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Natural immunity to Sindbis virus is influenced by host tissue sialic acid content

(complement/alternative pathway/viral immunity)

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ABSTRACT **Recent studies have shown that the sialic acid content of Sindbis virus influences both its ability to activate the alternative pathway *in vitro* and its susceptibility to complement dependent clearance from the bloodstream *in vivo*. Other studies have shown that the sialic acid content of Sindbis virus is determined by the host in which it is propagated. Because individuals vary in their cell surface sialic acid content, it is possible they also vary in their ability to defend themselves against Sindbis virus infection by virtue of their ability to modify the virus sialic acid content and thereby the capacity of the virus to activate the alternative pathway. To test this hypothesis, outbred Swiss mice were injected subcutaneously with Sindbis virus. There was a significant positive correlation between the level of viremia 18 hr after infection and the sialic acid content of the host's erythrocytes. In addition, animals with erythrocyte sialic acid levels equal to or greater than the mean had a higher level of viremia than animals with erythrocyte sialic acid levels less than the mean. Finally, animals that had muscle sialic acid levels equal to or greater than the mean had a higher incidence of viremia than animals with muscle sialic acid levels less than the mean. These studies suggest that the amount of tissue sialic acid in an individual host influences its ability to resist Sindbis virus infection.**

Sindbis virus is an enveloped virus of the Togavirus group. After Sindbis virus is injected into the footpads of susceptible mice, it replicates locally in the muscle and then invades the bloodstream. A transient viremia ensues, which in turn results in infection of the central nervous system and an acute encephalitis. This animal model of Sindbis virus infection has proven useful in studying the role of the different components of the immune system in the pathogenesis of acute viral infections (1).

The complement system plays an important role in the host's defense against a number of different viral infections (2–5). In the case of Sindbis virus, nonimmune mice depleted of the third component of complement (C3) by cobra venom treatment, or mice genetically deficient in the fifth component of complement, demonstrate an increased susceptibility to infection (4, 6). The complement system appears to exert its major protective effects through the control of the viremia (4, 7, 8).

To subserve its protective functions, the complement system must first be activated, either via the classical or alternative pathway. Activation of the classical pathway usually requires the participation of antibody and is thus important in acquired immunity. Activation of the alternative pathway may occur in the absence of antibody and is therefore important in natural immunity. Recent studies have shown that the ability of a particle to activate the alternative pathway is inversely related to the amount of unacetylated sialic acid on its surface (9). Our own studies have shown that Sindbis virus with relatively small amounts of sialic acid on its envelope is better able to activate the alternative pathway than Sindbis virus with greater amounts of sialic acid on its surface (8). The sialic acid content of Sindbis virus is determined by the host in which it is grown (8, 10). Thus, the ability of Sindbis virus to activate the alternative pathway is, at least in part, determined by the host cell in which the virus is propagated (8, 11).

Because individuals vary in their cell surface sialic acid content, it is possible that they also vary in their ability to defend themselves against Sindbis virus infection by virtue of their ability to modify viral sialic acid content and thereby the capacity of the virus to activate the alternative pathway. We report here studies in nonimmune mice that demonstrate that the amount of tissue sialic acid in an individual host influences its ability to resist Sindbis virus infection.

MATERIALS AND METHODS

Buffers. Veronal-buffered saline (pH 7.4) with ionic strength 0.147 and containing 0.15 mM calcium, 1 mM magnesium, and 0.1% gelatin (GVB^{++}) was prepared according to the method of Kabat and Mayer (12). Veronal-buffered saline (pH 7.4) with ionic strength 0.074 and containing 0.15 mM calcium, 1 mM magnesium, 0.1% gelatin, and 2.5% dextrose was prepared as described (13). Tris-buffered saline with EDTA consisted of 0.01 M Tris·HCl, 0.15 M NaCl, and 0.001 M EDTA (pH 7.4).

Preparation of Mouse Embryo Fibroblasts. Embryos from BUB/BnJ, C57BL/6J, and B10.SM mice (The Jackson Laboratory) were obtained at 15 days of gestation. To prepare mouse embryo fibroblasts (MEF), embryos were eviscerated, minced, and dissociated with trypsin. Cells were plated at 10^7 cells per 150-cm^2 flask in minimal essential medium containing 10% fetal calf serum. Cells were used for virus production at the first passage.

Virus Production and Purification. Confluent monolayers of MEF were infected with stock virus that had been propagated in chicken embryo fibroblasts (CEF). After gentle rocking of the flasks for 1 hr at 37°C, the inocula were removed and the cells were rinsed with warm minimal essential medium, which was replaced with minimal essential medium containing 0.5% fetal calf serum. After 48 hr, the medium was removed and virus was purified on sucrose gradients (14).

