

Immunoglobulin Response and Viremia in Dengue Vaccinated Gibbons Repeatedly Challenged with Japanese Encephalitis Virus

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DESCRIPTION: The background and preliminary results of this project can be found in SMRL Annual Report, 1970-1971. Briefly we have studied the humoral immunological response of gibbons previously infected with dengue viruses to repeated challenge with JEV. The purpose of the study was to attempt to improve serological specificity in secondary group B arbovirus infections. The study also provided a unique opportunity to examine whether previous dengue infections protected against JEV infection. With this report the study is concluded.

RESULTS: First JEV Challenge.

Table 1 records the prior dengue exposure of the eight gibbons, the presence or absence of detectable JE viremia following JEV inoculation, and the antibody response as measured by the three standard serological tests. None of the gibbons evidenced clinical illness after JEV challenge. JE viremia was detected in 5 of 8 animals. Two gibbons (S-70, S-71) had preexisting JEV and dengue neutralizing (Nt) antibody on day 0, and one had antibody to one or more dengue serotypes without detectable JEV Nt antibody; 4 of these gibbons had viremia. Thus the presence of JEV or dengue Nt antibody in acute sera did not protect against JE viremia. No attempt was made to titer the amount of virus present in viremic serum.

Listed in Table 1 are the serological results in preinfection serum (day 0) and in a representative convalescent serum (day 21). The serological responses of the 8 gibbons were remarkably uniform and were characteristic of the broad serological response seen in secondary group B arbovirus infections; all showed diagnostic HI, CF, and Nt antibody titer rises to JEV and to two or more of the 4 dengue serotypes. The JEV HI titers were significantly higher (≥ 4 -fold) than the highest dengue HI titers in only 2 gibbons (S-92, S-94), and JEV CF and Nt titers in all 8 gibbons were equal to or less than the highest dengue CF and Nt titers. Thus we could not reliably identify the most recent group B infection as JEV in these dengue-vaccinated gibbons using routine serological tests.

In an attempt to improve serological specificity for recent JEV infection, we tested for the presence and immunospecificity of IgM contained in acute and convalescent serum. The acute and convalescent whole serum from 2 gibbons was first treated with 2-ME. As seen in Table 2, the HI titers against D1-4 and JEV were not reduced after 2-ME treatment. Thus IgM antibody could not be detected in whole serums. Nevertheless we suspected that IgM might exist, but was masked by high titered, 2-ME resistant IgG antibody in whole serum. Attempts were therefore made to isolate IgM and IgG by sucrose density gradient centrifugation (S-DGC) of serum specimens.

Representative S-DGC assay results on a convalescent serum (gibbon S-94, day 28) are listed in Table 3. Fractionation of this serum revealed that all of the IgM detectable by radial immunodiffusion was concentrated into fractions 4 and 5, whereas IgG was concentrated into fractions 7-10, and IgA fraction 8. 2-ME sensitive HI antibody, limited to fractions 3-5, was found to react with JEV but not dengue 1-4. In contrast HI antibody in fractions 7-12 reacted in high titers with both JEV and dengue 1-4 antigens; was resistant to 2-ME treatment, and was principally IgG by radial immunodiffusion. The presence of

JEV—monospecific IgM antibody in the convalescent serum (Table 3) and the absence of such antibody in the acute serum (day 0; not shown) provide strong serological confirmation of a recent JEV infection.

Sera of the 8 test gibbon sera obtained before and after JEV infection were fractionated and the results of these assays are tabulated for IgM in Table 4. Newly produced JEV—monospecific IgM antibody was first detected 14 and 21 days after JEV infection in 7 of 8 gibbons; it was not detected in any gibbons by day 90.

The S—DGC fractionation technique permitted us to approximate the time of appearance of IgG as well as IgM. As shown in Table 5, serum IgG HI heterospecific antibody appeared coincident with or before IgM monospecific antibody in all of the gibbons.

