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TECHNICAL MANUSCRIPT 159

CROSS-PROTECTION MECHANISM BETWEEN SINDBIS AND SEMLIKI FOREST VIRUSES IN MICE

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TECHNICAL MANUSCRIPT 159

CROSS-PROTECTION MECHANISM BETWEEN SINDBIS AND SEMLIKI FOREST VIRUSES IN MICE

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ABSTRACT

The mode of action and site for cross-protection among Group A arboviruses has been studied in mice using Sindbis and Semliki Forest (SF) viruses as a working model.

Swiss mice immunized with Sindbis virus by the intracerebral route demonstrated substantial resistance to intraperitoneal challenge with lethal doses of SF virus. Serologic examination by hemagglutination-inhibition, complement fixation, and neutralization revealed that antibody to SF virus first appeared in the serum of Sindbis-immune and normal mice four days after challenge. SF antibody attained maximum titers in Sindbis-vaccinated mice 10 days after challenge with SF virus and slowly declined thereafter. Antibody responses to SF virus in normal mice paralleled responses found in Sindbis-immune mice, but all normal mice succumbed to SF infection seven days after challenge.

Periodic titration of the blood, brain, liver, and spleen in 10- to 14-gram mice showed a detectable quantity of SF virus in the organs of both groups 24 hours after challenge. Maximum titers of SF virus appeared in the blood, liver, and spleen by the third day after challenge. Mean virus titers in the brain increased progressively in both groups of mice from the first through the seventh day, the last day of assay; on the seventh day, however, the titer of SF virus in the brains of Sindbis-immune mice was two to three logs less than that in the brains of normal mice.

The results imply (a) that an anamnestic response of circulating antibody to SF virus could not be demonstrated and thus could not account for cross-protection observed in mice immunized with Sindbis virus, and (b) that the brain appears to be the site for possible localized cross-production resulting from previous experience with a related virus.

CROSS-PROTECTION MECHANISM BETWEEN SINDBIS AND SEMLIKI FOREST VIRUSES IN MICE

Cross-protection among arboviruses has been demonstrated by numerous investigators; however, certain aspects of these immunological overlaps are not understood and require further elucidation. Past experience in this laboratory has shown Sindbis and Semliki Forest (SF) viruses to be acceptable models for further studies of variables encountered in cross-protection. In adult mice Sindbis virus elicits antibodies that do not cross react with Semliki virus antigen by hemagglutination-inhibition or neutralization; also, mice immunized with Sindbis virus demonstrate substantial resistance to intraperitoneal (IP) challenges with Semliki virus. Experiments were designed to determine (a) if there was an anamnestic response from antibodies that were not detectable in serum prior to challenge, and (b) location and length of time that challenge virus remained in the Sindbis-immune and normal hosts.

Swiss mice* (10 to 14 gram) were immunized by intracerebral (IC) inoculation with 10^5 $SMLD_{60}$ of Sindbis virus. Twenty-eight days after immunization equal numbers of Sindbis-immune and normal mice were challenged IP with 0.1 ml of a 10^{-3} dilution of Semliki Forest virus seed. Ten of the challenged mice were picked at specified intervals and exsanguinated. The blood was collected in three pools, each containing the blood of three to four mice.

Each serum pool was assayed by hemagglutination-inhibition (HI), complement fixation (CF) and neutralization (NT) procedures. The HI and CF tests were performed in micro-plates against eight units of antigen using serological techniques similar to those described by Clarke and Casals.** NT tests were conducted in suckling mice using the constant virus-serum dilution technique. The NT titer was defined as that dilution of serum capable of protecting 50 per cent of the assay mice against 150 suckling mouse intraperitoneal LD_{50} of SF virus.