Determination of Sialic Acid Content. The sialic acid content of MEF, erythrocytes, and muscle was determined by the

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Abbreviations: C3, the third component of complement; GVB^{++}, Veronal-buffered saline (pH 7.4) with ionic strength of 0.147 and containing 0.15 mM calcium, 1 mM magnesium, and 0.1% gelatin; MEF, mouse embryo fibroblasts; CEF, chicken embryo fibroblasts; C2DHS, serum from a patient with genetically determined C2 deficiency; pfu, plaque-forming units.

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thiobarbituric acid method (15).

Serum. Serum was obtained from a child with a genetically determined deficiency of the second component of complement (C2DHS) (16) and was stored at −70°C. The C2DHS had normal levels of the other components of the classical pathway, a normal factor B level, and normal alternative pathway function when tested in a rabbit erythrocyte lysis assay (16). The titer of C3 in the C2DHS was ≈2,160 units/ml (13).

Complement Activation Studies. The desired concentration of purified Sindbis virus was incubated for 30 min at 37°C in C2DHS diluted 1:10 in GVB^{++}. Controls consisted of serum diluted 1:10 in GVB^{++} with no added virus. The percentage of functional C3 that was consumed was calculated as described (13, 14).

Animal Inoculation. Outbred adult (5- to 6-week-old) Swiss mice were obtained from Buckburg Farms (Thomkins Cove, NY). They were injected in their rear footpads with a total of 10^4 plaque-forming units (pfu) of CEF Sindbis virus. The amount of virus in whole blood from individual animals was determined by plaque assay of serial 1:10 dilutions on monolayers of BHK-21 cells (4).

RESULTS

Activation of the Alternative Pathway by Sindbis Virus Propagated in MEF from Different Strains of Mice. Our previous studies have shown that Sindbis virus propagated in fibroblasts from different species activates the alternative pathway in an inverse relationship to the amount of viral and cellular sialic acid content (8). The following experiments were performed to determine if intraspecific variation in cellular sialic acid content also was able to influence the ability of Sindbis virus to activate the alternative pathway. Sindbis virus was propagated in primary MEF derived from three different inbred strains of mice, which vary considerably in the sialic acid content of their cells (17). As can be seen in Table 1, there was an inverse relationship between the ability of the individual preparations of Sindbis virus to activate the alternative pathway and the sialic acid content of the MEF in which the virus was propagated.

Relationship of Host Tissue Sialic Acid Content and Level of Viremia in Individual Mice. Outbred Swiss mice were injected in their rear footpads with 10^4 pfu of Sindbis virus and the level of viremia was determined 18 hr after infection. In addition, the sialic acid content of the erythrocytes and muscles of the same mice was measured. As can be seen in Fig. 1, there was a positive correlation between the amount of host tissue (erythrocyte) sialic acid and the level of viremia. In addition, animals with erythrocyte sialic acid levels equal to or greater than the mean (0.116 μmol/mg of protein) had a significantly higher mean (±SEM) level of viremia (366 ± 95 pfu/ml) than animals with erythrocyte sialic acid levels less than the mean (139 ± 35 pfu/ml) ($P < 0.01$ with Student's t test).

The relationship of the viremia to the level of sialic acid in the muscle, the initial site of viral replication, also was studied.

Table 1. Relationship between host cell sialic acid content and the ability of Sindbis virus to activate the alternative pathway

Origin of MEF	Sialic acid, μmol/mg of cell protein	% C3 consumption in C2DHS*
BUB/BnJ	0.028	79.8
C57BL/6J	0.048	29.5
B10.SM	0.093	18.5

* Purified Sindbis virus produced in the indicated MEF was incubated at a concentration of 625 μg/ml in C2DHS for 30 min at 37°C and the amount of C3 consumed was determined.

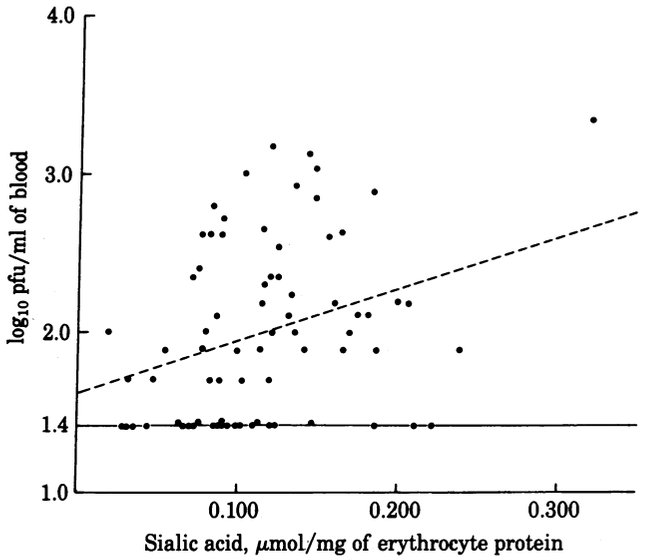

FIG. 1. Outbred Swiss mice were injected in the rear footpads with 10^4 pfu of Sindbis virus. At 18 hr after injection, the level of viremia and the level of erythrocyte sialic acid were assessed in individual mice. A positive linear correlation (Pearson $r = 0.379$, $P < 0.005$, dashed line) was observed between these two parameters. The solid horizontal line represents the lower limit of detection.

