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Herpes simplex virus – induced interferon production and activation of natural killer cells in SM/J mice. Relation to antiviral resistance

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We have studied the susceptibility of SM/J mice to intraperitoneal (i.p.) infection with herpes simplex virus type 1 (HSV) and have searched for correlations of susceptibility with the activation of Natural Killer (NK) cells and with local induction of interferon. SM/J were exceedingly susceptible to virus infection as they could be killed by < 10 plaque forming units (PFU). The NK cell system of these mice, as measured by the activity of spleen cells against YAC-1 lymphoma cells, was hyperreactive, which is in agreement with previous findings of others. The peritoneal exudate cells (PEC) of uninfected mice had no activity. However, 18 h after i.p. injection of HSV NK cell activity was detected in the PEC population, which was at least as high as that in C57BL/6 mice that are resistant to HSV infection. Thus it appears as if the NK cell system does not play a major role in antiviral resistance in our experimental system. In contrast, from our previous work it would rather appear that the magnitude of the early local interferon response is important for resistance. The current data obtained in SM/J mice are in accordance with this, in that these highly susceptible mice are deficient in their early interferon response. Homozygous beige mice were found to be as resistant to infection with HSV as C57BL/6 mice. While the NK cell activity in their PEC population after injection of HSV was low, the titers of locally induced interferon were as high as those in the controls.

herpes simplex virus; inbred mouse strains; NK cells; interferon; natural resistance

Introduction

Natural Killer (NK) cells may play a role in antiviral resistance (see ref. [8,15] for recent reviews). Studies in a mouse model of murine cytomegalovirus infection have suggested such a role [2], whereas little further evidence in support of this has come

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from other virus models [6,7,16]. NK cell activity in mice is genetically controlled. For example, homozygous beige mice are defective in NK cell activity [13]. More recently, it has been found that SM/J mice have a hyperreactive NK cell system [3]. This has been found for 'uninduced' NK cell activity whereas, to our knowledge, virus-induced NK cell activity has not been investigated as yet in SM/J mice. We have investigated NK cell activity in SM/J mice after injection of herpes simplex virus type 1 (HSV) and have, in addition, analyzed HSV-induced interferon induction. Both parameters were analyzed in the perspective of resistance of SM/J mice against intraperitoneal (i.p.) infection with HSV. A few experiments were performed with additional strains of mice, including beige mice.

Materials and methods

Virus

HSV Type 1 strain WAL (subsequently referred to as HSV) was prepared as described [18]. One pool of virus was used in all experiments which contained 10^8 plaque forming units (PFU)/ml and which was kept frozen at -70°C in small samples. Freshly thawed aliquots were used in each experiment. The virus pool was repeatedly tested for mycoplasma and found to be clean.

Mice

Male SM/J mice (Jackson Laboratories, Bar Harbor, Maine) were used at the age of 8–12 wk. For comparative experiments mice from a number of inbred strains of the same age and sex were used. These have included C57BL/6, DBA/2 (Bomholtgard, Ry, Denmark) as well as AKR/J and SJL/2 mice (Jackson). C57BL/6-bg/Ola mice were obtained from Olac (Shaw's Farm, Blackthorn, Bicester, Oxon, United Kingdom) together with age- and sex-matched C57BL/6 Ola mice that served as controls.

Experimental protocol

Mice were injected i.p. with different doses of HSV as indicated in the respective experiments. Except for the experiments in which lethality was determined, mice were divided into different groups. From one group of mice the peritoneal fluid was recovered to determine the interferon titer and the titer of HSV as described [17]. From another group of mice, the peritoneal cell population was washed out for testing NK cell activity.

Testing of NK cell activity

A 4-h ^{51}Cr-release assay was used with YAC-1 lymphoma cells as targets and effector/target cell ratios between 50:1 and 5:1 exactly as described [5]. As effector cells, both spleen cells and peritoneal exudate cells (PEC) were used, both of which were either recovered from untreated mice or from mice 18 h after i.p. injection of 1×10^5 PFU of HSV.

