Cross Protection among Togaviruses in Nude Mice and Littermates

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SUMMARY

After immunization with Sindbis virus, T-cell deficient nude mice, compared to normal littermates, were equally protected against challenge with Sindbis virus. However, the nude mice showed about one-tenth the protection observed with normal littermates after challenge with Semliki Forest virus at a dose of 100 LD₅₀. This is consistent with our previous interpretation that sensitized T-cell populations play a major role in cross protection between the two togaviruses. The remaining low level of specific cross protection in nude mice (detectable only at a challenge dose of 10 LD₅₀) could not be attributed to an anamnestic response of neutralizing antibody to the challenge virus or to an effective antibody-dependent, complement-mediated cytolysis of infected cells in vivo. Other possible compensatory mechanisms to explain the low level of specific cross protection in nude mice are discussed.

INTRODUCTION

Recovery from many virus infections including togaviruses, may sometimes involve a cell-mediated process (Griffin, 1975; Notkins & Lodmell, 1975). Based on adoptive transfer experiments, we have suggested that cell-mediated immunity plays a major role in heterologous protection among Group A togaviruses (Peck et al. 1975). In such experiments, mice were protected against challenge with Semliki Forest virus after receiving a preparation of spleen cells from mice that had been immunized with Sindbis (heat resistant - HR strain) virus. This cross protection was evident when B cells were depleted from the spleen cell population but was abrogated when T cells were removed prior to adoptive transfer.

The nude mouse (nu/nu) has been shown to be athymic (Flanagan, 1966) and to have a profound immunological deficiency. This animal model has been used extensively to study the immune response (Pantelouris, 1968; Wortis, 1974), in order to evaluate not only the role of thymus-dependent activities but also to assess residual (thymus-independent) and compensatory mechanisms operative in the virtual absence of thymic influence (Jutila, 1977).

Antibody responses to a number of viruses have been evaluated in the athymic nude (nu/nu) mouse and compared with those found in normal littermates (nu/+ or +/+; Burns et al. 1975). Sindbis HR virus was one of the viruses used to study these responses. In the primary response, neutralizing antibody production paralleled that found in littermates. Furthermore, the titre was sustained for 2 weeks after injection, suggesting that homologous protection against togaviruses could be thymus independent with the antibody responsible being IgM. However, the level of production was relatively low and secondary
antibody responses could not be demonstrated in nudes, as opposed to the significant response in the littermates (Burns et al. 1975).

In the present communication, we have chosen the athymic nude model to investigate further the importance of thymic-dependent immunity in cross protection among togaviruses. In contrast to observations on homologous protection, we demonstrate a greatly diminished, yet still significant, level of protection in the nude mice but we argue that this level of protection is accounted for by a compensatory non-thymic-dependent immune mechanism.

METHODS

Animals. All experiments were done with nude (nu/nu) mice and their normal littermates (nu/+, +/++) established in NIH/Swiss background. The mice were supplied by Life Sciences Research Laboratory (St. Petersburg, Florida, U.S.A.) at 4 weeks of age and were maintained with autoclavable mouse chow. Bedding, chow and water were sterilized by autoclaving and the animals were kept in plastic pans covered with sterilized filter tops. The temperature of the housing unit was maintained at 21 ± 1°C throughout the study period and the mice were handled aseptically in a biological laminar flow cabinet.

Cell cultures and media. Chick embryo (CE) fibroblast cultures were prepared from minced, trypsinized (0.025% trypsin), 10-day-old embryos as described by Dulbecco & Vogt (1953). Five ml samples of 5 × 10⁶ cells/ml were dispensed into 60 × 15 mm plastic Petri dishes (Falcon Plastics, Oxnard, Calif.) and confluent cell monolayers were evident after 18 to 24 h at 37°C in a 5% CO₂ atmosphere. Growth medium consisted of medium 199 supplemented with 10% heat-inactivated (56°C, 30 min) calf serum, 0.075% sodium bicarbonate, 100 units/ml of penicillin, 100 μg/ml of streptomycin, vitamins, glutamine and other essential amino acids.

