

Dengue Infection at Children's Hospital of Bangkok

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OBJECTIVE: To collect specimens for specialized dengue virus isolation and serology.

BACKGROUND:

1. Dengue virus infections are an increasing public health problem for both military and civilian populations in the tropics and subtropics. Among US military troops stationed in the Pacific Theater it is an annoying and potentially critical problem. In Thailand and elsewhere in the Southeast Asia, dengue hemorrhagic fever (DHF) is a major cause of morbidity and mortality among children.

A need exists to develop vaccines to control this disease. In the past dengue vaccination has been attempted and was possibly effective in modifying a dengue epidemic in Puerto Rico in 1963; however, the vaccine used in this case was manufactured by a method which is unacceptable by present-day standards. Development of virus vaccines presently calls for the original isolation and propagation of candidate viruses on cell lines certified by the United States Department of Health Education and Welfare, Division of Biological Standards. With a view towards isolation of candidate dengue strains, acute plasma was collected from patients presenting with symptoms compatible with dengue hemorrhagic fever at the Children's Hospital of Bangkok.

2. Recent evidence has suggested that both viral and non-viral antigens may be involved in the host response to viral disease. Technology for isolating, purifying and studying many of these antigens has been developing over the past several years. With dengue, several antigens have been identified and at least one has been purified to the point where study is possible. This antigen is a soluble complement fixing antigen, which develops in dengue infected suckling mice. Preliminary evidence suggests that humans recovering from secondary dengue develop humoral antibody against this antigen late in their convalescence. Sera were collected at specified intervals after diagnosis of DHF for study of the temporal development of antibodies to this and other viral and non-viral antigens.

DESCRIPTION: Patients suspected of having dengue virus infections were selected from the outpatient clinic and the infectious disease wards of Bangkok Children's Hospital. A standardized chart of pertinent signs, symptoms, and laboratory findings was instituted on each patient. Blood was obtained on the day of diagnosis and on days 3, 5, 15 and 30 after the day of selection. Blood drawn on the first day was divided into plasma and serum portions. On subsequent collections blood was allowed to clot and serum was collected.

Plasma was used for isolation of viruses using a direct and delayed plaque technique. Isolates were identified by plaque reduction neutralization test using monkey antisera.

Sera were used for standard serology; hemagglutination inhibition (HI) tests were performed using suckling mouse brain antigens prepared from dengue 1 (Hawaii), dengue 2 (New Guinea C), dengue 3 (H-87), dengue 4 (H-241), Japanese encephalitis (Nakayama) and Chikungunya (Ross). Sera were extracted with acetone and tested against 8 units of antigen. All sera were tested simultaneously. Aliquots of plasma

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and acute and convalescent sera were stored at -70°C for reisolation on certified cells and for specified dengue serologic tests against viral and non-viral antigens. At the conclusion of collection, clinical, isolation and serological data were used to identify individuals infected with dengue and to determine the type of antibody response and the severity of the illness. Patients were considered to have had dengue infection if a four-fold rise in antibody titer to at least two of the group B antigens was found between acute and convalescent sera or if convalescent antibody titers to at least two antigens equaled or exceeded 1:640. Criteria for the identification of primary or secondary dengue infected patients have been previously reported. Patients with HI antibody titers of $> 1:640$ to at least two dengue antigens were considered to have secondary infections while those with convalescent antibody of $\leq 1:640$ were considered to have primary infections. Grading of severity of DHF used criteria established by one of us (SN) and used in the past:

Grade I: Fever accompanied by non-specific constitutional symptoms; the only hemorrhagic manifestation is a positive tourniquet test.

Grade II: Fever and skin hemorrhage or other bleeding such as epistaxis or gingival hemorrhage.

Grade III: Circulatory failure manifested by rapid, weak pulse with narrowing of pulse pressure (< 20 mm Hg) or hypotension (systolic pressure < 90 mm Hg).

Grade IV: Moribund patients with undetectable blood pressure or pulse.

PROGRESS: Specimens were collected from 134 patients. These patients were seen and diagnosed as having illness compatible with dengue infection. Twenty-seven were collected in the outpatient clinic and 107 were obtained on hospital wards. Acute and at least one convalescent sera were obtained from 123 patients and 95 of these were diagnosed as dengue by viral isolation, by serological criteria or both. Of these 95, 91 were hospitalized for their illness. Twelve patients exhibited low level antibody responses characteristic of primary infection, and 8 of these were hospitalized. Sixty-two patients showed a four-fold rise in antibody titer and another 19 had high fixed titers. All of them were considered to have had secondary infections.

