Activation of Natural Killer Cells and Induction of Interferon 
After Injection of Mouse Hepatitis Virus Type 3 in Mice

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High levels of natural killer (NK) cell activity and high titers of interferon were observed in the peritoneal exudate of C57BL/6 mice 20 to 30 h after injection of mouse hepatitis virus type 3 (MHV3) but not during the first 10 h after infection. C57BL/6 mice were susceptible to MHV3 infection and showed high titers of MHV3 in the peritoneal exudate 48 h after infection. A/J mice, in contrast, were resistant to the dose that killed 100% of the C57BL/6 mice (10 macrophage-infecting doses) and showed considerably lower virus titers than those shown by C57BL/6 mice. NK cell activity and interferon titers were significantly lower in the peritoneal exudate of A/J mice than in that of C57BL/6 mice. Serum interferon titers were also lower in A/J mice. Thus, our data show an inverse relationship between resistance and the levels of these two parameters. The data suggest that, in contrast to the situation observed with certain herpesviruses, interferon and NK cells may not be of overwhelming importance in the defense of mice against MHV3.

Natural killer (NK) cells are a novel class of lymphoid cells that are defined functionally by their capacity to lyse certain target cells without obvious preimmunization of the host (for recent developments in this field of research, see reference 4). One of the most interesting recent findings is that NK cell activity may be considerably increased by interferon or interferon inducers (4). Viruses are the most potent inducers of interferon, and thus it is not surprising that activation of NK cells occurs after injection of viruses in mice. It has been suggested that NK cells play a role in primary antiviral resistance (12). This suggestion has found support in an experimental system of murine cytomegalovirus infection (2). Further data were obtained from a mouse model of herpes simplex virus (HSV) infection. In this system, resistance of mice is genetically controlled (8), and there is some correlation between resistance and degree of NK cell activation (1, 6). However, there are certain strains of mice to which this correlation does not apply (H. Engler, R. Zawatzky, H. Kirchner, and D. Armerding, manuscript in preparation). SJL mice, for example, which are deficient in NK cell activity, are relatively resistant to HSV infection. In contrast, a perfect correlation appears to exist between the magnitude of the local interferon production and resistance (Engler et al., in preparation).

In the present paper, we analyze interferon production and NK cell activation in the experimental infection of mice with mouse hepatitis virus type 3 (MHV3), yet another model in which primary antiviral resistance is under genetic control (3).

MATERIALS AND METHODS

Virus. MHV3, originally prepared as previously described (9) was passaged one time in C57BL/6 mouse peritoneal macrophages which were then cultivated in 24-well plates (code 76-033-05 Linbro, Flow; Bonn, Federal Republic of Germany). After 48 h, the cell-free supernatant was recovered. One pool of virus containing 10^5 macrophage-infecting doses (MID) was used throughout the experiment (the virus pool caused a typical cytopathic effect in vitro up to a dilution of 10^{-3}).

Virus titration. Original peritoneal exudate cells (PEC) of C57BL/6 mice were used to titrate MHV3 (9). However, later during our studies, macrophages which had been derived from mouse bone marrow and which can be conveniently grown in large numbers were used for viral titration. This method is based on a technique described by Klimetzek and Remold (7) and will be reported in detail elsewhere (L. Schindler and H. Kirchner, manuscript in preparation). Briefly, bone marrow was obtained by flushing femora and tibiae with sterile saline. Bone marrow cells were grown in media containing conditioning factors derived from L-cell supernatants (7); the media were kept in 24-well plates, and the cells were grown for 8 to 10 days. After this time, the population, consisting of at least 95% macrophages, was infected with MHV3 and incubated for 2 additional days. MHV3 caused the formation of giant cells in these cultures. Virus titers were expressed as the reciprocal value of the virus dilution that still caused the formation of giant cells.

Mice. Male A/J/Bom and C57BL/6j/Bom mice were
obtained from Gl. Bomholtgard Ltd., Ry, Denmark, at the age of 8 weeks and used in the experiments within 2 to 3 weeks of purchase.

Experimental protocol. Mice usually were injected with 10 MID of MHV3. This dose was chosen because it kills 100% of the susceptible C57BL/6 mice but less than 5% of the resistant A/J mice. At various times, 2 ml of balanced salt solution was injected intraperitoneally (i.p.), and the PEC were collected. The cells were washed twice by centrifugation and then counted. The PEC from each mouse were tested separately, and at least three mice were tested in each group of each experiment. For controls, PEC from uninfected mice were used.

In additional experiments, a different protocol was used. Mice were injected i.p. with virus, and at different times thereafter, a small amount of fluid was injected. After being gently massaged, the abdomens were opened and fluid was recovered. The fluid was analyzed for its interferon content, and (in some experiments) for its virus titer.

Interferon determination and NK cell assay. For interferon testing, a one-step assay with L cells and vesicular stomatitis virus was used, all details of which have been described elsewhere (14). For testing NK cell activity, PEC were used immediately after recovery. NK cell activity was tested in a 4 h test in which mycoplasma-free YAC-1 lymphoma cells were used as targets. All details of the assay are described elsewhere (3a).

RESULTS

Measurement of NK cell activity in the PEC population after injection of MHV3. PEC of C57BL/6 mice had low or undetectable levels of NK cell activity against YAC-1 target cells.