Viruses. Sindbis virus (HR) has been maintained in this laboratory for several years; the stock was grown in CE cells, to a yield of 5 × 10⁶ p.f.u./ml. This prototype alphavirus was prepared for use by inoculating a low dose (10 to 100 p.f.u./plate) on to CE cell monolayers after purification by three plaque passages. The parent HR strain is not lethal for weanling mice by any route of inoculation. Lethal, brain-adapted variants (HRB) have been obtained after passage of the parent strain in suckling mice (Boone & Brown, 1976). These lethal strains were used for intracerebral challenge of mice that were previously immunized with an intracerebral inoculation of the stock HR strain. Semliki Forest virus (SFV) was originally obtained from Dr W. P. Allen as a mouse brain suspension. A stock of SFV was prepared as described above for Sindbis virus. The stock was assayed on CE cell monolayers and found to contain 10⁸ p.f.u./ml.

Titration of virus in vitro. Virus stocks were titrated by plaque assay on CE cell monolayers (Dulbecco & Vogt, 1953). Growth medium was removed from monolayers, cells rinsed with saline A (containing 0.85% NaCl, 0.1% glucose, 0.04% KCl and phenol red; buffered to pH 7.4 with sodium bicarbonate), then infected with various concentrations of virus. Dilutions were made in Brain Heart Infusion broth (BHI, Difco, Detroit, Michigan). Virus was allowed to adsorb to cells for 30 min at 37°C and the monolayers overlaid with lactalbumin hydrolysate agar (0.5% lactalbumin hydrolysate supplemented with 0.1% yeast extract, 1.1% agar, 125 units/ml of penicillin, 125 μg/ml of streptomycin, 0.14% sodium bicarbonate and 10% Hanks' balanced salt solution (HBSS). Cultures were then incubated for 48 h at 37°C in 5% CO₂. After incubation, infected cultures were stained with neutral red (a dilution of 10⁻⁴ in sterile water), incubated for 6 h and plaques then counted.

Titration of virus in vivo, immunization and challenge. Stocks of HRB or SFV were diluted in BHI and injected into groups of ten mice for each dilution either i.p. (0.2 ml/mouse) or
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Deaths were recorded twice daily so that the average day of death and the 50% lethal dose (Reed & Muench, 1938) were calculated for each virus. Animals dying within the first 24 h were not included in the calculations since such death was considered non-specific.

Other animals were immunized with Sindbis virus i.c., and after 2 weeks, they were challenged with 10 or 100 MIPLD<sub>50</sub> (intraperitoneal dose lethal for 50% of animals) of HR<sub>6</sub> i.c. or SFV i.p. Serum samples were collected on days 0, 4, 6 and 8 post-challenge from some animals to determine neutralizing antibody titres while other animals were observed for survival and average day of death.

Antisera. Mice were inoculated i.c. either with spent medium (sham control) or Sindbis virus. The medium or Sindbis virus stock was diluted 1:10 with BHI and 0.025 ml was injected at a point between the eye and ear directly into the brain. Fourteen days later, blood was taken, serum separated and samples stored at −20 °C until used.

Neutralization assay. A standard plaque neutralization test was used to detect the presence of neutralizing antibody. Approx. 400 p.f.u. of virus contained in 0.2 ml of BHI were mixed with 0.2 ml of doubling dilutions of antiserum or control serum and incubated at 27 °C for 1 h. The number of infectious virus particles were then assayed on CE cell monolayers.

