

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 4 Serologic Response to Thai Hemorrhagic
Fever Virus Infection

Project No. 3A 025601 A 811 Military Medical Research Program
S.E. Asia

Task 01: Military Medical Research Program
S.E. Asia

Subtask 01: Military Medical Research Program
SEASIA (Thailand)

Reporting Installation: US Army-SEATO Medical Research Laboratory
APO 146, San Francisco, California

Division of Medical Research Laboratories
Department of Virology

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Major Scott B. Halstead, MC

Associate Investigators: Dr. Suchinda Udomsakdi
Dr. Ananda Nisalak
Dr. Pairatana Sukavachana

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

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The objective is to study the serologic response of humans and laboratory animals to viruses associated with Thai hemorrhagic fever. This project is concerned with the serologic response of humans and laboratory animals to viruses associated with Thai hemorrhagic fever. Techniques studied include the HI, CF and Neutralization tests. Studies during the report period have shown that of all dengue prototypes commonly employed in the SMRL virus laboratory, dengue 1 is the most broadly reactive in the HI and CF tests. Following HF infection caused by dengue viruses the HI antibody response is prompt and of great magnitude. The mean HI titer

of 31 sera obtained 8-14 days after onset of fever was 1:40,960. Beginning at 1-3 months after infection HI antibody begins to decline and 7 months after infection mean values are below 1:320. CF antibody after dengue infection follows an almost identical pattern except that values are approximately 10-100 fold lower. Serologic response to chikungunya virus is quite different. HI antibody develops less rapidly than following dengue infection with average titers of 1:900 achieved 15-30 days after infection. Development of complement-fixation antibody is definitely delayed with most sera not showing any CF activity until 15-30 days after infection. Thus, sera having high HI antibody but low or no CF antibody to chikungunya suggest recent infection.

BODY OF REPORT

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Objective: To study the serologic response of humans and laboratory animals to viruses associated with Thai hemorrhagic fever. Techniques employed include the hemagglutination-inhibition, complement-fixation and the neutralization test.

Description: Sera collected from hemorrhagic fever patients and from suitably exposed laboratory animals are studied at intervals following infection.

Sera to be tested by prototype high mouse passaged dengue and chikungunya viruses and by virus prototypes selected from Thailand. The sensitivity and specificity of various antigens and serologic techniques are compared.

Progress: Serologic study of hemorrhagic fever has posed a number of unique challenges to the virologist. Listed below are some of these problems together with pertinent observations made during the course of studies:

1. Patient with typical clinical history of hemorrhagic fever, adequate virus specimen collection but negative serologic response. Using as criteria of negativity a fixed low HI or CF titer below 1:640 and 1:32, respectively, the number of such cases occurring in the 1962 and 1963 study is shown in Table 26. The increase in percent of no diagnosis in 1963 is unexplained. It should be emphasized that careful clinical analysis of these cases with negative serology showed them to resemble all other HF cases in clinical findings and severity. Convalescent sera were obtained 20-25 days after onset of illness.

2. Simultaneous diagnostic antibody response to dengue and chikungunya viruses. Up to 7.7% of patients studied in 1962-1963 had simultaneous HI response to chikungunya and dengue.

In three out of 17 such cases, dengue viruses have been recovered. No chikungunya viruses have been isolated. It is difficult to believe that up to 8% of dengue HF patients are infected with chikungunya virus immediately after they have had dengue infection. At present there is no explanation for this phenomenon.

3. The extremely rapid and high antibody response following dengue infection. Figure 3 shows the mean titers of dengue 1 HI antibody in 46 patients studied at various intervals to 7 months after infection. The very rapid rise of HI antibody to mean values of 1:20,480 occurring between days 2 and 5 frequently results in high - fixed titers in acute and convalescent serum pairs collected at normal intervals. In 15 out of 46 sera a HI titer of 1:163,840 was observed. Note that HI antibodies decline rapidly and that by 3 months after infection mean values have declined to 1:640. This suggests that a late convalescent serum collected 2-3 months after infection may be compared with acute serum titer to diagnose recent dengue infection. CF antibody response to dengue closely resembles HI response.

The evanescent character of high HI antibody following dengue infection has led to the adoption in this laboratory of a titer of 1:640 or greater as being suggestive of recent dengue infection. Note that titers of this magnitude rarely occurred in surgical patients admitted concurrently with hemorrhagic fever patients (Table 27).

