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TECHNICAL MANUSCRIPT 16

CROSS-PROTECTION IN ANIMALS
INFECTED WITH GROUP A
ARTHROPOD-BORNE VIRUSES

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ABSTRACT

Experiments were carried out to investigate the phenomenon of cross-protection among Venezuelan equine encephalomyelitis (VEE), Eastern equine encephalomyelitis (EEE) and Semliki Forest (SF) viruses in a variety of laboratory animals after immunization by the intraperitoneal, subcutaneous, or respiratory route; the last was effected by exposing the animals to aerosols of virus. One injection of an attenuated strain of VEE (9t) protected guinea pigs against a lethal challenge dose of EEE or SF virus in guinea pigs and mice, respectively. Two injections of live SF virus protected guinea pigs against small doses of VEE or EEE virus. Mice vaccinated with 9t responded by demonstrating resistance mechanisms that appeared to operate in series. This consisted of, first, an early nonspecific interference phase, followed by a second, specific phase. The second phase also included a partially specific mechanism of resistance of unknown origin and of relatively long duration, manifested as cross-protection in the group A viruses.

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I. INTRODUCTION

An attenuated strain of Venezuelan equine encephalomyelitis (VEE) virus was recently isolated and described.¹ When this strain (9t) was used as an immunizing agent, it was found to induce not only a solid resistance in laboratory animals against a challenge with homologous virulent strains but also an immunity against the heterologous Eastern equine encephalomyelitis (EEE) virus to a substantial degree.^{2,3} The latter occurred without evidence of cross-neutralizing antibody. In this report, further investigations designed to characterize the cross-protective phenomenon are described. Attention was given to the time of onset, the level, and the persistence of the acquired homologous and heterologous immunity.

II. MATERIALS AND METHODS

A. VIRUS STRAINS

The attenuated VEE virus, 9t, used to immunize mice, guinea pigs, or rhesus monkeys, the virulent VEE seeds designated PES and CES, and the EEE virus seeds have been described elsewhere.^{1,3} Semliki Forest (SF) virus* was obtained from Dr. W.P. Allen of this laboratory.

B. VIRUS IMMUNIZATIONS

Twelve- to fourteen-gram mice and 200- to 300-gram guinea pigs were injected with $10^{3.4}$ MICLD₅₀ of 9t by the intraperitoneal (IP) or subcutaneous (SC) route. Mice received maximum doses of $10^{3.4}$ and guinea pigs $10^{4.4}$ MICLD₅₀ of 9t by the respiratory route, using a method described previously.³ Monkeys received $10^{5.2}$ MICLD₅₀ of 9t by the respiratory route in the same manner. Immunizations with EEE and SF viruses were carried out by injecting approximately 10^7 and $10^{5.8}$ MICLD₅₀ of each strain, respectively, by the IP route.

C. VIRUS CHALLENGES

Challenge doses of virus were administered to immunized guinea pigs 21 to 25 days post-immunization. Eight additional days were allowed in instances in which a second challenge was employed. Controls consisted of unimmunized animals that came from the same group as those that were immunized. The number of MICLD₅₀ in each challenge was selected to

* This strain, Sen 1 MB, was obtained through the courtesy of Drs. H.M. Fowell and L.A. Baker, Eli Lilly Laboratories, as a 10 per cent mouse brain preparation. It had received three additional brain passages in this laboratory prior to use.

demonstrate the maximum or near-maximum dose that could be withstood after immunization, except for the challenge dose of $10^{5.8}$ MICLD₅₀ of DES given by the intracerebral (IC) route to monkeys that were immunized by 9t aerosols. Virus challenges were carried out in mice immunized with 9t by titrating PES, EEE, or vaccinia virus (IHD strain) at various intervals. Challenges with vaccinia virus were given only by the IC route. PES and EEE viruses were administered by either the IC or IP route. All aerosol exposures were carried out in a modified Henderson Apparatus as described previously.³

III. RESULTS

A. RESPONSE IN GUINEA PIGS TO VEE, EEE, OR SF VIRUS

The data in Table I (lines 1 and 2) reveal that normal guinea pigs were highly susceptible to infections with the virulent VEE virus strain administered either by injection or by exposure to aerosols. Symptoms in guinea pigs included a febrile response within 18 to 24 hours, followed by prostration and death within several days. One hundred MICLD₅₀ given by the IP or SC route was sufficient to elicit a typical response. Their response to EEE virus on line 3 differed, however, in that 10^8 MICLD₅₀ were necessary to induce a fatal illness, which was preceded in the majority of cases by a diphasic temperature response; the peaks occurred at 24 to 48 hours and again between 72 and 120 hours. In contrast to the equine encephalomyelitis viruses, SF virus neither caused a febrile nor a lethal response in guinea pigs. In this host, therefore, SF virus was suitable only for limited use as a live-immunizing antigen. Mice were used for tests in which it was necessary to employ SF virus as a lethal agent. These studies are discussed below.