Skin grafts. In view of the preliminary evidence which suggested a primary role for T-cells in cross-protection among togaviruses (Peck et al. 1975) and that nude mice should lack T-cells and T-cell function (Kindred, 1971; Manning et al. 1973; Wortis, 1974), it was necessary to test for the presence of functional T-cells in our stock of nude mice, since some cross-protection was found in these animals. Host versus graft response is considered to be the most demanding single in vivo test to detect T-cell activity (Kindred, 1978; Rygaard, 1978). Donor mice were killed by cervical dislocation while recipient mice were anaesthetized with ether. Hair of graft donors and recipients was removed with Nair hair remover (Carter-Wallace, New York, NY, U.S.A.) applied directly on the back for 5 min. After washing and treatment with 70% ethanol, an 8 to 12 mm piece of skin was removed from the donor and this graft was placed within a skin crater on the recipient, formed by removing a similar piece of skin. Grafts were held by light but firm pressure using skin closures (3M Company St. Paul, Minn., U.S.A.) applied directly on the grafts and the area was wrapped with bandages (3M). One week later, after the primary union was completed, the bandages were removed and the animals inspected daily thereafter.

It was found that nudes accepted grafts of DBA/2 skin for longer than 12 weeks (latest time examined) whereas littermates reject grafts within 14 days, an observation supporting the conclusion that the nude mice used were T-cell deficient.

<sup>51</sup>Cr release assays. The antibody-dependent, complement-mediated cytotoxicity of infected CE cells by nude or littermate antisera was done as described previously (King et al. 1977). Briefly, CE cells were labelled with <sup>51</sup>Cr-sodium chromate (New England Nuclear, Boston, Mass., U.S.A.) for 45 min at 37 °C. The suspension was washed and diluted to 2 to 3×10<sup>6</sup> viable cells/ml in growth medium. Linbro plates (24 well, Linbro Scientific Inc., Hamden, Conn., U.S.A.) were seeded with 1 ml/well of the labelled cell suspension and this was allowed to form a monolayer at 37 °C under 5% CO<sub>2</sub> for 16 to 18 h. The monolayers were washed twice with HBSS and infected with 0.1 ml of the virus at a m.o.i. of 10. Virus was allowed to adsorb for 30 min at 37 °C. The monolayers were washed four times and growth medium finally added. The supernatant fluids of one set of monolayers were removed at designated times after infection to determine the <sup>51</sup>Cr released as the result of c.p.e. of the virus. At various times p.i. a second set of monolayers was washed and layered with 0.5 ml of the appropriate serum dilution and 0.5 ml of a 1/7.5 dilution of freshly reconstituted guinea pig complement (Flow Laboratories, Rockville, Md., U.S.A.).
Table 1. Homologous and heterologous protection in nude and littermate mice against 100 LD$_{50}$ of challenge virus

<table>
<thead>
<tr>
<th>Immunization*</th>
<th>Challenge†</th>
<th>Nude mice</th>
<th>Littermates</th>
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<tr>
<td></td>
<td></td>
<td>$P$</td>
<td>$\ast$ T</td>
</tr>
<tr>
<td>Sham</td>
<td>HR$_{B-14}$</td>
<td>&lt; 0.005</td>
<td>8.8</td>
</tr>
<tr>
<td>HR</td>
<td>HR$_{B-14}$</td>
<td></td>
<td>19.5</td>
</tr>
<tr>
<td>Heterologous; i.p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>SFV</td>
<td>&lt; 0.05</td>
<td>6.1</td>
</tr>
<tr>
<td>HR</td>
<td>SFV</td>
<td></td>
<td>8.4</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>SFV</td>
<td>&lt; 0.1</td>
<td>5.7</td>
</tr>
<tr>
<td>HR</td>
<td>SFV</td>
<td></td>
<td>7.3</td>
</tr>
</tbody>
</table>

* Immunized with 10$^8$ p.f.u. Sindbis virus (HR), i.c. route, 25 mice per group.
† HR$_{B-14}$ challenge by i.c. route; SFV challenge by i.p. route.
‡ Average survival time, a calculation of the average day of death assuming half the survivors die the day after the last day of observation and the others survive indefinitely.
§ Survivors at 15 days post challenge.