A breakdown of clinical and laboratory findings for these 95 patients is shown in Tables 1, 2 and 3. The findings were essentially similar to those of previous clinical studies. Comparison of patients showing primary and secondary responses demonstrates that DHF occurred in both groups. All but one patient with shock had secondary dengue infections. The exception was an 8 month old baby from whom only an acute plasma was obtained. Dengue 2 virus was isolated from this plasma at a time when no antibody was detectable; the mother unfortunately was not tested for antibody to dengue. Since no convalescent serum was collected, this patient could not be classified on the basis of antibody titer. He was considered to be a primary case because the acute plasma taken on the 5th day of illness had a titer of $\leq 1:20$ to all group B antigens tested.

Four patients were sampled early in the course of illness before developing thrombocytopenia. Figure 1 shows the relationship of platelet counts, antibody titer and hematocrit to the time course of disease for one patient. There appeared to be a temporal relationship between the time of platelet decrease and the development of shock.

Between July and December of 1973, 22 strains of dengue were isolated from the 95 patients with evidence of dengue infection, representing an isolation rate of 23% (see Table 4 and 5). Nine strains were isolated from thirteen patients with serological evidence of primary disease; isolation was successful in 69% of primary cases. At the time plasma was obtained for isolation, only one individual had dengue antibody. In this case the titer for the homologous strain of dengue virus was four-fold lower than that of the other dengue types tested, suggesting antigen-antibody complexes had been formed. The 82 secondary cases yielded 13 (16%) isolates. Dengue antibody was absent from the acute plasma in only three secondary cases, all of which had plasma obtained within the first two days of illness. The remaining ten had initial antibody titers ranging from 1:20-1:320 for the homologous virus (see Table 6). In the

majority of isolations from secondary cases (11/13) the antibody to the homologous types was lower than or equal to that of other types. Two had four-fold lower antibody titers to the infecting dengue type than to other dengue types tested, again possibly indicating complex formation. Dengue types 1, 2 and 3 were isolated; see Table 4 for a breakdown by antibody response and Table 5 for a breakdown by grade of disease.

Of the 22 isolates, plasma containing 4 dengue-1, 4 dengue-3 and 7 dengue-2 were shipped to Walter Reed Army Institute of Research for reisolation as candidate strains on certified cell lines for vaccine development.

Follow-up specimens were obtained on 41 patients, approximately 3, 5, 15 and 30 days after the diagnosis of DHF (see Table 7). Of these, 34 were secondary patients and 6 were primary patients. In 30 of the primary patients and 5 of the secondary patients dengue virus was isolated. Of the secondary patients, 4 were Grade I, 18 Grade II, 10 Grade III and 2 Grade IV. The primary patients included 1 undifferentiated fever (UF), 4 Grade I and 2 Grade II. These acute and longterm follow-up specimens will be tested for antibody to viral and non-viral antigens.

Table 1. Summary of Clinical and Laboratory Findings in Thirteen (13) Primary Dengue Patients

| Findings | UF* (5)** | Dengue Hemorrhagic Fever | | | |
|-------------------------------|---------------|--------------------------|--------------|---------------|--------------|
| | | Gr I (3) | Gr II (4) | Gr III (1) | Gr IV (0) |
| Fever | 5/5 (100)**** | 3/3 (100) | 4/4 (100) | 1/1-***** | 0/0- |
| Hepatomegaly*** | 0/5 (0) | 1/3 (33) | 2/4 (50) | 1/1- | 0/0- |
| Positive tourniquet test | 0/5 (0) | 3/3 (100) | 4/4 (100) | 1/1- | 0/0- |
| Petechiae | 0/5 (0) | 0/3 (0) | 4/4 (100) | 1/1- | 0/0- |
| Other signs of bleeding | 1/5 (20) | 0/3 (0) | 0/4 (0) | 0/1- | 0/0- |
| Hemoconcentration | 0/5 (0) | 0/3 (0) | 2/3 (67) | 1/1- | 0/0- |
| Platelet counts $\leq 50,000$ | 0/5 (0) | 0/3 (0) | 2/4 (50) | 1/1- | 0/0- |

- * UF indicates undifferentiated fever
- ** Number of patients
- *** ≥ 2 cm below costal margin
- **** Percentage of cases
- ***** 8 month old infant

