May 15, 1969

Dr. Albert B. Sabin  
Department of Pediatrics  
College of Medicine  
The Children's Hospital Research Foundation  
Cincinnati, Ohio  45229

Dear Albert:

Received your communication regarding Leon's request about the history of dengue patients Smith, Thomas, and Barnes.

I have scoured the books and unfortunately do not have the clinical data or protocols on the inoculation of these Longview patients. I think you kept these data filed in a book entitled "Longview Patients." Going back over the old papers on "Diagnosis of Dengue," I came across the following statements....Four mental patients with tertiary syphilis of the nervous system....as far as it is known, none of the patients had been in areas where dengue was endemic, although one of the patients (Thomas) had developed Sandfly Fever following experimental inoculation in 1944.... Two of the four patients (Allendorf and Smith) received intravenously 2 ml of acute phase Type 1 (Hawaiian) human dengue serum which had been stored in the lyophilized or frozen state since 1944. The other two patients (Thomas and Barnes) received intravenously 2 or 3 ml of a mixture of acute phase Type 2 (New Guinea C) sera which had been stored in the lyophilized state at 4°C since 1945....

We have all the information on the individual's subsequent responses, including serology and virus isolation, and monkey inoculation with their acute phase sera.

We have completed testing the Type 2 specimens you sent, and they, too, were inactive by the mouse test.

In regard to the notebooks, the accompanying sheets list them and the material they contain. I cannot over-emphasize what an invaluable help they are, especially with the current work that we have been doing.
You may be interested to know of our progress. We are devoting a great deal of effort to characterizing unadapted Type 1 virus, so that we can establish definite markers which may be of assistance when compared to tissue culture propagated "attenuated" virus. Although we could not produce clinical diseases in juvenile African Green monkeys by the intracutaneous route, they all became infected and developed high levels of circulating antibody which tended to be type specific but heterotypic antibodies were in evidence. Antibodies persisted for at least 90 days. When challenged with early passaged or late passaged Type 1 virus, a marked booster response was seen as early as five days, and by our testing procedures the responses were so broadly cross-reactive we could not distinguish between the 4 dengue types.

On the other hand, animals inoculated with approximately the same dose of mouse-adapted strains responded very poorly to primary inoculum, titers were significantly lower, and did not persist, and there was no heterotypic response. When re-challenged, the response was still poor; although heterotypic antibodies did appear, differentiation was still possible.

We had hoped to be able to measure the viremic patterns, but unfortunately in spite of intensive efforts and the fact that viruses used were highly pathogenic for mice, we could not detect a significant pattern of viremia following inoculation with unadapted or mouse-adapted virus.

However, most exciting was the fact that we could get infection without clinical signs with unadapted virus when the virus was given orally. The animals responded serologically as well and in some cases, better than those given the virus intracutaneously. We are about to follow up these studies with mouse-adapted virus at different passage, to determine their behavior as well as excretion patterns.

The development of tissue culture strains starting with acute phase sera is proceeding at a very slow rate. Thus far propagation has only been achieved in HEK, and we have had negative results with WI-38, chick and duck embryo, and primary hamster kidney. The biggest problem is the development of a rapid assay system so that we can know what is going on. We have developed a fairly good plaquing system in Y15 (porcine kidney) for mouse-adapted strains, but are not sure of its susceptibility for unadapted strains.

Your new position came as a pleasant surprise, and best of luck in what will turn out to be a tremendous but exciting challenge! Perhaps in the future there may be the opportunity to work with you again in Israel.

Sincerely,

B. H. Sweet, Ph. D.
Director Medical Microbiology
and Cell Biology

BHS:ds
Att.
NOTEBOOKS

Book 1 - Dengue '53 - Isolation, Serology, Laboratory Diagnosis

1) Detection and quantitation of virus in mice from acute phase sera of Type 1 and Type 2 patients.
2) Serological responses of patients receiving Type 1 and Type 2 unadapted virus. (Neut, CF, HI) vs dengue and other Group B viruses.
3) Immunogenic effect of JB vaccine in patients previously infected with dengue or sandfly fever viruses.
4) Survey of Borneo, Malayan, and Mexican residents and patients with epidemic HF for dengue HI antibodies.