Dilutions were made in medium 199 without supplements. After 1 h at 37°C, the supernatant fluid was removed, the $^{51}$Cr released measured in an Intertechnique gamma spectrometer and the specific $^{51}$Cr release determined as previously reported (King et al. 1977).

Statistical treatment of data. Survival patterns of each experimental group were compared and statistically analysed using the conversational programme EXPVAL, provided by Dr Steven Vas (Toronto Western Hospital, Toronto, Ont., Canada). The EXPVAL programme applies an analysis of variance by negative exponential transformations and T tests (Liddel, 1978). A statistical transformation is a method of censoring data based upon a mathematical function of observed survival time. This programme employs a transformation assuming that one half of the surviving animals in each group die the day after the last day of observation; the other half is assumed to survive indefinitely. Statistically, the transformation represents an assumption of the worst possible case.

RESULTS

Determination of the MIPLD$_{50}$ for SFV in nude and littermate mice

The possibility that nude mice may be more susceptible to SFV infection than their normal littermates was examined. The MIPLD$_{50}$ was found to be 10$^8$ p.f.u. in nudes and 10$^3.6$ p.f.u. in the littermates. This difference is generally considered to be of marginal significance and is not distinctly different from data obtained when comparing different strains of mice (Brand & Allen, 1964).

Homologous and cross protection

A single dose of 10$^7$ to 10$^8$ p.f.u. of Sindbis HR was inoculated i.c. into either nudes or littermates. Spent culture medium was used in the sham inoculation. Two weeks later, all animals received lethal doses of challenge virus, either HR$_{B-14}$ (passaged in suckling mouse brain for 14 passages) i.c. or SFV i.p. This course of immunization and challenge was adopted primarily because the usual multiple i.p. immunization schedule (Peck et al. 1975) requires an extra 10 days, which we wished to avoid in the nude mouse due to the accelerated aging process (Jutila, 1977; Pantelouris, 1978). Furthermore, a single i.c. injection has been demonstrated to be as effective as, or even superior to, repeated i.p. injections for inducing cross protection (Fine et al. 1974). The results presented in Table 1 indicate that
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HR Sindbis virus induces homologous protection against the lethal variant HRB-14, supporting our previous findings in other mice (Peck et al. 1975). Interestingly, the response of nude mice was comparable to that of the littermates and homologous protection was maximal for both sets of animals (100% survival). In contrast, survival after heterologous virus challenge of nudes differed noticeably when compared to litter mates (12% versus 60%). Cross protection in the littermates was evident by the increase in the number of animals surviving a lethal dose of SFV in the actively immunized group compared to those sham inoculated (60% versus 20%). However, nude mice showed no appreciable increase in percent survival in the Sindbis immunized compared to the sham inoculated groups (Table 1). Another parameter of protection is the average day of death. Both actively immunized nude mice and littermates showed an increase in the average survival time after challenge with SFV compared with sham-inoculated mice although this increase was less than 2 days for nudes but greater than 5 days for littermates. This extension of mean time to death noted in these studies and elsewhere (Fine et al. 1974; Woodman et al. 1975) is considered as a second valid index of protection. The statistical significance supporting the conclusions, based on a combination of % survival and average survival time is evident in the Table. The results on the very much lower level of cross protection in athymic mice compared to littermates support the conclusion that T-cells are required for maximum cross protection.

Effect of varying dose of challenge virus

As can be seen in Table 1, cross protection in nude mice was shown as only a slightly delayed time of death in actively immunized compared to sham-inoculated animals. It was possible that the nude mice possessed a low level of immunity to the heterologous virus but this was insufficient to affect survival to a typical challenge dose of 100 MIPLD₉₀. The search for a remaining low level specific immunity was tested by comparing the survival of animals at a low dose level, namely 10 MIPLD₉₀. The results are presented in Table 2 and indicate that cross protection was observed with the low challenge dose in nude mice as defined by both % survival (70% versus 38%) and average day of death (15.1 versus 10.5) although such protection was not as significant as that seen with littermates (survival of 90% versus 30% and average day of death 18.0 versus 9.9). These experiments were done with 20 mice per group and repeated twice with almost identical results.