Table 2. Summary of Clinical and Laboratory Findings in 82 Secondary Patients

| Findings | UF* (2)** | Dengue Hemorrhagic Fever | | | |
|-------------------------------|---------------|--------------------------|---------------|----------------|--------------|
| | | Gr I (14) | Gr II (38) | Gr III (22) | Gr IV (6) |
| Fever | 2/2 (100)**** | 14/14 (100) | 38/38 (100) | 22/22 (100) | 6/6 (100) |
| Hepatomegaly*** | 1/2 (50) | 6/11 (56) | 15/30 (50) | 16/19 (84) | 5/5 (100) |
| Positive tourniquet test | 0/2 (0) | 12/13 (92) | 36/36 (100) | 22/22 (100) | 6/6 (100) |
| Petechiae | 0/2 (0) | 0/13 (0) | 29/36 (81) | 16/22 (73) | 4/6 (67) |
| Other signs of bleeding | 0/2 (0) | 0/13 (0) | 14/36 (39) | 7/22 (32) | 5/6 (83) |
| Hemoconcentration | 0/2 (0) | 7/11 (54) | 18/36 (50) | 18/19 (95) | 6/6 (100) |
| Platelet counts $\leq 50,000$ | 0/2 (0) | 8/14 (57) | 24/38 (63) | 21/22 (95) | 6/6 (100) |

* UF indicates undifferentiated fever

** Number of patients

*** ≥ 2 cm below costal margin

**** Percentage of cases

Table 3. Hemagglutination Inhibition Antibody Levels in Convalescent Sera from 94 Patients with Dengue

| Grade (s) of Disease | Primary Infection (Titer < 1 : 640) | Secondary Infection (Titer \geq 1 : 640) |
|----------------------------|---|--|
| UF* | 5 | 2 |
| I & II | 7 | 52 |
| III | 0 | 22 |
| IV | 0 | 6 |
| TOTAL | 12 | 82 |

* UF indicates undifferentiated fever

Table 4. Dengue Strains Isolated from Human Infections : Dengue Type and Convalescent Hemagglutination Inhibition Antibody Responses

| Antibody Response | Number of Patients | Total Isolations | % Isolation | Strains Isolated | | | |
|-----------------------------|--------------------|------------------|-------------|------------------|--------|--------|---------|
| | | | | Type 1 | Type 2 | Type 3 | Untyped |
| Primary (< 1 : 640) | 13 | 9 | 69 | 3 | 2 | 3 | 1 |
| Secondary (\geq 1 : 640) | 82 | 13 | 16 | 1 | 9 | 1 | 2 |
| TOTAL | 95 | 22 | 23 | 4 | 11 | 4 | 3 |

Table 5. Dengue Strains Isolated from Human Infections : Dengue Type and Clinical Syndrome

| Clinical Syndrome (No. of cases) | Number of Strains Isolated | | | |
|-------------------------------------|----------------------------|--------|--------|---------|
| | Type 1 | Type 2 | Type 3 | Untyped |
| UF* (7) | 2 | 1 | 2 | 1 |
| G I (17) | 1 | 0 | 0 | 0 |
| G II (42) | 1 | 4 | 2 | 2 |
| G III (23) | 0 | 5 | 0 | 0 |
| G IV (6) | 0 | 1 | 0 | 0 |
| TOTAL | 4 | 11 | 4 | 3 |

* UF Indicates undifferentiated fever

Table 6. Distribution of Dengue Isolations by Hemagglutination Inhibition Titers to Dengue 2 or to the Homotypic Virus in the Acute Serum

| Reciprocal Titer | No. of Patients | Dengue Isolates | |
|------------------|-----------------|-----------------|-----|
| | | No. | % |
| < 20 | 20 | 13 | 65 |
| 20 | 5 | 4 | 80 |
| 40 | 2 | 2 | 100 |
| 80 | 6 | 0 | 0 |
| 160 | 4 | 2 | 50 |
| 320 | 14 | 1 | 7 |
| ≥ 640 | 44 | 0 | 0 |
| TOTAL | 95 | 22 | 23 |

Table 7. Patients from whom Convalescent Specimens were Collected on approximately Day 3, 5, 15 and 30 following Clinical Diagnosis

| Antibody Response | No. of Patients | No. of Isolations | Grade of Disease | | | | |
|-----------------------|-----------------|-------------------|------------------|---|----|-----|----|
| | | | UF* | I | II | III | IV |
| Primary (< 1 : 640) | 7 | 3 | 1 | 4 | 2 | | |
| Secondary (≥ 1 : 640) | 34 | 5 | | 4 | 18 | 10 | 2 |
| TOTAL | 41 | 8 | 1 | 8 | 20 | 10 | 2 |