Titration of interferon

Peritoneal fluid was rendered cell-free by centrifugation. Virus was inactivated by dialysis against acid buffer (pH 2). The one-step plaque reduction assay using mouse L cells and vesicular stomatitis virus for interferon determination has been described [17]. Interferon titers are expressed in international units (IU).

In vivo and in vitro replication of HSV

All viral determinations were done by a plaque assay using RITA cells of monkey kidney origin, exactly as described previously [18]. In vivo replication of HSV was monitored by testing the viral titers in samples of peritoneal fluid at various times after i.p. infection with HSV. For the studies of in vitro replication of HSV in peritoneal macrophages a system was adapted from the paper of Lopez and Dudas [12] which will be described in detail elsewhere (J. Brücher and H. Kirchner, to be published). Briefly, PEC from previously untreated mice were collected by lavage of the peritoneal cavity and grown for several days on plastic Petri dishes in the presence of conditioned media [10]. After 7 days, the cells represented a monolayer of virtually 100% macrophages as determined by esterase staining and phagocytosis of carbon particles.

Results

HSV-induced NK cell activity

Spontaneous and HSV infection induced NK cell activity against YAC-1 lymphoma cells was determined in three strains of mice: C57BL/6, SM/J and SJL. Groups of three mice were infected by i.p. inoculation (10^5 PFU) and cells were collected after 18 h. The averaged results of three experiments are shown in Table 1. As described by others [3], spleen cells of untreated SM/J mice showed high levels of NK cell activity against YAC-1 cells. In contrast, no NK cell activity was observed in the PEC population. However, 18 h after infection with HSV these cells had acquired high NK cell activity. This activity was at least as high as in C57BL/6 mice which are high responders in this system, whereas SJL are low responders.

HSV-induced interferon production in vivo

Mice were infected i.p. with HSV and at various times the peritoneal fluid was recovered and tested for interferon activity. In Fig. 1, the interferon titers of SM/J mice in comparison with C57BL/6 and DBA/2 are shown. The data are derived from one individual experiment (three mice of each strain at each time point) but principally identical results were obtained in two additional experiments. It can be seen that SM/J mice showed only low interferon titers when tested 4 or 8 h after infection. In contrast, high interferon titers were observed in C57BL/6 mice already 4 h after virus injection, as previously described [6]. DBA/2 mice also did not produce significant interferon titers during the first 4 h after injection of HSV. Both DBA/2 and SM/J mice, however, showed high interferon titers in the peritoneal fluid later during the course of the infection.

TABLE 1

NK cell activity of different mouse strains after infection with HSV[a]

Mouse strain	Effector cell population[b]	NK cell activity (% specific lysis)[c]
SM/J	Spleen, non-induced	24
	Spleen, HSV-induced	52
	PEC, non-induced	3
	PEC, HSV-induced	34
C57BL/6	Spleen, non-induced	20
	Spleen, HSV-induced	45
	PEC, non-induced	5
	PEC, HSV-induced	31
SJL	Spleen, non-induced	6
	Spleen, HSV-induced	17
	PEC, non-induced	2
	PEC, HSV-induced	7

[a] 18 h after infection with HSV-WAL Type 1 (infective dose 10^5 PFU/animal i.p.).
[b] Ratios: spleen 50:1; PEC 10:1.
[c] Mean values of 3 experiments, each with 3 mice.