Injection of MHV3 in C57BL/6 mice resulted in the activation of NK cells in the PEC population. This effect was not detectable during the first 10 h, but it reached its peak at about 20 h and did not substantially increase thereafter (Fig. 1). A comparison was made between susceptible C57BL/6 and A/J mice resistant to MHV3 infection. NK cell activity was significantly lower in A/J mice than in C57BL/6 mice (Fig. 1). This difference was observed for all doses of MHV3 and for all times tested after injection of MHV3. The reproducibility of the documentation of the difference between A/J and C57BL/6 mice is shown in Fig. 2.

Measurement of interferon titers in sera and peritoneal fluid. Small samples of peritoneal fluid were recovered at various times after injection of MHV3 as described in Materials and Methods. Interferon could first be detected at 24 h after viral injection (Fig. 3). Earlier than that, little or no interferon could be detected, and there was no interferon in the peritoneal fluid of untreated mice. Similar kinetics were seen when the presence of serum interferon was determined (Fig. 3). Significantly higher titers of interferon were observed in the peritoneal exudate and sera of C57BL/6 mice than in those of A/J mice (Fig. 4).

Determination of virus titers in the peritoneal fluid. Twenty-four hours after injection of 10^3 MID of MHV3, high virus titers were detected in the peritoneal exudate of C57BL/6 mice, but significantly lower titers were detected in the exudate of A/J mice (Table 1).

DISCUSSION

In the above-reported experiment, we studied a murine model of viral infection with MHV3 in which resistance to infection is genetically con-
controlled. As firmly established by Bang and Warwick (3), C57BL/6 mice are highly susceptible to MHV3 and die of hepatitis 3 to 4 days after infection. A/J mice, in contrast, are resistant. Resistance is expressed by the level of the macrophages in that macrophages of resistant mice are unable to replicate MHV3 (3). Resistance, however, is not absolute, since very high doses of virus also kill A/J mice.

It has been suggested that NK cells play a role in antiviral defense. Ideal for testing the relevance of NK cells in antiviral defense are models in which resistance to infection is genetically controlled. In a murine model of cytomegalovirus infection, a clear-cut correlation has been observed between high NK cell activities after virus infection and high resistance (2). Recently, we studied a murine model of HSV infection.
(3a). Some resistant mouse strains demonstrate high levels of NK cell activity after injection of HSV, and some susceptible strains are low in NK activity. However, there are mouse strains that do not fit into this scheme.

In terms of the MHV3 infection system, data have been presented by Tardieu et al. (10) that suggest a role of effector bone marrow-dependent cells in resistance. These so-called "M cells" share some similarities with NK cells. In the report of Tardieu et al., the conclusions were derived from transfer experiments in which cell populations were transferred from resistant adult mice to susceptible newborn mice. The conclusions of our paper are based on a completely different approach and obviously are at variance with the conclusions of Tardieu et al. We observed an inverse correlation between resistance and NK cell activity. Thus, C57BL/6 mice that were highly susceptible to infection showed high levels of NK activity, whereas resistant A/J mice showed very low activity.

MHV3 is known to replicate readily in the peritoneal macrophages of genetically susceptible mice. In our experiments, high titers of MHV3 in the peritoneums of C57BL/6 mice were observed at 48 h (Table 1). This time coincided with the peak of interferon production. It is tempting to speculate that interferon production is induced simultaneously with viral replication, which then leads to NK cell activation. A/J mice are resistant to MHV3 infection because their macrophages are resistant to infection. Thus, considerably less virus is produced. Therefore, little interferon is produced and no activation of NK cells occurs.

This situation is in marked contrast to the data which we have obtained for the murine model of HSV infection. It is noteworthy that this contrast was seen despite the fact that the same mice were used and that the techniques were exactly the same: the experiments were done by the same investigators at the same time.

In the HSV infection system, we have previously observed a positive correlation between resistance and high titers of early interferon produced in the peritoneal exudate (3a). This correlation was without exception for the eight strains of mice tested. Mouse strains susceptible to HSV, in contrast to resistant C57BL/6 mice, showed no measurable titers of interferon 2 h after infection. It is noteworthy to recall that in the MHV3 system early titers of interferon in the peritoneal exudate could not be found.

This paper suggests that interferon production at the local site is of less importance in the MHV3 infection system, since significantly higher titers of interferon were produced in genetically susceptible mice than in resistant mice. This conclusion is at variance with the data of Virelizier and Gresser (11), who found that injection of an antiserum against interferon in mice results in increased mortality after infection with MHV3. The reasons for this discrepancy are not clear, and we plan to do additional experiments with antisera against interferon.

In conclusion, our data for the MHV3 infection system are comparable to those of Hirsch for a Sindbis virus infection system (5) and those of Welsh and Kiessling (13), which suggest that NK cells do not play a role in antiviral resistance. Our data are in contrast to those for a murine cytomegalovirus infection system (2) and a murine HSV infection system (1), in which a positive correlation between genetically determined resistance and levels of NK cell activity has been observed. However, we believe that at present, no final conclusion on whether NK cells play a role in antiviral resistance can be reached. The different viral infection systems are hardly comparable, and different experimental approaches have been used by different investigators. Perhaps NK cells play a role in the defense against some viral infections and not others.

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LITERATURE CITED

role in the recovery of mice from Sindbis virus infection. Immunology 43:81-89.