The SF virus dilution used for the challenge was also titrated in Sindbis-immune and normal mice at the time of challenge. Table I shows the per cent of mice in the titration that succumbed to SF virus. The greatest resistance within the Sindbis-vaccinated group occurred among mice inoculated with the 10^{-3} dilution of SF virus seed. Progressively less resistance was observed among immunized mice that received higher dilutions of SF virus. This decrease of resistance as the challenge dose decreases is somewhat

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* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

** Clarke, D.H., and Casals, J. "Techniques of hemagglutination and hemagglutination-inhibition with arboviruses," Am. J. Trop. Med. Hyg. 7:561-573, 1958.

TABLE I. TITRATION OF CHALLENGE DOSE OF SEMLIKI FOREST VIRUS IN SINDBIS-IMMUNE AND NORMAL MICE

Dilution of SF Virus Inoculated	Per Cent of Deaths in Mice	
	Sindbis-Immune	Normal Control
10^{-3} a/	30	80
10^{-5}	40	70
10^{-6}	20	40
10^{-7}	30	20
10^{-8}	10	0

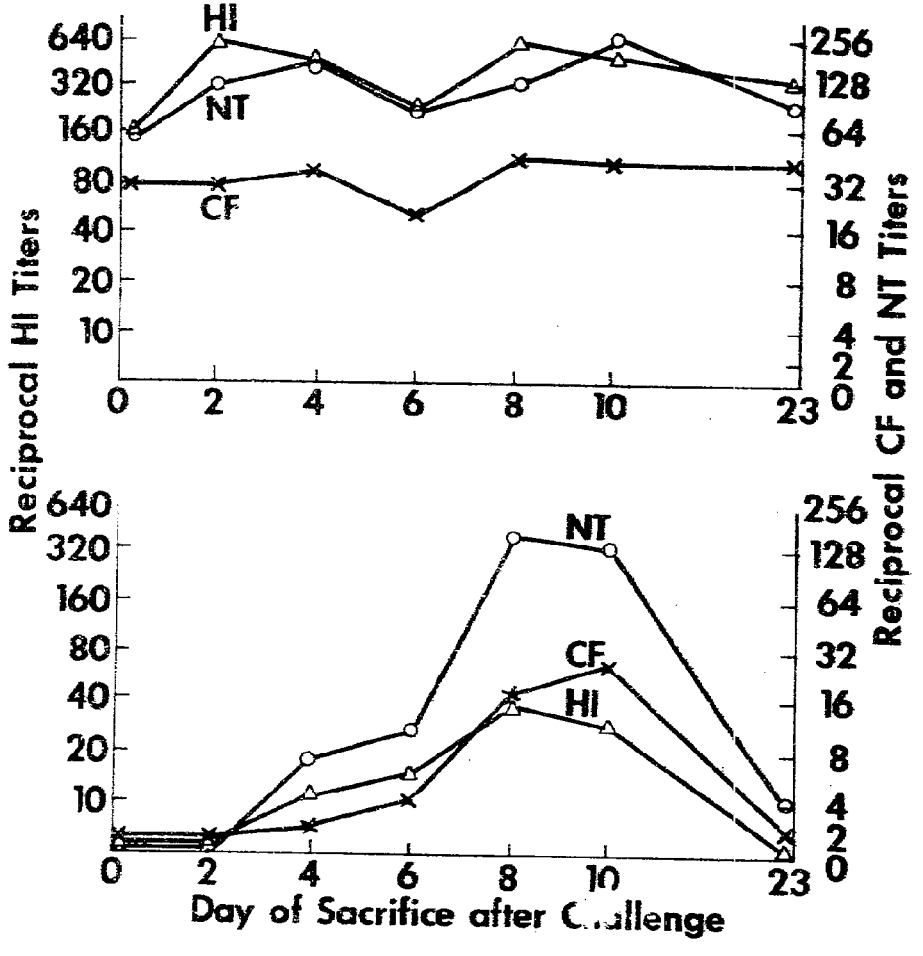
a. Challenge dilution of Semliki Forest virus.

characteristic of this heterotypic challenge system. The normal mice exhibited the expected end point with an LD_{50} titer of 5.6 log.