Although the results above provide support for our hypothesis that T-cells and cell mediated immunity are largely responsible for cross protection, we were curious about the specificity of the remaining low level of cross protection observed in the athymic mice (Table 2). It was possible that the cross protection was due to the stimulation of a non-specific mechanism induced by immunization with Sindbis virus. This was tested in two ways. Mice immunized with Sindbis were challenged with 10⁷ p.f.u. of herpes simplex virus, type 1 (HSV-1), i.p. Seventy-five % of nude mice, sham-immunized or immunized with Sindbis virus died at 12±2 days with no statistical difference. No littermates died at this dose level. In a second series of experiments, nude mice were immunized with HSV or vaccinia virus and challenged with SFV. Again, no protection was observed in these animals over sham-immunized controls. On the basis of these results, the low level of cross protection of mice immunized with Sindbis virus to SFV challenge was considered specific.

Antibody response

One obvious explanation for cross protection in the immunized nude mouse is enhanced production of antibody to SFV challenge following immunization with Sindbis virus. Although it is established that there is no cross neutralizing antibody between Sindbis virus and SFV (Brand & Allen, 1964), some antigenic relationships do exist, such as those which elicit antibody that inhibits virus haemagglutination (Brand & Allen, 1964). We therefore
Table 2. Heterologous protection in nude and littermate mice against 10 LD_{50} of SFV

<table>
<thead>
<tr>
<th>Immunization*</th>
<th>Nude mice</th>
<th>Littermates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P ASST% survivors| P ASST% survivors|</td>
<td></td>
</tr>
<tr>
<td>Sham HR</td>
<td>&lt; 0.01 10.5 38 4.4</td>
<td>&lt; 0.04 9.9 30 0.05</td>
</tr>
<tr>
<td></td>
<td>15.1 70 18.0 90</td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

* Immunized with 10^6 p.f.u. Sindbis virus (HR), i.c. route, 20 mice per group.
† Average survival time.
‡ Survivors at 15 days post challenge.

Table 3. Lack of an anamnestic (cross) neutralizing antibody response to SFV after SFV challenge in nudes and littermates immunized with Sindbis (HR)*

<table>
<thead>
<tr>
<th>Days after challenge with SFV</th>
<th>Neutralizing antibody titres to SFV</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Nude mice</td>
</tr>
<tr>
<td></td>
<td>Sham-inoculated</td>
</tr>
<tr>
<td>0</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>4</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>NT†</td>
</tr>
</tbody>
</table>

* Titre = extrapolation from triplicate determinations of the reciprocal of antiserum dilutions reducing the number of SFV plaques by 50%. Mice, in groups of ten, were immunized with 10^6 p.f.u. of Sindbis virus HR i.c. At 14 days, 10 MIPLD_{50} of SFV were given to immunized or sham-inoculated controls and the animals were killed on days 0, 4, 6 and 8 to obtain sera for titration.
† NT = not tested since no mice survived in this group after day 7.

tested whether challenge with SFV would cause an anamnestic (heterologous) response to SFV in HR-immunized animals which would include the production of neutralizing antibody. The neutralizing antibody titres to SFV of immunized and sham-inoculated nude mice and littermates after challenge with SFV are presented in Table 3. Within the time when animals were dying from the SFV challenge (up to 8 days), the neutralizing antibody titres between sham-inoculated and Sindbis HR immunized nude mice or littermate mice were not significantly different.