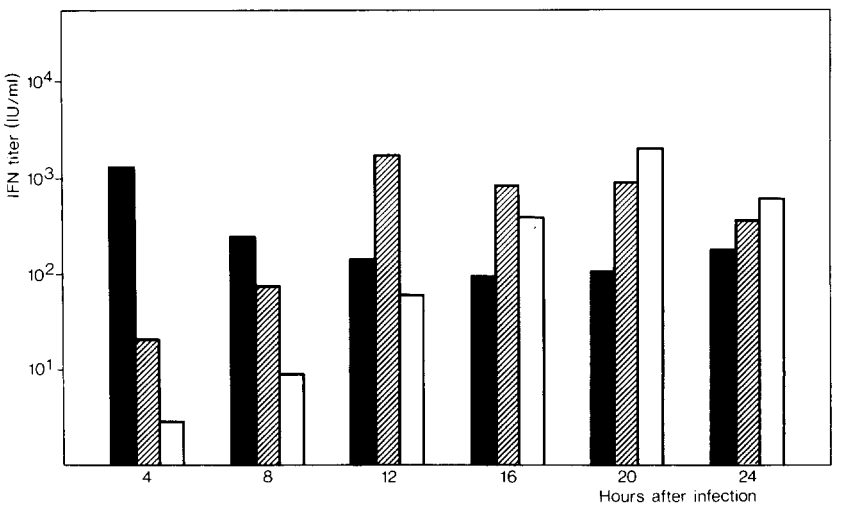

Fig. 1. Interferon titers in the peritoneal fluids of mice at different times after i.p. infection with HSV. Black bars: C57BL/6 mice; shaded bars: DBA/2 mice; white bars: SM/J mice. Each point represents the mean value of three mice tested individually.

Susceptibility of SM/J mice to i.p. infection with HSV

To find out if there was a correlation between in vivo NK cell activation and genetically determined resistance to virus infection, the LD_{50} of HSV was established for SM/J mice after i.p. injection with HSV. It was then compared to the LD_{50} in 4 additional strains of inbred mice (Table 2). The LD_{50} value was lowest in SM/J mice: < 10 PFU were required to kill the mice.

Titers of HSV in the peritoneal fluid and in cultures of macrophages

To correlate in vivo virus replication with in vivo interferon production, titers of HSV were determined in the peritoneal fluid of SM/J, C57BL/6 and DBA/2 mice. As can be seen in Table 3, 24 h after infection titers of 7×10^5 PFU/ml were observed in SM/J mice, i.e. considerably higher than in C57BL/6 mice and about as high as in susceptible DBA/2 mice.

In addition, the replication of HSV was tested in vitro in macrophage cultures of different strains of mice. In cultures of PEC of SM/J mice, again HSV replicated to high titers. These were about as high as those observed in PEC cultures of DBA/2 mice and 1000-fold higher than those observed in PEC cultures of C57BL/6 mice (Table 4).

TABLE 2

Lethal $dosis_{50}$ (LD_{50}) of HSV in different mouse strains

Mouse strain	LD_{50} (PFU, i.p.)	Level of resistance
C57BL/6	2.2×10^5	High
SJL	8×10^4	High
DBA/2	6.4×10^2	Low
AKR	3×10^1	Low
SM/J	2×10^0	Very low

TABLE 3

HSV yield in cultured macrophages of different mouse strains[a]

Mouse strain	Virus titer[b] (PFU/ml $\times 10^{-5}$)
C57BL/6	0.02–0.07
DBA/2	8–30
SM/J	7–60

[a] PEC were recovered by lavage of the peritoneal cavity of previously untreated mice and cultured for 7 days in the presence of conditioned medium. After this time they consisted of nearly 100% pure macrophages and formed a confluent monolayer.

[b] Virus yield 48 h after infection with 10^3 PFU/ml (equivalent to a m.o.i. of 0.001); range in 5 experiments.

TABLE 4

Titers of HSV in the peritoneal fluids of different strains of mice[a]

Mouse strain	Time	Titer (PFU/ml × 10^{-3})
C57BL/6	8	0.1
	16	2.0
	24	9.0
DBA/2	8	0.7
	16	8.0
	24	50.0
SM/J	8	1.0
	16	9.0
	24	70.0

[a] Infecting dose: 10^6 PFU/mouse i.p.

HSV virulence, NK cell response and interferon production of beige mice

Homozygous beige mice became available to us after completion of the studies already described. The lethality of beige mice did not differ from that of C57BL/6 mice. Since only a limited number of mice was available, determination of the LD_{50} could not be done. However, our data did show that the lethality was identical in both types of mice, both when 10^5 PFU of HSV or 10^4 PFU were injected.

At best borderline NK cell activity of PEC could be induced by infection with HSV in beige mice. Nevertheless, the interferon titers in the peritoneal fluids did not differ from those obtained in C57BL/6 control mice (Table 5).