Second JEV Challenge

Seven gibbons were challenged a second time with JEV 13 months after the first JEV inoculation. The purpose was to determine 1) whether a first JEV infection is associated with prevention or attenuation of a second infection as measured by viremia and serological response and 2) whether JEV monospecific IgM antibody is produced after a second JEV challenge.

Table 6 records the presence or absence of detectable viremia following the second challenge and the antibody response measured by the three standard serological tests. As noted after the first JEV challenge, none of the gibbons displayed clinical illness.

Viremia was not detected in these rechallenged animals on days 2, 3, 4, and 6, after infection. The high-titered JEV Nt antibody induced 13 months earlier had fallen to lower titers by day 0 of the second challenge, but in only one gibbon (S—81) had Nt antibody declined to titers of less than 1:10. In marked contrast to the antibody response noted after first JEV challenge when all gibbons demonstrated titer rises to dengue and JEV in the 3 serological tests, only one gibbon (S—51) demonstrated a broadly cross-reactive serological response after second challenge. Further comparison of antibody responses revealed attenuated titer rises with depression of titer levels in convalescent sera following second challenge. The blunting of the immune response was particularly marked against the dengue antigens.

No IgM HI antibody was found in sera drawn 14, 21 & 28 days after second challenge.

SUMMARY: The response of primates to serial group B arbovirus infections was investigated by inoculating gibbons with live Japanese encephalitis virus (JEV) 17 to 21 months after infection with multiple dengue virus serotypes. Five of 8 gibbons developed detectable JE viremia, and all 8 animals had high-titered cross-reactive serum antibody titer rises to group B antigens, indicating JEV replication in these dengue-vaccinated gibbons. The immunoglobulin response was characterized by early production of high-titered cross-reactive IgG antibody, and later production of low-titered IgM antibody reacting monospecifically with JEV. Thirteen months later 7 of these gibbons were reinoculated with JEV. In contrast to the first JEV challenge, no viremia was detected and the serological response, including IgM antibody production, was partially or completely aborted. This study suggests that prior infection with JEV, but not dengue, can protect gibbons against JEV inoculated more than one year later. In addition, serum IgM antibody induced by JEV infection in these gibbons previously infected with dengue serotypes is shown to be serologically specific for JEV. Sucrose—density gradient fractionation of serum and measurement of IgM antibody may thus provide a more precise serological procedure with which to investigate group B arbovirus infections in previously infected populations.

Table 1.
Serological response and viremia in dengue vaccinated gibbons[§] challenged with Japanese encephalitis virus (JEV)

Gibbon	Days after	Reciprocal antibody titer																
		Previous virus	JEV	HI					CF					Nt+				
				challenge	Viremia*	challenge #	D1	D2	D3	D4	JEV	D1	D2	D3	D4	JEV	D1	D2
S-36..... D-2,3,4	day 3	0		20	40	40	20	20	4	4	4	4	4	115	160	<40	65	<10
		21		160	320	640	160	640	32	32	32	128	32	500	675	<160	350	1000
S-51..... D-2,2,	day 3,5	0		0**	0	0	0	0	0##	0	0	0	0	<40	50	<40	<40	<10
		21		320	320	640	320	1280	32	32	64	32	64	450	1000	<160	650	800
S-61..... D-4,1,2	neg	0		20	20	20	40	0	4	8	4	4	0	375	50	<40	50	<10
		21		160	160	320	160	640	64	64	64	64	32	400	2600	<40	500	1000
S-70..... D-2,3,4,2	day 3	0		20	40	80	40	20	16	8	4	8	4	90	200	25	70	15
		21		160	160	320	160	640	32	128	32	128	128	250	950	100	95	1200
S-71..... D-4,2,3	neg	0		0	20	20	20	0	4	4	4	8	0	30	160	<10	110	20
		21		80	160	160	80	320	16	32	32	32	16	25	1050	90	380	500
S-81..... D-3,2,4	neg	0		40	40	80	20	20	4	4	4	4	4	40	80	70	20	<10
		21		80	80	1280	80	320	16	16	32	32	32	130	400	40	600	200
S-92..... D-3,4,1	day 5	0		20	20	20	20	20	4	4	4	4	4	<40	60	<40	<40	<10
		21		320	320	640	320	2560	32	64	256	512	128	400	2000	30	2560	3050
S-94..... D-3,4,2	day 3	0		0	40	0	0	0	0	0	0	0	0	25	35	<40	<40	<10
		21		160	160	320	160	1280	8	16	32	64	32	230	600	40	1380	2950

