

Isolation of Dengue Viruses from Leukocytes and Plasma of Dengue Hemorrhagic Patients

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OBJECTIVE : To continue studies on isolation of dengue viruses from dengue hemorrhagic fever (DHF) patients of the Children's Hospital; to determine if virus can be isolated from leukocytes during natural dengue infections and to identify the cells infected.

BACKGROUND : Previous studies have shown that dengue viruses can be isolated from leukocyte preparations taken from patients with dengue hemorrhagic fever (1); these studies were extended.

With this report we adopt the convention of reporting all isolations for a single calendar year, in this case January 1st, 1977 to December 31st, 1977. This period of reporting encompasses a single "dengue season" and will considerably facilitate year to year comparisons of DHF epidemics. Approximately half of specimens included in this report were concluded in last year's report.

METHODS : Clinical histories and heparinized blood samples were obtained from patients admitted to Bangkok Children's Hospital. The first day of fever was defined as the first day of illness. A follow-up serum sample was requested approximately 15 days after admission.

Heparinized blood specimens were separated into plasma and cell fractions by centrifugation. The cell fraction was resuspended in a solution of Dextran T-250, the red cells were allowed to sediment, and subsequent slow centrifugation pelleted the leukocytes. Viruses were isolated from plasma or leukocyte populations using the direct or delayed plaque method on LLC-Mk₂ cells.

Characterization of infected cells as "adherent" or "non-adherent" was accomplished by adding leukocyte suspensions to tissue culture flasks and incubating the cells for 2 hours at 37°C. Media and non-adherent cells were withdrawn, and the adherent cells in the flask washed once. LLC-Mk₂ cell suspensions were added to the flask, incubated overnight, and overlaid with agar. The non-adherent cells which had been withdrawn from the flask were mixed with a suspension of LLC-Mk₂ cells, put in a second flask, incubated overnight and

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overlaid with agar.

RESULTS : A total of 81 viruses were isolated from the plasma and/or leukocytes of 336 patients hospitalized with hemorrhagic fever during 1977 (Table 1). Chikungunya virus was isolated from 2 patients; one was obtained from plasma while the other was isolated from leukocytes obtained from blood drawn on the eighth day of illness. The dengue virus isolates consisted of 67 strains which could be classified as Den - 2, 3 or 4 and 12 other viruses that could not be identified. The latter viruses were considered to be dengue, as the serum of the patients from which these viruses were isolated showed a significant rise in dengue HI antibody titers. Nine of the dengue viruses were isolated from plasma alone, 14 from plasma and leukocytes and 56 from leukocytes alone (Table 2). Viruses were not isolated from plasma specimens with HI antibody titers against homologous antigens greater than 1:2560; however, viruses were isolated from the peripheral blood leukocytes (PBL) of patients with homologous HI antibody titers of 1:10240 or greater (Table 3).

Most dengue virus isolations were obtained from plasma and leukocytes during the first five days of illness (Table 4), and the majority of the isolates were obtained during the febrile period.

Direct plaquing methods showed a range of 2 to 100 infectious centers per 3×10^5 leukocytes. The small number of infectious centers indicated that relatively few of the leukocytes produced infection in LLC-Mk₂ cell cultures. In initial trials to ascertain the type of leukocytes infected, virus isolations were attempted from cells adherent to plastic tissue culture flasks and from non-adherent cells. Dengue viruses were isolated from twenty-one sets of adherent and non-adherent cell populations (Table 5). Eleven sets yielded virus from adherent cells only, 3 sets from non-adherent cells and 7 sets from both adherent and non-adherent cells. Investigations are underway to further characterize the infected cell populations.

Table 1. Virus isolations from plasma and/or leukocytes

Virus	Isolation	
	No.	%
Chikungunya	2	2.5
Dengue-1	0	0.0
Dengue-2	37	45.7
Dengue-3	10	12.3
Dengue-4	20	24.7
Dengue-?	12	14.8
Total	81	100

Table 2. Dengue virus isolations from plasma and/or leukocytes

Specimen	Isolations	
	No.	%
Plasma only	9	11.4
Leukocytes only	56	70.9
Both Plasma and Leukocytes	14	17.7
Total	79	100

Table 3. Identified dengue virus isolations from plasma and leukocytes tabulated against the patients homologous HI titers

Homologous ¹ Titers	Isolations			
	Plasma	Leukocytes	Both	Total
10	-	2	2	4
20	-	4	4	8
40	2	4	4	10
80	1	6	-	7
160	-	7	3	10
320	-	5	-	5
640	-	3	1	4
1280	3	4	-	7
2560	2	3	-	5
5120	-	2	-	2
10240	-	5	-	5
Total	8	45	14	67

¹ Reciprocal HI antibody titer

Table 4. Isolation of dengue viruses from plasma and leukocytes of dengue hemorrhagic fever patients.

Day of Disease	Total Studied	Virus Isolation			
		Plasma	Leukocytes	Both	Total
2	4	-	3	1	4
3	25	1	4	5	10
4	76	3	17	6	26
5	93	2	22	1	25
6	58	1	4	-	5
7	44	1	4	1	6
8-10	36	1	2	-	3
Total	336	9	56	14	79

Table 5. Isolation of dengue viruses from adherent and non-adherent leukocytes.

Leukocytes	Isolation	
	No.	%
Adherent cells only	11	52.4
Non-adherent cells only	3	14.3
Both adherent and non-adherent cells	7	33.3
Total	21	100

REFERENCE :

1. Scott, R.M., Nisalak, A., Usa, C., Seridhoranakul, S., Nimmannitya, S., Isolation of Viruses from Leukocytes of Dengue Patients. AFRIMS Annual Progress Report, April 1976-September 1977, pp. 55-62.