Antibody-dependent, complement-mediated cytolysis

Although the Sindbis HR immunized nude mouse produce lower titres of neutralizing antibody to SFV than do sham-inoculated littermates following challenge, the possibility exists that antibody produced in the nude mouse may function in a complement-mediated cytotoxicity of virus infected cells better than does antibody from littermates. We have reported that hyperimmune antisera to Sindbis virus HR shows cross cytolysis of SFV-infected chick embryo fibroblasts (King et al. 1977). Antibody-dependent, complement-mediated (ADCM) cytolysis of chick embryo fibroblasts infected with Sindbis virus HR or SFV by nude mouse or littermate antisera was determined and the results are presented in Fig. 1. The only ADCM cytolysis observed was with littermate antisera in the homologous system. No cross cytolysis was found with littermate or nude antisera. Furthermore, no homologous ADCM cytolysis was observed for the nude antiseria. It should be noted that in our previous report (King et al. 1977), hyperimmune antisera were used while, in the present work, antisera were obtained with the single i.c. immunization dose. This difference in the preparation of the antisera is taken to explain the absence of any cross cytolysis with littermate antisera.
Fig. 1. Antibody-dependent, complement-mediated cytolysis of chick embryo fibroblasts infected with Sindbis virus HR (○ - - ○) or SFV (● - - ●) by nude mice (a) or normal littermate (b) antisera. CE cells, \(2.5 \times 10^6\), were labelled with \(^{51}\)Cr and infected with Sindbis virus HR or SFV. Dilutions of antisera, as noted, and guinea pig complement (final dilution, 1:15) were added and incubated for 1 h at 37 °C. The supernatant was removed and \(^{51}\)Cr release measured. The percentage of specific \(^{51}\)Cr release was determined by the following formula:

\[
\text{% specific release} = \left( \frac{\text{ct/min in supernatant with immune serum}}{\text{total ct/min in supernatant plus cells in immune serum}} \right) - \left( \frac{\text{ct/min in supernatant with normal serum}}{\text{total ct/min in supernatant plus cells in normal serum}} \right) \times 100
\]

Controls included: 1, infected cells plus complement only; 2, infected cells plus heated (56 °C for 30 min) antiserum only, at the highest concentration used; 3, infected cells plus unimmunized mouse serum plus complement; 4, infected cells only; 5, non-infected cells plus antiserum and complement. There were about 40000 total counts and the controls showed 4 to 6 % (1600 to 2400 ct/min) background release into the supernatant. The experiment was repeated with identical results.

**DISCUSSION**

Based on the studies of Burns *et al.* (1975), who showed comparable levels of antibody in nude mice and littermates after one injection of Sindbis virus, we suggest by inference that the homologous protection may be mediated, at least in part, by neutralizing antibody. Neutralizing antibody in normal mice is generally accepted as playing the major role in homologous protection involving Group A togaviruses. However, a sensitized T-cell response in infected mice has been shown in the *in vitro* tests and a specific mononuclear cell infiltration (T-cell?) was demonstrated in studies on the specific inflammatory response observed in infected and surviving weanling and adult mice (Griffin, 1975; McFarland & Johnson, 1975).

We have previously reported that heterologous, or cross protection, among Group A togaviruses may be primarily a sensitized T-cell dependent process (Peck *et al.* 1975). Athymic nude mice were used in the present study with the expectation, therefore, that cross protection to SFV after immunization with Sindbis virus would be significantly reduced or
absent in them, as opposed to littermates, thus indirectly supporting our earlier work. There was indeed a much lower level (about one-tenth) of cross protection in nude mice compared to littermates. This is based (Table 1) on no difference in the % survival of immunized nude mice, compared to sham-inoculated nude mice at a challenge dose of 100 LD50. Yet there was a significant increase in % survival in immunized littermates compared to sham-inoculated littermates. Only by the average survival time data could one find a bare suggestion of cross protection in the immunized athymic mice (less than two days) whereas littermates showed an average survival time greater than four days. Thus, the reduction in cross protection in nude mice is coincident with a lack of T-cell function in such animals and is consistent with the hypothesis that T-cell mediated immunity plays the major role in cross protection in normal mice. Differences (p < 0.01) of both average survival time and percent survival were observed with nude mice only when a minimal (10 LD50) challenge dose of SFV was used. However, the remaining low level of cross protection observed in nude mice could be explained by postulating either a residual non-T-cell mechanism present exclusively in nude mice, or also present, but obscured, in normal mice.