A comparison of HI, CF and NT results are shown in Figure 1 for sera of mice challenged with a heterotypic virus. Attention is first directed to data obtained from tests with Sindbis antigen (Figure 1 a).

Examination of the Sindbis-immune serum against Sindbis antigen (Figure 1 a) shows a continued increase in antibody titer through Day 4, particularly for the HI and NT reactions; however, by the sixth day the Sindbis-immune mice experienced a two- to four-fold loss and recovery in Sindbis antibody. Maximum antibody titers appear in 8 to 10 days with a gradual drop in HI and NT reactions by Day 23. Again, all three test procedures appear to agree on the general course of antibody response.

In Sindbis-immune mice, SF antibody (Figure 1 b) could be demonstrated on Day 4 by all three assay procedures; a maximum antibody titer was attained between 8 and 10 days. All three procedures closely agree on the course of antibody rise.



Sindbis antibody response in Sindbis-immune mice after challenge with SF virus

Semliki antibody response in Sindbis-immune mice after challenge with SF virus

Figure 1. HI, CF, and NT Serum Titers against Sindbis and Semliki Forest Virus Antigens.

The SF antibody response in nonimmunized was similar to that found in Sindbis-immune mice. The HI, CF and NT titers each appeared by Day 4 and increased through Day 6; after the seventh day all normal mice succumbed to SF virus.

From the data just examined, cross-protection was shown to be present in Sindbis-immune mice challenged with SF virus. No significant differences could be demonstrated between the rates of antibody formation to SF virus in normal and Sindbis-immunized mice, and it is concluded that the anamnestic response hypothesized for antibody to SF virus was not in evidence to account for the demonstrated cross-protection.

In a second experiment, Sindbis-immune and normal control mice were challenged IP with $10^{3.5}$ LD_{50} of Semliki Forest virus 28 days after immunization. Six mice were picked at random from each group and sacrificed at one hour, six hours, and one, two, three, five, and seven days after challenge. The blood, brain, liver and spleen were aseptically removed, and the individual organs were prepared as a 10 per cent suspension in brain-heart infusion broth. Each tissue suspension was titrated in 10- to 14-gram mice by the IC route to determine the LD_{50} of each sample.

The data obtained from assay of the individual organs and blood are recorded on Tables II, III, and IV and show the range of virus titers per six mice and the geometric mean per period of assay.

In Table II the viremias of Sindbis-immune and normal mice appear almost identical. Each group of mice demonstrated the presence of SF virus by Day 1 and a maximum virus titer by Day 2. SF titers diminished considerably from Day 3 through Day 7; however, some virus was still present in the immunized mice on Day 7.

Titration of liver suspensions showed essentially the same virus response as described for the blood, and since virus titers in the liver were usually lower than titers in the blood, it is probable that virus found in the liver was a reflection of the virus present in the blood in this organ.

As shown in Table III, SF virus was demonstrable in the spleens of Sindbis-immune mice 24 hours after challenge and reached a maximum titer by Day 2. For reasons not yet understood, titration samples taken on Day 5 proved to be toxic for mice and this toxicity prohibited a valid measurement of the virus. Some SF virus was still present in spleen samples on the seventh day after challenge.

TABLE II. VIRUS TITERS IN MOUSE BLOOD AFTER INTRAPERITONEAL INOCULATION OF SEMLIKI FOREST VIRUS

Time of Sacrifice	Sindbis Immune		Normal Control	
	Range	Mean[a]	Range	Mean
1 Hr.	0[b]	0	0	0
6 Hr.	0	0	0	0
1 Day	0-3.5	<1.0	0-3.7	1.1
2 Days	0-3.9	2.7	0-6.5	2.8
3 Days	0-4.5	2.0	0-4.5	1.9
5 Days	0-3.0	<1.0	0-1.4	1.0
7 Days	0-1.5	<1.0	0	0

a. Geometric mean of titers of tissues from 6 mice.
b. Titers represent log LD_{50} of virus per 0.03 ml of tissue. "0" titer means no virus was detectable in 0.03 ml of a 10 per cent suspension of tissue, the lowest dilution tested.