4. Antibody formed following dengue virus infection is not type specific or dengue group specific regardless of serologic system used. In the 1962 sera it has been observed that dengue 1 HI and CF antigens reacted most broadly with paired human HF sera. Thus, using dengue 1 antigen, 67% of 46 HF sera showed a 4 fold or greater rise in both the CF and HI test (Table 28) and the balance of paired sera showed a high fixed titer (Table 28). Using the criteria of rising antibody or high fixed titers in paired sera in the HI test any antigen was equally satisfactory. By CF, however, dengue 1 and 2 resulted in a positive diagnosis more frequently than other dengue antigens. Only dengue 1 CF antigen gave results comparable to HI antigens.

The greater frequency of dengue 1 CF antibody response following HF, particularly compared with other dengue viruses, suggests this virus predominated in 1962. However, from 1962 isolation data (SMRL 2) dengue 1 and dengue 2 infections occurred with equal frequency in this epidemic. It is concluded that dengue 1 is a broader antigen or is more antibody avid than other types. The lower incidence of dengue 3 and 4 CF antibody parallels the isolation data for these types.

Table 30 illustrates the multiplicity of reaction of acute-convalescent human sera with HI and CF antigens. It is obvious that monovalent or bivalent response is rare and that multiple reactions are the rule. Not shown is data showing a similar frequency of reaction of JE - CF antigen with HF sera.

Figure 4 shows HI and CF antibody response to chikungunya virus. HI antibody response is slower and of lower magnitude than that following dengue infection while CF antibody response is definitely delayed. Decline of antibody titers in convalescence is rather slow.

Summary and Conclusions: HI antibody rises preceptitously following dengue hemorrhagic fever. Decline of HI antibody is also very rapid beginning as early as 1 month after infection. CF antibody response is also prompt although of lower magnitude. Antibody response to chikungunya virus is quite different; HI antibody rises fairly rapidly but with mean values 40 times lower than those following dengue. CF antibody is delayed, rarely being detectable until 15 or more days following infection. Data has been presented to show the broader reactivity of dengue 1 virus in testing HF sera. Using the HI test various dengue antigens may be used interchangeably, while most sera react with 4 or more CF antigens, high titered reactions with dengue 1 and 2 are most common. It is impossible to predict the type of infecting dengue virus by HI or CF tests of human sera. Studies are continuing.

Table 26. Hemorrhagic fever patients with adequate paired sera but with fixed HI and CF antibody titers to dengue 1 equal to or less than 1:320 and 1:16, respectively. Acute serum collected on 1st day of hospitalization and 2nd serum 14-21 days later.

	1962	1963
Number of "negative"	13	25
Total studied	143	147
Per cent	8.8	17.0

Table 27. Occurrence of HI antibody to dengue 1 at a titer of 1:640 or greater in 103 surgical patients admitted to hospital through the hemorrhagic fever season of 1962.

Cases	Total Studied	HI Titer 1:640 or greater on admission	%
Surgical cases	103	4	3.8

Table 28. Four-fold or greater antibody rise in 46 paired HF sera using dengue 1 - 6 HI and CF antigens.

		4x or greater antibody response with indicated antigen					
		D 1	D 2	D 3	D 4	D 5 (TH-36)	D 6 (TH-Sman)
HI	Number	31	39	27	19	27	25
	%	67.4	63.0	58.3	41.3	58.3	54.3
CF	Number	31	30	23	20	24	27
	%	67.4	65.2	50.0	43.5	52.2	58.3

Table 29. High-fixed antibody or four-fold antibody response in 46 paired HF sera using dengue 1 - 6 HI and CF antigens. HI titers of $\geq 1:640$ and CF titers of $\geq 1:32$ considered positive.

		High fixed titer or 4x antibody response with indicated antigen					
		D 1	D 2	D 3	D 4	D 5 (TH-36)	D 6 (TH-Sman)
HI	Number	46	46	46	45	45	46
	%	100	100	100	97.8	97.8	100
CF	Number	44	40	24	21	36	35
	%	95.7	86.9	52.2	45.6	78.3	76.1

Table 30. Multiplicity of "positive" HI and CF antibody response with dengue 1 - 6 viruses in 46 paired HF sera.