B. IMMUNIZATION WITH ATTENUATED VEE VIRUS

As shown in Table I (lines 5 and 6), guinea pigs responded to the IP or SC administration of $10^{3.4}$ MICLD₅₀, or to an aerosol exposure to $10^{4.4}$ MICLD₅₀ of 9t, by showing only a febrile response. These animals were found subsequently to be immune to challenges with a large lethal dose of the virulent homologous strain. PES challenge failed to elicit any clinical signs of illness when administered by either the IP or respiratory route (Table I, lines 7a, 8a, and 8b).

Immunization of guinea pigs with 9t altered significantly the course of illness after a challenge with EEE virus. Among unimmunized control animals, 72 per cent (26/36) showed a typical diphasic febrile response and succumbed. Only 14.2 per cent (5/35) of the animals immunized with 9t showed a diphasic febrile pattern and only 5.7 per cent (2/35) succumbed (Table I, line 7b). Approximately 68 per cent (24/35) exhibited

TABLE 1. RESPONSE OF GUINEA PIGS AFTER IMMUNIZATION WITH ATTENUATED (9t) VEE, EEZ, AND SF VIRUSES TO CHALLENGES WITH THE LETHAL VEE (VES) AND EEE VIRUSES

Line No.	Dose of Immunizing Virus (MICLD ₅₀)	Route of Immunization	Dose of Challenge (MICLD ₅₀)	Route of Challenge	Febrile Response ^{a/}		Number Dead Number Challenged	Per Cent Mortality
					none	mono		
1	None		10 ^{2.1} PES	IP or SC ^{b/}		20/20	20/20	100
2	None		10 ^{7.4} PES	Resp ^{c/}		10/10	10/10	100
3	None		10 ^{8.1} EEE	IP		10/36	26/36	72
4	None		10 ^{5.8} SF	IP	20/20		0/20	0
5	10 ^{3.4} 9t	IP or SC	None	None		40/40	0/40	0
6	10 ^{4.4} 9t	resp.	None	None		40/40	0/40	0
7a	10 ^{3.4} 9t	IP or SC	10 ^{8.7} PES	IP	15/15		0/15	0
7b	"		10 ^{8.1} EEE	IP	6/35	24/35	2/35	5.7
7c	10 ^{3.4} 9t and 10 ^{3.7} PES	IPd/	10 ^{8.1} EEE	IP	6/15	9/15	9/15	0
8a	10 ^{4.4} 9t	Respiratory	10 ^{8.7} PES	IP	9/30	1/10	0/10	0
8b	"		10 ^{7.4} PES	IP	20/20		0/20	0
8c	"		10 ^{8.1} EEE	IP	1/10	9/10	0/10	0
9	10 ^{7.1} EEE	IP	10 ^{8.7} PES	IP		7/7	0/7	0
10a	10 ^{5.8} SF	IP	10 ^{2.1} PES	IP	1/10	9/30	9/10	90
10b	"	IP 2x ^{e/}	10 ^{2.1} PES	IP	8/10	2/10	2/10	20
11a	10 ^{5.8} SF	IP	10 ^{8.1} EEE	IP	3/5	3/5	3/5	60
11b	"	IP 2x	10 ^{8.1} EEE	IP	5/5		0/5	0

- Elevations $\geq 104.5^\circ\text{F}$, which reached either one peak (monophasic) or two peaks (diphasic) separated by an interval of $<104.5^\circ\text{F}$ after immunization and/or challenge.
- Immunized by the intraperitoneal or subcutaneous routes, which produced comparable results.
- Immunized by the respiratory route by a five-minute exposure to a viral aerosol.
- Immunized with one dose of 9t, followed by a dose of PES two weeks later.
- Immunized by the intraperitoneal injection of two doses of virus one week apart.

a single temperature elevation and 17.1 per cent (6/35) showed no clinical signs. This modified effect was found to be more pronounced in the 9t-immunized animals (line 7c), which had received a second antigenic stimulus in the form of a challenge with the virulent PES prior to a challenge with EEE virus.