SF virus was present in the spleens of normal mice six hours after challenge; however, more distinct virus titers appeared by Day 1 and continued to increase through Day 3. Day 5 samples showed a decline in virus titer and did not appear to be toxic for assay mice. Again, some virus was present in the few remaining normal mice of Day 7.

The concentration of SF virus in the spleens of Sindbis-immune mice was frequently higher than those in blood or liver samples. This suggested that the spleen was probably a site of viral multiplication during the early phases of the infection. A comparison of the virus titers obtained in blood, liver and spleen showed considerable decline in virus multiplication after Day 3. In the previous experiment SF neutralizing antibody was shown to be present in immune and control groups of mice by Day 4 and could probably account for reduction in SF virus titers in the blood and viscera beyond Day 3.

TABLE III. VIRUS TITERS IN MOUSE SPLEEN AFTER INTRAPERITONEAL INOCULATION OF SEMLIKI FOREST VIRUS

Time of Sacrifice	Sindbis Immune		Normal Control	
	Range	Mean[a]	Range	Mean
1 Hr.	0[b]	0	0	0
6 Hrs.	0	0	0-2.2	< 1.0
1 Day	0-3.7	1.2	0-<1.5	< 1.0
2 Days	0-4.5	3.2	0-5.5	2.7
3 Days	0-4.2	2.7	2.5-4.6	3.7
5 Days	0-5.0	< 1.0	0-2.8	1.2
7 Days	0-3.1	1.0	0-1.0	< 1.0

a. Geometric mean of titers of tissues from 6 mice.
b. Titers represent log LD_{50} of virus per 0.03 ml of tissue. "0" titer means no virus was detectable in 0.03 ml of a 10 per cent suspension of tissue, the lowest dilution tested.

As in the other tissues examined, virus appeared in the brains of both groups of mice by Day 1 (Table IV). The average increase of virus in the brains of Sindbis-immune mice paralleled virus multiplication shown for normal control mice through Day 3 and may be an artifact caused by viremic blood in the brain. Unlike that in other tissues examined, virus titers in the brain continued to increase from Day 3 to Day 7, the last day of assay.

Although virus multiplication was still in progress in the brains of Sindbis-immune mice, a two- to three-log difference could be discerned between immune and nonimmune groups on Day 7. This can best be shown by Figure 2, which graphically demonstrates the suppression of virus multiplication in the immunized group and the unrestricted viral replication in normal mice. Dissemination of virus is believed to be initiated soon after

TABLE IV. VIRUS TITERS IN MOUSE BRAINS AFTER INTRAPERITONEAL INOCULATION OF SEMLIKI FOREST VIRUS

Time of Sacrifice	Sindbis Immune		Normal Control	
	Range	Mean[a]	Range	Mean
1 Hr.	0[b]	0	0	0
6 Hrs.	0	0	0	<1.0
1 Day	2.4	<1.0	0-1.0	<1.0
2 Days	1.0-3.2	1.8	0-4.5	1.8
3 Days	0-4.3	2.2	0-4.0	2.3
5 Days	0-6.5	3.3	0-6.3	5.0
7 Days	0-6.6	3.8	6.1-6.8	6.3

a. Geometric mean of titers of tissues from 6 mice.
b. Titers represent log LD_{50} of virus per 0.03 ml of tissue. "0" titer means no virus was detectable in 0.03 ml of a 10 per cent suspension of tissue, the lowest dilution tested.

intraperitoneal challenge and is probably carried via the peripheral circulation to liver, spleen and possibly the brain. The brain shows a continued increase in virus titer and is considered the "target" organ for viral infection.

From the data just examined, the blood and liver are only remotely involved in viral multiplication. Spleen samples show some evidence of increased viral replication; however, this is probably stifled by the appearance of neutralizing antibody on Day 4. Hence, the most prominent site of viral reproduction is the brain.

