

Nepuyo virus, a new group C agent isolated in Trinidad and Brazil

II. Serological studies[♦]

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Nepuyo virus was first isolated from *Culex (Eubonnea) accelerans* in Trinidad by Spence et al. in 1957¹. Shope and Causey, in reporting the serological relationships of 205 group C arbovirus strains from Belém, stated:

Five strains, isolated from a single mouse group, failed to conform to any of the prototype patterns [at that time recognized] and were set aside for future study as a possible new type².

The present paper gives details of the serological studies in which these five Belém strains plus the original Trinidad isolate were shown to represent a new serological type in group C.

MATERIALS AND METHODS

Four strains of Nepuyo virus were used: TRVL 18462, the original Trinidad isolate, and three Belém strains, BE An-10655, BE An-10656 and BE An-10709, each derived from a different baby mouse of the same sentinel group. Since serum of suckling mice infected with BE An-10709 yielded a hemagglutinating antigen, this strain was chosen as the Belém prototype.

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The previously described group C strains used were as follows: Oriboca, BE An-17 and BE An-913; Marituba, BE An-15; Apeu, BE An-848; Murutucu, BE An-974 and BE An-879; Caraparu, BE An-3994 and BE H-5546; and Itaquí, BE An-12797.

Complement-fixation (CF) testing was performed in plastic plates by a microtechnique modified from Fulton and Dumbell³. Approximately two units of complement were used, with primary incubation overnight at 4°C. Hemagglutinins were prepared from the sera of suckling mice by acetone extraction. Hemagglutination-inhibition (HI) testing was done in lucite trays using goose red blood cells, according to the techniques of Clarke and Casals⁴. Neutralization (N) testing was done in 3-day-old mice by the constant serum, varying virus dilution method. Serum-virus mixtures were incubated for one hour at 37°C and inoculated intraperitoneally (i.p.)

For HI and CF testing, both immune and hyperimmune sera were prepared in adult mice inoculated i.p. Hyperimmune sera were produced by injecting live virus, as indicated in Table 2. Immune sera were made as follows: For BE An-10709, Apeu and Marituba viruses, mice were given a single injection of 10% infected mouse liver and bled three weeks later. TRVL 18462 serum was made with two injections of infected mouse brain. For Oriboca, Murutucu, Caraparu and Itaquí viruses, a 10% liver suspension inactivated for one hour at 37°C with 0.5% beta-propiolactone was administered on days 1 and 3, live vaccine was given on day 10 and the mice were bled on day 21.

Immune sera for N testing were prepared in guinea pigs given a single i.p. inoculation of 10% infected mouse liver and bled between 13 and 20 days postinoculation.

RESULTS

TRVL 18462 and BE An-10709 viruses were independently recognized as group C agents in Trinidad and Brazil, respectively^{1,2}. Reciprocal cross CF and HI testing subsequently showed TRVL 18462 to react in a manner indistinguishable from that of BE An-10709.

Next, reciprocal cross-HI studies with the BE An-10709 strain hemagglutinin and immune mouse sera for group C prototype viruses demonstrated that Nepuyo virus differed from the known members of group C (Table 1). Cross-reactivity was noted with Murutucu serum but not with sera for other group C prototypes. When hyperimmune sera were used (Table 2), titers were higher in the homologous systems and a greater degree of cross-reactivity was noted.

The results of N testing (Table 3) closely followed those obtained in HI testing. Nepuyo virus was distinct by N test but cross-reacted to a significant degree with Murutucu, Marituba and Itaquei viruses. Again, the most marked cross-reaction occurred with Murutucu serum.

In CF testing (Table 4), Nepuyo virus also was distinct from the six group C types. Cross-reactions were observed with Marituba and Apeu viruses, and to a lesser degree with Murutucu and Oriboca.

Table 1 – HI relationships of Nepuyo virus to previously described group C prototype viruses using mouse immune sera

Antigen	Serum							
	Nepuyo BE An-10655	Nepuyo BE An-10656	Murutucu An-974	Marituba An-15	Apeu An-848	Caraparu An-3994	Oriboca An-17	Itaqui An-12797
Nepuyo, BE An-10709	>320*	>320	40	0	0	0	0	0
Murutucu, BE An-974	0	0	>320	0	0	0	0	0
Marituba, BE An-15	0	0		80				
Apeu, BE An-848	0	0			40			
Caraparu, BE An-3994	0	0				320		
Oriboca, BE An-17	0	0					80	
Itaqui, BE An-12797	0	0						80

* Reciprocal of the serum dilution inhibiting eight antigen units; 0 = less than ten.

Table 2 – HI relationships of Nepuyo virus to group C viruses using mouse hyperimmune sera

Antigen	Serum							
	Nepuyo BE An-10709 2 inj.	Nepuyo TR 18462 4 inj.	Murutucu BE An-879 2 inj.	Caraparu BE H-5546 2 inj.	Apeu BE An-848 1 inj.	Oriboca BE An-913 2 inj.		
Nepuyo, BE An-10709	2560*	1280	160	40	0	0		
Murutucu, BE An-974	320	160	640	640				
Caraparu, BE An-3994	160	80			640			
Apeu, BE An-848	160	80			640			
Oriboca, BE An-17	0	0				160		

* Reciprocal of the serum dilution inhibiting eight antigen units; 0 = less than 40.

Table 3 – N test relationships of Nepuyo virus to group C viruses using guinea pig immune sera

Serum	Virus						
	Nepuyo An 10709	Murutucu An 974	Marituba An 15	Apeu An 848	Caraparu An 3994	Itaqui An 12797	Oriboca An 17
Nepuyo, BE An 10709	≥5.1*	≤2.1	2.4	0	0	1.7	0
Murutucu, BE An 974	3.8	4.6					
Marituba, BE An 15	2.3		≥4.1				
Apeu, BE An 848	0			≥4.8			
Caraparu, BE An 3994	0.5				5.2		
Itaqui, BE An 12797	3.0					≥4.4	
Oriboca, BE An 17	0						≥6.7

* Log neutralization index.

Table 4 – CF relationships of Nepuyo virus to group C viruses using mouse immune

Antigen	Serum							
	Nepuyo TR 18462	Nepuyo BE An 10709	Marituba An 15	Apeu An 848	Murutucu An 974	Oriboca An 17	Caraparu An 3994	Itaqui An 12797
Nepuyo, TR 18462	16/≥128*	32/128	0/0	0/0	0/0	0/0	0/0	0/0
Nepuyo, BE An 10709	-	32/8	0/0	0/0	0/0	0/0	0/0	0/0
Marituba, BE An 15	8/64	8/16	64/128					
Apeu, BE An 848	8/128	8/64		64/128				
Murutucu, BE An 974	0/0	8/32			128/32			
Oriboca, BE An 17	0/0	4/32				64/128		
Caraparu, BE An 3994	0/0	0/0					32/64	
Itaqui, BE An 12797	0/0	0/0						32/≥128

* Reciprocal of the serum dilution over the reciprocal of the antigen dilution fixing complement. The first dilution used was 1:4. Results are a composite of two tests.

SUMMARY

Nepuyo virus, represented by one strain from Trinidad and five from Brazil, reacts by hemagglutination-inhibition (HI), neutralization and complement-fixation (CF) testing with previously described group C types but is distinct from them. By HI and neutralization testing Nepuyo is most closely related to Murutucu virus, and by CF testing to Apeu and Marituba viruses.

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