§ Last dengue inoculation 17-21 months before first JEV inoculation; dengue virus serotypes 1,2,3,4 = D1,2,3,4.

* Monitored 3,5 and 8 days after JEV challenge.

Each gibbon inoculated sc with 1×10^5 pfu JEV.

+ 50% plaque reduction neutralization test

** 0 = <1:20 for HI

0 = <1:4 for CF

Table 2.
Effect of 2-mercaptoethanol (2-ME) on the HI Antibody Titers in Whole Gibbon Serum

Gibbon	Day after JEV inoc.	Reciprocal HI Titer			
		Dengue-1,2,3,4		JEV	
		C ⁽¹⁾	2-ME ⁽²⁾	C	2-ME
S-51	0	<20	<20-20	<20	20
	21	320	320-640	1280	1280
S-94	0	<20-40	<20-40	<20	<20
	21	160-320	160-320	1280	1280

(1) Serum aliquot treated with buffer

(2) Serum aliquot treated with 2-mercaptoethanol

Table 3.

Fractionation* of convalescent serum from a dengue-vaccinated gibbon+ challenged with JEV

Serum—Sucrose	Immunoglobulin S			Reciprocal HI Antibody titer											
	IgM	IgG	IgA	JEV		D4		D3		D2		D1			
				C#	2ME@	C	2ME	C	2ME	C	2ME	C	2ME		
1	0	0	0	4	2	0	0	2	0	0	0	0	0	0	
2	0	0	0	4	2	2	0	2	0	0	0	0	0	0	
3	0	0	0	8	2	2	0	2	2	2	0	0	0	0	
4	+	0	0	32	2	2	0	2	2	2	2	2	2	2	
5	+	0	0	16	2	2	2	4	2	2	2	2	2	2	
6	0	0	0	4	2	2	2	8	4	4	2	4	4	2	
7	0	+	0	16	8	4	4	8	8	8	8	4	4	4	
8	0	+	+	64	64	32	16	32	32	32	16	16	16	16	
9	0	+	0	128	128	32	16	32	64	32	32	32	32	32	
10	0	+	0	16	16	8	8	32	32	16	8	8	8	16	
11	0	0	0	16	16	8	4	32	16	16	8	16	16	16	
12	0	0	0	32	16	16	8	64	32	32	16	32	32	16	

* Fractionation by sucrose density gradient centrifugation (S-DGC)

+ Serum obtained 28 days after JEV inoculation of gibbon S-94

S Presence of immunoprecipitable gibbon immunoglobulin in each fraction assayed by radial immunodiffusion in agar containing

anti-human IgM, IgG, or IgA; + = Ig detected, 0 = Ig not detected

C = aliquot of serum sucrose-fraction treated with buffer

@ 2-ME = aliquot of serum-sucrose fraction treated with 2-mercaptoethanol

Table 4.
**Production and persistence of JEV monospecific IgM antibody in dengue-vaccinated
gibbons following JEV infection**

Gibbon	Day after JEV inoculation						
	0	7	14	21	28	56	90
S-36	0 ⁽¹⁾	0	+ ⁽²⁾	+	+	+	0
S-51	0	0	0	+	+	0	
S-61	0	0	0	+	+	+	0
S-70	0	0	0	+	+	0	
S-71	0	0	0	+	0	0	
S-81	0	0	+	+	+	0	
S-92	0	0	0	0	0	0	
S-94	0	0	+	+	+	0	0