Immunization with 9t by the respiratory route (line 8c) produced essentially the same modified effect on EEE virus disease as when the IP or SC route was employed. In this case as well, the result was the conversion from a lethal, diphasic febrile response to a nonlethal monophasic febrile response.

Immunization with 9t also protected mice against large lethal doses of SF virus. These data appear in Table II and are discussed in greater detail in the section dealing with the immunization to SF virus.

Other data related to those presented above but not shown in Table I concern the response of monkeys to a lethal dose of VEE virus after immunization by the respiratory route. Exposure of these animals to aerosols of $10^{5.4}$ MICLD₅₀ of the 9t strain afforded complete protection against $10^{5.2}$ MICLD₅₀ of a lethal VEE strain (CES) given intracerebrally. None of the four monkeys treated in this manner succumbed. In contrast, two unvaccinated control monkeys developed illness and died within seven days post-challenge.

C. IMMUNIZATION WITH EEE VIRUS

Guinea pigs were immunized with EEE virus, administering large but nonlethal doses of virus by the IP route. This was followed by IP challenge with the virulent VEE virus strain and, as seen in Table I, line 9, resulted in a febrile response without lethality.

D. IMMUNIZATION WITH SF VIRUS

As indicated earlier, large doses of SF virus by the IP route failed to elicit any detectable clinical response in guinea pigs. As shown in Table I (lines 10a and 11a), one injection of $10^{5.8}$ MICLD₅₀ also failed to protect these animals against minimal lethal doses of either VEE or EEE virus. A second injection of SF virus, however, elicited a slight degree of resistance to the heterologous viruses, manifested by a reduction of the mortality rate with VEE virus from 90 to 20 per cent and from 60 per cent to no deaths with EEE virus following a challenge with a low dose of either agent (lines 10b and 11b).

In Table II, the results of cross-resistance studies between SF and 9t in mice and guinea pigs are compared. As mentioned previously, the fact that SF virus elicited no clinical response in guinea pigs necessitated the use of mice as hosts that could be lethally infected with this agent.

Mice immunized with 9t were protected solidly against 10^6 MICLD₅₀ of SF virus and, despite the poor immunogenic response of guinea pigs to SF virus, it was possible to demonstrate that they were protected against approximately 100-fold-greater VEE virus challenge than the nonimmunized animals.

TABLE II. CROSS-RESISTANCE IN MICE OR GUINEA PIGS VACCINATED WITH ATTENUATED (9t) VEE OR SF VIRUS TO VIRULENT VEE (PES) OR SF VIRUS

Challenge Virus	Titer expressed as LD ₅₀ (log ₁₀)				Protective Index ^c
	SF Immunized Guinea Pigs ^a	Nonimmunized Guinea Pigs	9t Immunized Mice ^b	Non-immunized Mice	
PES	7.9	9.1			1.8
SF			2.1	8.2	6.1

- Immunization was two doses of $10^{5.8}$ MICLD₅₀ given intraperitoneally seven days apart. One dose failed to elicit any protection against the challenge virus.
- Immunization dose was $10^{4.4}$ MICLD₅₀ given intraperitoneally.
- Difference in number of LD₅₀ resisted by immunized and nonimmunized animals.

2. TIME OF ONSET, LEVEL, PERSISTENCE OF IMMUNITY INDUCED BY 9t IN MICE

As seen in Figure 1, 24 hours after the IP administration of 9t to mice, resistance to VEE and EEE viruses, given as IP challenges, was well developed. Resistance to EEE virus remained at approximately the same level until the seventeenth day. Subsequent tests not represented in Figure 1 have revealed that this level of resistance was present also at day 60. Resistance to VEE virus increased to a state of maximum immunity by day 3 and was found to persist at least ten months.