In attempts to identify such a mechanism in the athymic mouse, we have eliminated: cross-neutralizing antibody, complement-facilitated cross-neutralizing antibody (data not presented), an anamnestic antibody response that neutralizes the challenge virus and a more effective antibody-dependent, complement-mediated cytolysis. This elimination is based on the fact that the levels of these humoral factors were the same as, or lower than, those found with sham-inoculated controls (nude mice or littermates) in test systems in vitro.

The elimination of the humoral factors tested to explain cross protection in the nude mouse suggests a non-T-cell mechanism that generates cytotoxicity for SFV infected cells. One possibility is the macrophage. Activated macrophages have been demonstrated in the resistance of nude mice to the bacterial pathogen Listeria monocytogenes (Nickol & Bonventre, 1977). They have also been implicated in the resistance of nude mice to herpes simplex virus type 2 virus induced hepatitis (Morgensen & Anderson, 1978). In preliminary experiments (data not presented), peritoneal or spleen macrophages from both non-immunized and immunized nude mice showed about twice the level of cytotoxicity, as did littermate macrophages, against cells infected with Sindbis virus HR, or SFV and the amount of cytotoxicity was not increased when nude or littermate anti-Sindbis virus HR sera were added in the assay. However, these experiments are difficult to repeat and expand in the present nude mouse system since we are using the nu gene in an outbred Swiss background. Thus macrophages from different mice cannot be pooled and our preliminary findings are based on comparisons of macrophages handled individually from different mice. These experiments will be repeated in nude mice with a BALB/c background, for we still consider the macrophage, with or without the help of antibody, as possibly playing a role in the low level of specific cross protection in the nude mouse.

A second possible non-T-cell mechanism that has been described as effective in the nude mouse is the subpopulation of non-adherent, non-phagocytic cells referred to as endogenous NK (Natural Killer) cells described in tumour systems (Herberman et al. 1975; Pross & Jondal, 1975) and virus induced NK cells (Welsh & Zinkernagel, 1977). These cells have the morphology of small lymphocytes and possess low avidity Fc receptors but no complement receptors. Furthermore, this cell is present in relatively higher numbers in nude mice as compared to thymus-bearing littermates (Herberman & Holden, 1978). NK cells have been reported to be cytotoxic for togavirus-infected cells (MacFarlan et al. 1977) and also may function in concert with macrophages (Miller et al. 1977). However, we are sceptical that NK cells without antibody can provide the compensatory mechanism for the low level of cross protection in nude mice since this mechanism should be operative equally well in the immunized and non-immunized nude mouse. In addition, interferon or augmentation of
natural or antibody-dependent cell-mediated cytotoxicity (NK cells) by interferon (Herberman et al. 1979) should be equally effective in either immunized or non-immunized nude mice after challenge of SFV (interferon inducer) and not be responsible for specific cross protection after immunization.

Another possible mechanism to explain the low level of cross protection in the Sindbis virus HR immunized nude mouse is antibody-dependent cellular cytotoxicity mediated by K cells and other leukocytes as described for tumour or virus systems (Herberman, 1978; Rouse & Babiuk, 1978). We believe that such cross cytolysis of SFV infected cells is possible based on our studies of ADCM cross cytolysis (King et al. 1977). We are examining this possibility in nude mice with BALB/c background where cytotoxicity is measured with effector cell populations pooled from different mice and tested on infected BALB/c fibroblasts (3T3 cells).

In conclusion, the evidence that we have presented in this study is consistent with our earlier reports that the primary immune mechanism for cross protection (as opposed to homologous protection) in mice among the togaviruses we studied is primarily T-cell mediated. We also found a low level of non-T-cell mediated specific cross protection in athymic mice that warrants further study.

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Z. LATIF AND OTHERS


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