vaccinia and ALVAC canary poxviruses. The NYVAC vaccinia was the basis of the candidate dengue vaccines.

Extensive preliminary studies showed that the prM gene was required for maturation of the Japanese encephalitis virus, and that recombinant vaccinia viruses with the prM/E genes secreted the proteins which formed 20 nm sub-viral particles that agglutinated goose cells and were immunogenic. No other flavivirus genes were needed to induce fully neutralizing antibodies.

The Japanese encephalitis and yellow fever recombinant vaccinia viruses immunized mice which resisted homologous virus challenge. The comparable NYVAC construct immunized pigs and markedly reduced viremia levels after challenge of the pigs.

The dengue NYVAC constructs induced antibody in mice, but at the end of the grant period had not yet been tested for ability to induce protection from challenge.

7. Low passage collection of arbovirus strains. A large collection of low passage arbovirus strains was developed and maintained lyophilized. Priority was given to yellow fever, dengue, chikungunya, California group encephalitis, Venezuelan encephalitis, St. Louis encephalitis, western encephalitis, eastern encephalits, Japanese encephalitis, and other human and veterinary disease arboviruses. The original (or as close to original as was available) material was passaged once in C6/36 mosquito cells or in Vero cells. The resulting stock was lyophilized in aliquots. These were stored and distributed to any and all persons requesting material for study. The collection now contains in excess of 400 strains.

8. Distribution of reagents. Virus stocks, antigens, antibodies, cell lines, and live insects were distributed to laboratories in 24 countries. Data on reagents available for distribution were entered in a dBase-3+ data bank. More than 4,000 entries have been made.

CONCLUSIONS

The World Reference Center for Arboviruses received agents from the U.S. and foreign countries for characterization and identification. Sabia virus and Guanarito virus were two new arenaviruses that caused hemorrhagic fever in Brazil and Venezuela respectively. A new genotype of Japanese encephalitis virus was discovered from Indonesia. Batai virus was identified in sera of febrile humans for the first time from the Sudan. The classification of the Venezuelan encephalitis virus complex and the New World arenaviruses was revised. Outbreaks of arenavirus hemorrhagic fever in Venezuela and Brazil, of Rift Valley fever in Egypt, and of Venezuelan encephalitis in horses in Colombia and Mexico were investigated. Study of the epidemiology of Guanarito virus causing Venezuelan hemorrhagic fever implicated the cotton rat as a reservoir. Serosurveys found Japanese encephalitis and other flavivirus antibody in Korean watchdogs, hantavirus antibody in inhabitants of Sao Paulo, Brazil, and high prevalence of mosquito- and sand fly-borne viruses in Egyptians inhabiting a flooded village. Rapid PCR-based diagnostic techniques were developed for eastern encephalitis and St. Louis encephalitis. Neutralization tests of Army volunteers supported the licensing of the inactivated mouse brain vaccine for Japanese encephalitis. Recombinant vaccinia viruses expressing the prM/E genes were developed for Japanese encephalitis, yellow fever, and the four dengue serotypes. The Japanese encephalitis is a candidate vaccine and the dengue recombinants are potential candidate vaccines. The low passage collection of arboviruses was augmented, and reagents were distributed to laboratories in 24 countries.

PERSONNEL SUPPORTED BY THE PROJECT

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