Book 2 - Hawaiian Dengue - 1952-1955

1) Studies of unadapted, MP15, MP20, MP118, egg-passaged Hawaiian virus in intracerebrally inoculated monkeys and mice (clinical, pathology, paralytogenesis, and serology).
2) Early passage 1-7 of Type 1 dengue virus, Faber strain in mice.

Book 3 - New Guinea C Dengue - 1955

1) Studies of unadapted MP18, MP25, New Guinea "C" in intracerebrally inoculated monkeys and mice (clinical, pathology, paralytogenesis and serology).
2) Inoculation of Longview patients with MP18 New Guinea "C" (clinical course, CF and Neut. responses).
3) Attempted adaptation of MP20 to embryonated eggs.

Book 4 - Dengue Viruses in Monkey Kidney Tissue Culture

1) Adaptation of mouse passage 20 and unadapted Type 1 and Type 2 viruses to Cynomolgus tissue culture.
2) Stability studies on frozen and lyophilized tissue culture material.
3) Behavior of Tissue Culture passaged material in mice and monkeys (clinical, pathology, serology).

Book 5 - Miscellaneous Dengue

1) Attempted adaptation of Type 1 MP18 dengue to embryonated eggs.
2) Active cross-immunity studies in mice between Type 1 and Type 2 unadapted viruses.
3) Identification and characterization (HI) of Gordon Smith's Chia agent (Malaya) as dengue 1.
4) Studies with Uganda viruses (strain A and B) from Ross (probably Chikungunya).
5) Tests on dengue material sent to the V&R registry.

Book 6 - Dengue Vaccine Preparation and Tests in Patients and Medical Students

1) Protocol for preparing Type 1 and Type 2 vaccines.
2) Studies on various additives as stabilizers for frozen or lyophilized live vaccines.
3) Behavior of Lederle lyophilized vaccines (Types 1 and 2) in monkeys and mice (clinical, pathology, and serology).

4) Tests for possible interference with immunogenic capacity of Type 1 and Type 2 dengue viruses when both are administered as a mixture in mental patients (clinical, serology).

5) Sequential immunization of mental patients with Type 1 and Type 2 vaccines (clinical and serology).

6) Viremic patterns in mental patients receiving Type 2 virus only.

7) Tests of lyophilized Type 1 and Type 2 vaccines in medical students (clinical records, serology, viremia studies).

Book 7 - Sicilian Sandfly Fever Adaptation to Mice

1) Isolation, passage, and identification in suckling hamsters and mice.

2) Characterization of mouse-passaged Sicilian at different passage levels in mice:
   a. Behavior at different passage levels; route of infection; etc.
   b. Animal host range and serological responses.
   c. Particle size by Gradiol filtration.
   d. Attempted adaptation to embryonated eggs.
   e. Attempted adaptation to monkey kidney tissue culture.

Book 8 - Sicilian Sandfly Studies in Patients

1) Pathogenicity of MP10 and MP20 in human beings (clinical, viremia, serology).

2) Challenge of patients who received mouse-passaged virus with unadapted virus (clinical, viremia, serological responses).

3) Passage of unadapted virus in human beings (clinical, viremia, and serology).

4) Development of CF test for Sicilian Sandfly, and application to human specimens.

Book 9 - Sicilian Sandfly Hemagglutination

1) Methods of preparation and physicochemical characteristics of HA.

2) Studies on removal of normal serum inhibitors for HAI test.

3) Development and application of HI test for diagnosis and immunological relationships.

Book 10 - Naples Virus

1) Passage of unadapted virus in human beings (clinical, viremia, serology).

2) Isolation and adaptation to mice.

3) Characterization in mice and host range studies (clinical and serological responses).

4) Particle size by Gradiol membrane filtration.

5) Quantitative viremia studies in patients inoculated with unadapted and adapted viruses.
6) Behavior of adapted viruses in human beings and resistance to challenge with unadapted virus (clinical, viremia, serology).
7) Immunological relationship to Sicilian Sandfly Fever.
8) Attempted propagation in tissue culture.
9) Attempted preparation of HA antigen.