(1) 0 = IgM JEV-specific antibody not detected in the serum drawn on that day

(2) + = IgM JEV-specific antibody detected in the serum drawn on that day

Table 5.
 Appearance of IgG and IgM HI antibody in the serum of dengue—vaccinated
 gibbons following JEV inoculation

Gibbon	Day Ig first detected ⁽¹⁾	
	IgG ⁽²⁾	IgM ⁽³⁾
S-36	14	14
S-51	14	21
S-61	14	21
S-70	14	21
S-71	7	21
S-81	14	14
S-92	7	not detected
S-94	7	14

(1) Data obtained from HI tests of S-DGC infections of serum drawn on days 0,7,14,21

(2) IgG antibody reactive against JEV and Dengue 1-4 antigens

(3) IgM data summarized from Table 4

Table 6.

Serological response and viremia in dengue-vaccinated gibbons challenged a second time with JEV

Gibbon	Days after		Reciprocal antibody titer																				
	previous virus	JEV	HI						CF						Nt ^{xx}								
challenge	Viremia* rechallenge #		D1	D2	D3	D4	JEV	D1	D2	D3	D4	JEV	D1	D2	D3	D4	JEV	D1	D2	D3	D4	JEV	
S-36	0		80	160	40	40	160	0#	4	4	4	4	0	0	80	0 ^S	45	20					
D-2,3,4, JEV+	14	Neg	40	80	80	40	1280	0	8	4	4	4	4	4	—	—	—	—					
	28		40	80	40	40	320	0	8	4	8	4	4	4	75	20	60	300					
S-51	0		20	40	20	20	80	0	0	0	0	0	0	0	35	10	45	30					
D-2,2, JEV	14	Neg	40	80	80	80	2560	0	4	4	4	8	32	32	—	—	—	—					
	28		40	40	40	40	1280	0	4	4	4	4	4	4	100	10	20	3300					
S-61	0		80	80	80	160	320	4	8	8	16	16	4	4	40	0	125	365					
D-4,1,2, JEV	14	Neg	40	40	40	160	320	8	8	8	16	16	4	4	—	—	—	—					
	28		40	40	40	80	320	8	8	8	16	16	4	4	125	60	80	530					
S-70	0		40	40	80	80	160	8	16	8	8	8	0	0	120	25	215	45					
D-2,3,4,2, JEV	14	Neg	40	40	80	80	640	8	16	8	8	8	8	8	—	—	—	—					
	28		40	80	80	80	640	8	16	8	16	16	8	8	60	30	30	30					
S-81	0		20	20	40	10	80	0	0	0	0	0	0	0	150	50	—	0					
D-3,2,4, JEV	14	Neg	20	20	40	20	320	0	0	0	4	4	4	4	—	—	—	—					
	28		20	20	40	20	320	0	0	4	4	4	4	4	45	20	0	90					
S-92	0		40	40	80	80	320	0	8	4	4	4	4	4	70	35	20	25					
D-3,4,1, JEV	14	Neg	40	40	80	40	320	0	8	8	8	8	8	8	—	—	—	—					
	28		40	80	80	80	320	0	8	8	8	8	8	8	20	10	20	200					
S-94	0		10	10	20	20	40	0	0	0	0	0	0	0	20	20	50	45					
D-3,4,2, JEV	14	Neg	40	40	80	40	1280	0	4	0	8	64	64	64	—	—	—	—					
	28		40	40	80	40	1280	0	4	4	8	32	32	32	20	15	10	13200					

* Monitored 2,3,4 and 6 days after JEV rechallenge.

Each gibbon inoculated sc with $2 \times 10^{4.5}$ pfu JEV.

xx 50% plaque reduction neutralization test; D1 not tested.

+ First JEV inoculation 13 months before 2nd inoculation.

0 = <1:4 for CF

S 0 = <1:10 for PRNT