Reactivity* to	HI		CF	
	Number	%	Number	%
All types (D1-D6)	44	95.6	10	21.7
Five types	2	4.4	12	26.1
Four types	0	0	13	28.3
Three types	0	0	6	13.0
Two types	0	0	4	8.7
One type	0	0	1	2.2

* Titers in paired sera either show 4 fold or greater rise or fixed at 1:640 (HI) or 1:32 (CF)

FIGURE 3. HI AND CF ANTIBODY RESPONSE FOLLOWING DENGUE CAUSED HEMORRHAGIC FEVER IN 46 PATIENTS

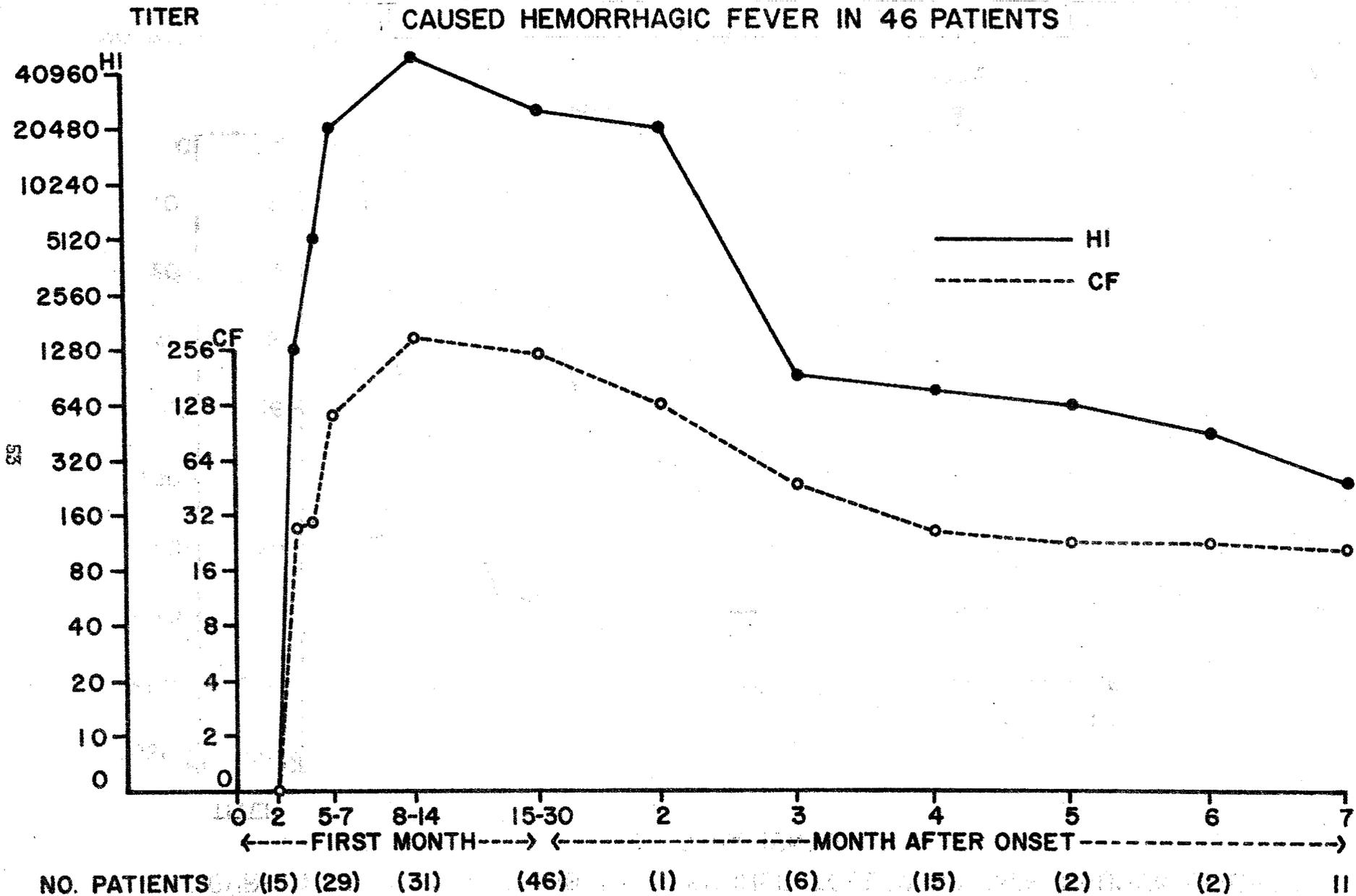


FIGURE 4. HI AND CF ANTIBODY RESPONSE FOLLOWING CHIKUNGUNYA DISEASE IN 16 PATIENTS