After IC challenges, resistance to EEE virus reached a peak at day 3 and remained at approximately the same level thereafter. Resistance to VEE virus, however, which equalled that of EEE virus at day 3, rose sharply to a peak at day 5, at which time the level of the resistance to the VEE challenges made by either the IC or IP route appeared to be nearly comparable. A peak of resistance to vaccinia virus was attained at day 3 and was followed by a sharp decline. Because vaccinia was nonlethal by other than the IC route, no attempt was made to study its infectivity by peripheral routes in mice immunized with 9t.

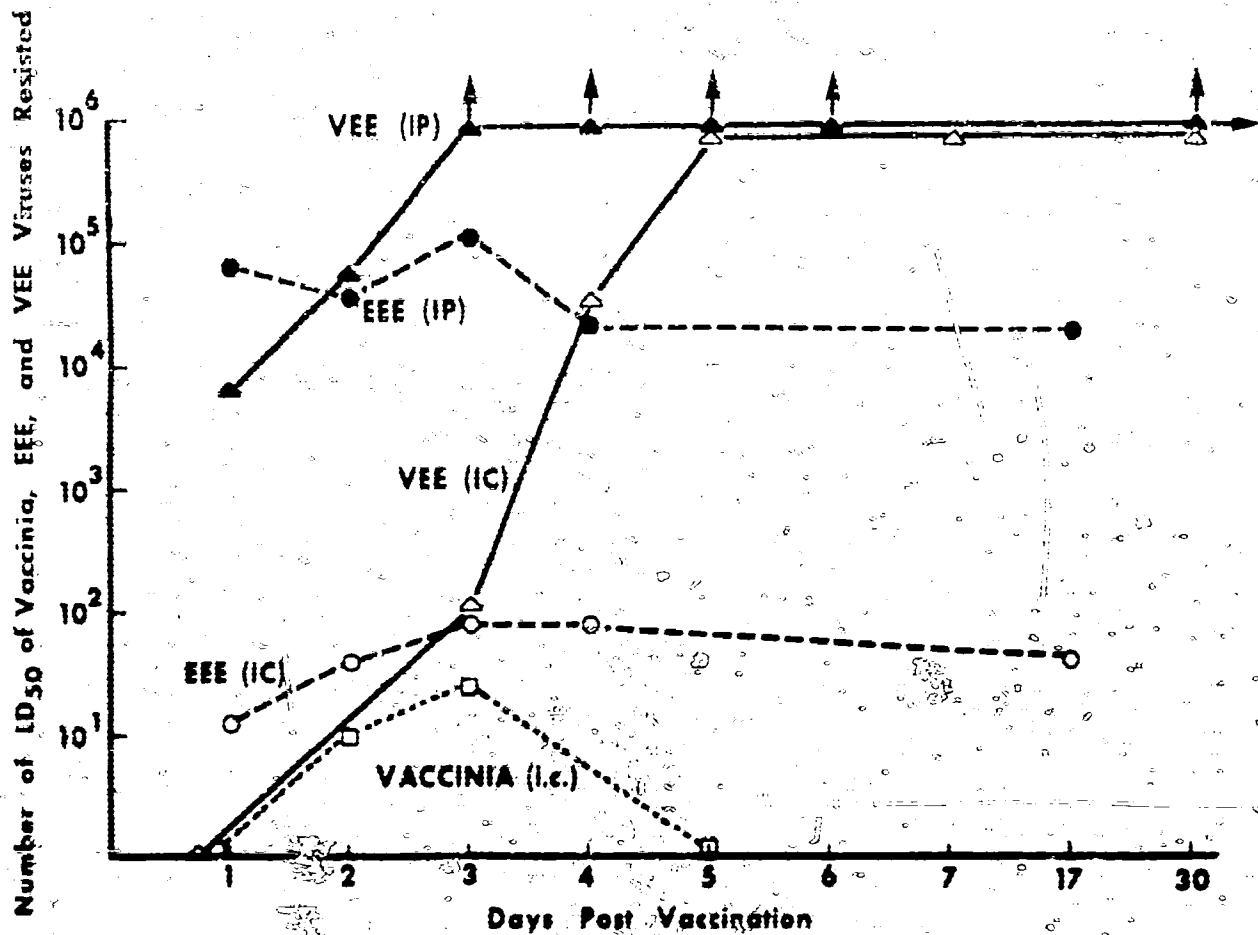


Figure 1. Resistance of Mice to Vaccinia (IHD), EEE, and Parent VEE Viruses after Vaccination with Attenuated VEE Virus.