* UF indicates undifferentiated fever

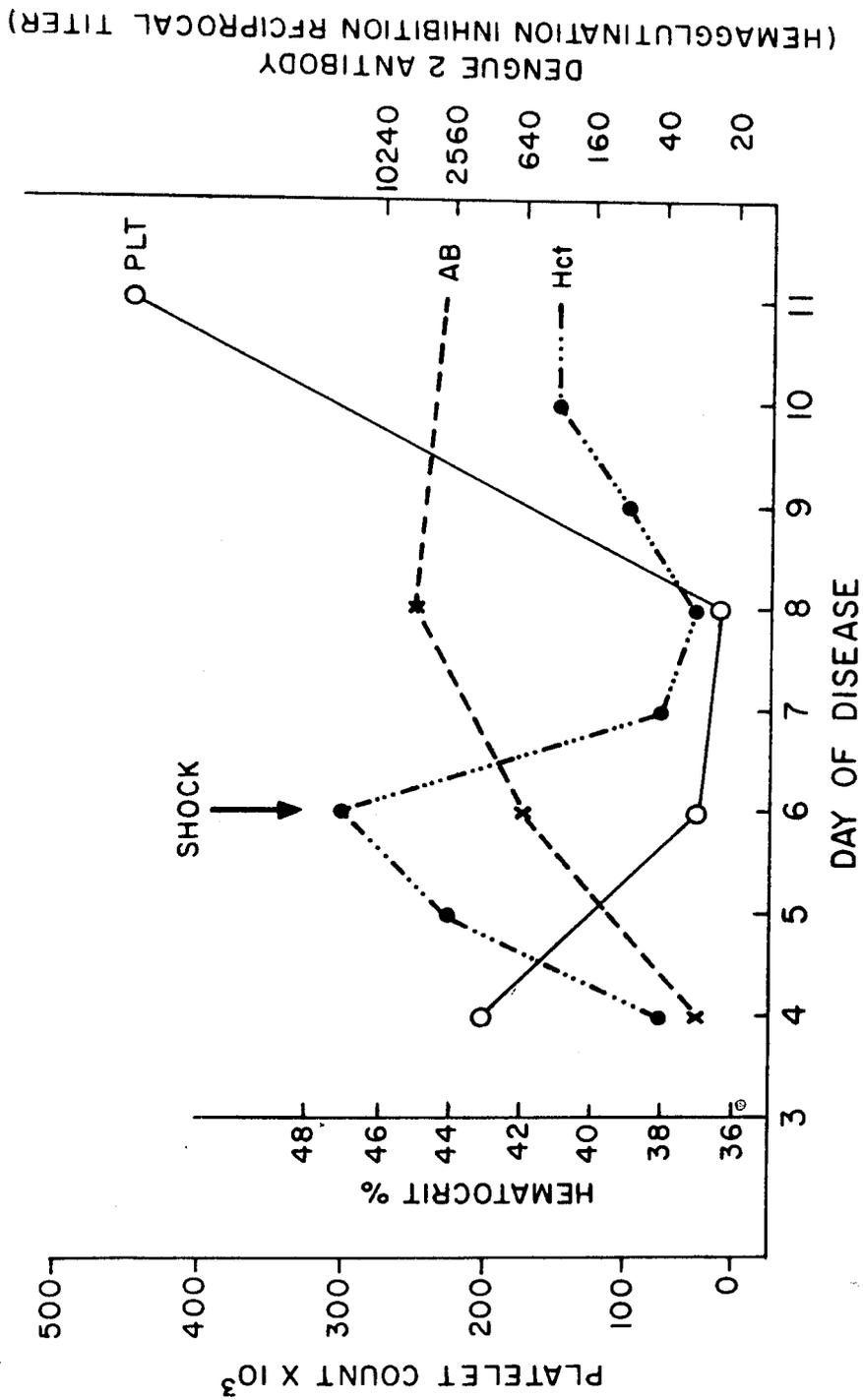


Figure 1. Platelet counts, hematocrits and antibody titers from one patient, showing the relationship of these parameters to the development of shock. PLT indicates platelet counts, AB indicates Dengue-2 antibody titer, Hct indicates hematocrit