Animals with muscle sialic acid levels equal to or greater than the mean (0.055 μmol/mg of protein) had a higher incidence of viremia than did animals with muscle sialic acid levels less than the mean (Table 2).

Finally, the levels of erythrocyte sialic acid and muscle sialic acid in individual mice correlated with each other (Pearson $r = 0.226$, $P < 0.05$).

DISCUSSION

Resistance to viral infection involves the successful integration of a number of different components of the immune system. Recent studies have shown that the terminal complement components, C3–9, play a significant role in the host's defense against a number of different viral infections (2–5). Our own studies have shown that C3–9 exert their major protective effect by limiting the viremia in an experimental Sindbis virus infection of nonimmune mice (4, 7, 8).

Sindbis virus is capable of activating C3–9 through both the classical and alternative pathways (14). Recent studies have clarified the mechanism by which particles activate C3–9 via the alternative pathway (18). Assembly and maintenance of the alternative pathway C3-cleaving enzyme, C3bBb, depends on a

Table 2. Relationship between incidence of viremia and muscle sialic acid levels

Sialic acid, μmol/mg of muscle protein	Ratio
<0.055	20/30
≥0.055	30/35

Outbred Swiss mice were injected in the rear footpads with 10^4 pfu of Sindbis virus. At 18 hr after injection, muscle from the hind limbs of individual mice was removed and prepared for sialic acid determinations. The frequency of animals with and without viremia was analyzed with respect to the mean level of muscle sialic acid (0.055 μmol/mg of protein) for the population. A significant correlation (ϕ coefficient = 0.23, $P < 0.05$) between the incidence of viremia and a muscle sialic acid content equal to or greater than the mean was observed. Ratio = number of mice with viremia/number of mice injected.

number of opposing forces. One of these, alternative pathway factor H, acts to inhibit the assembly of the enzyme and accelerate its decay. The binding of factor H to a particle, and thus its inhibitory effects on the alternative pathway C3-cleaving enzyme, is favored by the presence of sialic acid residues on the particle. Thus, particles with high levels of sialic acid on their surface are poor activators of the alternative pathway, whereas particles with low levels of sialic acid are good activators of the alternative pathway.

Our previous studies have shown that, like other particles, the amount of sialic acid on the Sindbis virus envelope determines the degree to which it can activate the alternative pathway (8). The content of sialic acid on the Sindbis virus envelope is determined by the host cell in which it is propagated (8, 10). Thus, virus propagated in fibroblasts from mosquito cells, which have little if any sialic acid, has little sialic acid on its envelope and is a good activator of the alternative pathway. Conversely, virus propagated in baby hamster kidney cells, which have relatively more sialic acid, is a relatively poor activator of the alternative pathway. The current experiments extend these observations by demonstrating that intraspecific variation in cellular sialic acid content also is able to influence the ability of Sindbis virus to activate the alternative pathway. Virus propagated in fibroblasts from an inbred strain of mice with relatively low levels of cellular sialic acid activates the alternative pathway better than virus propagated in fibroblasts from an inbred strain of mice with relatively high levels of cellular sialic acid.

The above observations suggested that it is possible that hosts vary in their ability to defend themselves against Sindbis virus infection by virtue of their ability to modify viral sialic acid content and thereby the capacity of the virus to activate the alternative pathway. The present studies demonstrated a direct correlation between the level of viremia after subcutaneous injection of Sindbis virus and the individual host's level of tissue sialic acid. Thus, it appears that the host's level of tissue sialic acid is an important determinant of natural immunity to Sindbis virus infection. In individual hosts with relatively low levels of tissue sialic acid, virus with a relatively low sialic acid content would be produced. This virus would be a relatively good activator of the alternative pathway and effective clearance or neutralization of the virus, or both, would occur. Conversely, in hosts with relatively high levels of tissue sialic acid, virus with relatively high sialic acid content would be produced. This virus would be a relatively poor activator of the alternative pathway and less effective clearance or neutralization of the virus, or both, would occur.

Several components of the immune system participate in the control of virus dissemination (1). It is likely that the variation in the level of viremia found in different animals with similar levels of tissue sialic acid reflects the influence of other components of the host's defense against the virus. The present studies suggest that the ability of a host to prevent dissemination of an enveloped virus may be dependent, at least in part, upon the sialic acid content of the host. Because the sialic acid content of the host is, in part, genetically determined, it is possible that the host's tissue sialic acid level represents one mechanism of genetically determined natural resistance to virus infection.

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