IV. DISCUSSION

A consideration of the current evidence, together with that presented previously,³ indicates that the phenomenon of cross-protection among VEE, EEE, and SF viruses can be induced in a variety of laboratory animals after immunization by the IP, SC, or respiratory route. The cross-protection was reciprocal among the strains tested. Conceivably, immunization with a live-virus vaccine prepared with one agent might be expected to elicit a durable protective effect that may reduce the virulence of other infectious agents within the group A arthropod-borne viruses. This seems especially pertinent in view of a recent communication by Allen,⁴ who successfully protected mice against a wide variety of group A viruses using a number of selected viruses as immunizing agents.

As might be expected, however, the differences among the levels of the protective responses were found to vary depending upon whether the antigen-viral challenge system was homologous or heterologous. Immunized animals were capable of withstanding far greater challenge doses of homologous virus than of heterologous virus. In cases where lethality did not occur, this could be readily demonstrated when the febrile response was used as an indicator of infection. For example, guinea pigs that were immunized with 9t and challenged with a virulent strain of VEE (PES) failed to show any detectable evidence of infection. In contrast, when animals immunized with 9t were challenged with EEE virus, a limited infection ensued. A monophasic febrile response that occurred shortly after challenge usually replaced the typical diphasic type of response. Febrile responses were also found in EEE-vaccinated guinea pigs challenged with VEE virus.

Differences among the levels of immune responses were found to be dependent not only upon the agent employed for challenge but also upon different protective mechanisms that were stimulated in the host. For example, the data obtained by challenging mice at various intervals post-vaccination with 9t suggest the presence of at least two mechanisms of resistance that operated in sequence. One of these, a nonspecific resistance, which may be similar to that recently described by Traub,⁵ appeared to be largely, if not entirely, responsible for the resistance of mice to VEE, EEE, and vaccinia viruses during the early post-immunization interval. This is most clearly demonstrated by the response of immunized animals to a challenge with the completely unrelated vaccinia virus. Mice resisted this virus most successfully at day 3 and then showed a rapid return to susceptibility. This may be due to the presence of a highly avirulent form of the immunizing virus, which has been detected up to 96 hours in the brains of 9t-immunized mice.⁶ Such a particle may have blocked attachment sites and/or elicited interferon-like substances that were capable of imparting a protective effect for short durations. The second, a specific resistance, was most readily demonstrated, starting after 48 hours in the case of an IP challenge and 72 hours in the case of an IC challenge, at which times the resistance of mice to VEE virus rose sharply to a degree of maximum immunity. This roughly coincided with the detection of specific neutralizing antibody, which has been observed five to seven days after vaccination.²

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That portion of the second mechanism that was involved in the persistent resistance to EEE virus is the least understood. Early post-vaccinal resistance to this virus was demonstrable. However, after the third day, unlike the pattern of resistance to vaccinia virus, which showed a decrease, or to VEE virus, which showed an increase, the resistance of mice to EEE virus persisted at a constant level in the case of either an IP or an IC challenge. The data suggest that a partial specificity was involved, since resistance to EEE persisted beyond that of vaccinia virus. Moreover, the fact that the level of resistance to EEE did not vary after 72 hours suggests, in this case, that a single persistent mechanism may have been responsible. Previous studies have failed to disclose any cross-neutralizing antibody.⁴ Possibly a nonlethal particle, biologically similar to the provirus postulated by Traub⁷ may have established a "cell-associated" cross-resistance mechanism that cannot be accounted for in terms of circulating antibody but is readily demonstrable upon a challenge with heterologous virus. In such a situation, the live-immunizing virus itself might become latently established within strategically located cells, i.e., cells that might be within the normal line of attack by the challenge virus but protected by the "cell-associated" particle.

The possibility exists, however, that shortly after a heterologous challenge, an anamnestic-like response similar in character to the cross-HAI antibodies demonstrated by Casals⁸ was invoked in immunized animals. For example, as mentioned above, EEE virus disease appeared to have been initiated successfully in guinea pigs immunized with 9c, but the disease was terminated soon after the first temperature elevation. The later second temperature elevation that usually followed in the unimmunized animals was absent. This could indicate that 9c may have immunized the animals so that their antibody-producing cells may have become "pre-conditioned" in such a manner as to produce rapidly EEE virus antibody upon challenge with that virus.

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