Plasma and Serum as a Source of Dengue Virus in Dengue Hemorrhagic Fever Patients

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BACKGROUND: In studies reported in last year's Annual Report (pages 88–92), dengue virus was isolated from DHF patients more commonly from plasma than from serum. This difference was evident especially in children presenting with high dengue–2 HI antibody titers ($\geq$ 1 : 160). The significance of this finding was lessened by the small numbers of patients with isolates and by technical limitations in design. Isolations from serum and from plasma were handled by different technicians.

Studies of the role of individual dengue antigens recently described by WRAIR and other laboratories in the pathogenesis of DHF are planned. Interpretation of these projected studies will depend upon identification of the serotype of dengue virus currently infecting a DHF patient. By conventional isolation techniques using serum, isolates have been obtained from 15–20% of DHF patients and only from those patients admitted with low ($<$ 1 : 160) dengue HI titers. More efficient methods of virus isolation, especially from patients presenting with high antibody titers are desired. Consequently, during the 1972 DHF season, the isolation efficiency of plasma versus serum was studied.

METHODS: Studied were 68 patients admitted to Children's Hospital with a clinical diagnosis of DHF. Serum obtained on admission and discharge was tested against dengue 1–4, JEV, and Chikungunya antigens in HI tests. Patients were considered to have dengue infections if a 4–fold or greater rise in titer was shown to at least one dengue antigen or if titers were fixed but $\geq$ 1 : 640 to at least 2 dengue antigens.

For isolation studies, 10 ml of blood was obtained on admission. A 2 ml aliquot was allowed to clot and serum was separated for isolation. The remaining 8 ml aliquot was placed into 15 ml centrifuge tubes coated with heparin (80 units); heparinized blood was centrifuged at 600 rpm for 5 minutes at 4°C. The buffy coat layer was gently aspirated. Remaining whole blood was centrifuged at 1500 rpm for 15 minutes. Plasma was aspirated and pooled with the buffy coat layer to be used as cell–rich plasma for isolation studies.

The remaining whole blood was centrifuged at 5000 rpm for 15 minutes and the upper two–thirds of the supernatant aspirated with care to avoid platelet contamination. This supernatant was recentrifuged at 5000 rpm for 15 minutes, the upper two–thirds of the supernatant was aspirated and served as cell–free plasma for isolation attempts.

Each specimen was inoculated onto 3 flasks of LLC–MK2 cells; isolation was performed by the standard method of direct and delayed plaques. All isolation attempts were performed by the same technician. White blood cell and platelet counts were carried out on all cell–rich and cell–free plasma specimens used for isolation.

RESULTS: Of the 68 patients studied, 13 lacked serologic evidence of dengue infection; no isolates were obtained from these patients. Of the 55 patients with serologically confirmed dengue infections, 12 dengue isolations were obtained; all were dengue 2. Table 1 shows results of isolations from serum, cell–rich plasma, and cell–free plasma from the dengue patients. Dengue 2 virus was isolated from 6 of the 7 patients with admission HI titers $\leq$ 1 : 80 and from 7 of the 13 patients studied with HI titers $\leq$ 1 : 160. In these patients, isolation was equally efficient from serum or plasma specimens.

Dengue 2 virus was isolated also from 5 of 33 patients admitted with dengue 2 HI titers of 1 : 320–1 : 1280. No isolates were obtained from serum of these patients, but 5 isolates were from cell–free and 4 from cell–rich plasma.

No isolates were obtained from the 9 patients with initial dengue 2 HI titers $\geq$ 1 : 2560.

Little difference in isolation efficiency between cell–rich and cell–free plasma was found, even in those patients without virus in serum. Generally, these cell–free plasma preparations were indeed cell–free. White blood cells were not detected in any cell–free plasma preparation yielding virus, while platelet contamination ($1–3x10^3/mm^3$) was detected in only 5 of 12 cell–free preparations yielding virus. In the 5 patients with serum negative for dengue virus, WBC and platelets were not detected in cell–free plasma yielding virus in 3 patients.

DISCUSSION: These results confirm the limited data from the 1972 study in defining a small but important difference in the efficiency of isolation of dengue virus from plasma and serum of DHF patients. No difference in isolation efficiency was found in patients with low ($\leq 1:160$) admission dengue 2 HI titers, but all 5 isolates from patients with initial titers $\geq 1:320$ were obtained from plasma only. Our initial hypothesis that an increased isolation rate from plasma might be due to the presence of cell or platelet associated virus in plasma is not supported since no differences between cell–rich and cell–free plasma was found in patients lacking virus in serum. That the decrease in isolation efficiency might be due to adherence of virus to fibrin in the clot does not seem likely in view of the differences between isolation from serum in patients with low and high admission HI titers. Possibly, heparin itself influences neutralization of virus in high–titered plasma, permitting incompletely neutralized virus to infect LLC–MK2 cells. Further studies will be required to test this hypothesis. Since virus was isolated from only 22% of the patients with DHF by the most efficient specimen (plasma), other approaches to increase the efficiency of dengue isolation in DHF patients are indicated.

Table 1. Dengue 2 Isolations from Serum, Cell–rich Plasma, and Cell–free Plasma Related to Dengue 2 HI Titer in Serum Used for Isolation

Dengue 2 HI Titer (Reciprocal)	Number of Patients	Number of Isolates			
		Total	Serum	Plasma	
				Cell–rich	Cell–free
≤ 20	3	3	2	3	3
40	3	3	3	2	2
80	1	0			
160	6	1	1	1	1
320	5	1	0	1	1
640	16	2	0	1	2
1280	12	2	0	2	2
2560	4	0			
5120	2	0			
10240	0	0			
≥ 20480	3	0			
Total	55	12	6	10	11