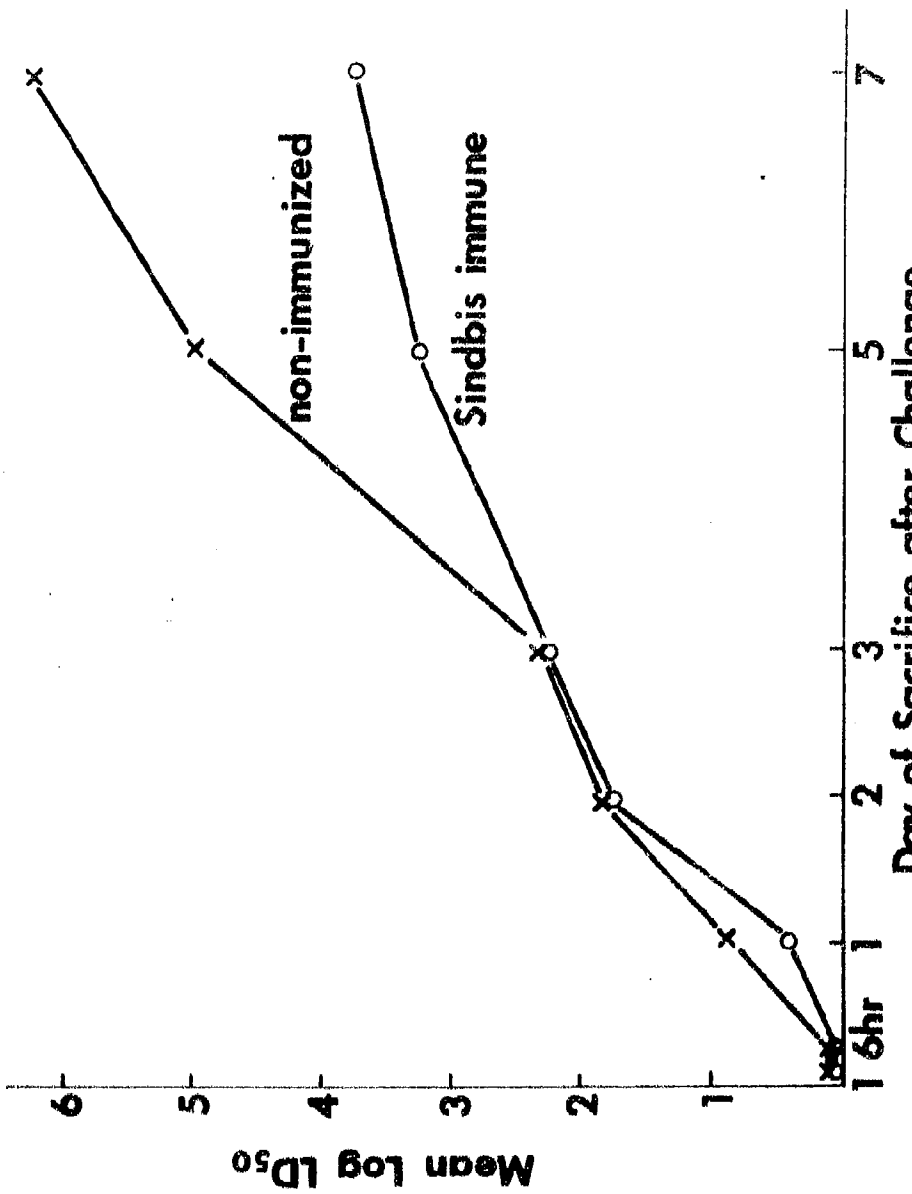


Figure 2. Average LD_{50} Titers for Semliki Virus in Mouse Brain.

Differences observed in SF brain titers (Figure 2) between Sindbis-immune and nonimmune mice appear to be the result of a localized resistance from previous experience with a related virus in the brain. The nature of this localized resistance is presently unknown; however, it might be speculated that the initial immunization sensitizes the tissues of the central nervous system in such a way that they suppress the multiplication of related viruses either by a localized production of antibody or by a refractory state of cell susceptibility.