Discussion

Resistance of mice to infection with HSV is genetically controlled [11] and originally it has been suggested that the magnitude of the NK cell response after injection of HSV – which is also genetically controlled – may be associated with resistance [1]. However, situations have been described where HSV-induced NK cell activity was not correlated with resistance. For example, BALB/c mice that are susceptible to HSV have a marked NK cell response and SJL mice are resistant despite the fact that their NK cell response after infection with HSV is low [6].

In the present study we have investigated SM/J mice previously shown to possess a hyperreactive NK cell system [3]. We have confirmed that spleen cells of 'uninduced' SM/J mice show high NK cell activity against YAC-1 lymphoma cells. PEC, however, of untreated mice show no killing, as it has been observed previously in our laboratory for a considerable number of other mouse strains [6]. When HSV was injected, high NK cell activity was measured in PEC of SM/J mice. In fact, this activity was as high as the one observed in C57BL/6 which is the strain with the highest response available to us.

TABLE 5

Activation of NK cells and interferon induction in the peritoneal cavity of homozygous beige mice after infection with HSV[a]

Exp. no.	Mice		NK cell activity[b] (% specific ^{51}Cr release)	Interferon titer[c] (IU/ml)
I	C57BL/6 bg/bg	1	3	780
		2	7	920
		3	4	840
	C57BL/6	1	31	630
		2	29	720
		3	23	750
II	C57BL/6 bg/bg	1	6	1140
		2	7	1230
		3	5	1310
	C57BL/6	1	18	1420
		2	28	1310
		3	21	1220

[a] Mice were injected i.p. with 5×10^5 PFU of HSV and individual mice were tested in two separate experiments.

[b] NK cell tests were performed at 18 h after injection of HSV (effector/target ratio 10 : 1).

[c] The peritoneal fluids were recovered from individual mice 4 h after injection of HSV.

Previously, Lopez has shown that among a large number of mouse strains AKR is most susceptible to HSV [11]. Our data indicate that SM/J mice are at least as sensitive as AKR mice, as they can be killed by i.p. injection of < 10 PFU of HSV. This high virulence is also reflected by the virus titers that are measured in vivo or in vitro in macrophage cultures.

Hence, it appears unlikely that NK cells play a major role in defense in the HSV model we have investigated. SM/J mice, that mount the highest NK cell response in the peritoneal cavity, are extremely sensitive to the lethal outcome of the infection. These conclusions are reiterated by our data obtained in beige mice. These have a genetic defect in the NK cell system [13] and accordingly we have found that there is only borderline NK cell activity in their PEC after infection with HSV. Nonetheless, their sensitivity to HSV did not differ from that of C57BL/6 controls.

It has been reported that the interferon production in mice after injection of certain viruses such as Newcastle Disease Virus (NDV) is genetically controlled [4]. We have recently found that the early circulating interferon response after i.v. injection of HSV in mice is also under genetic control [19]. This experimental situation was somewhat different from the earlier work since NDV was a virus that is apathogenic for mice whereas HSV under the experimental conditions chosen was able to kill adult mice. Furthermore, we have observed a correlation between resistance to HSV and the magnitude of the early local interferon response [6]. Thus, resistant mice produced

high interferon titers whereas susceptible mice showed borderline levels of early interferon if any.

In the present study we have tested the interferon response in SM/J mice. The data are congruent with the previous work since they showed that SM/J mice were incapable of producing early interferon at the local site in response to infection with HSV. However, as previously observed for DBA/2 mice [6], late interferon responses were also high in SM/J mice, but these later interferon titers appeared to be incapable of halting the lethal outcome of the infection.

It is established that interferon is causing an enhancement of NK cell activity. Thus, one may assume that the interferon produced after virus infection will lead – among other effects – to NK cell activation. It is, therefore, somewhat surprising to find high NK cell activities in the peritoneal cavity of SM/J mice. Perhaps, locally only small quantities of interferon are required to activate NK cells and perhaps these low concentrations escape detection in the peritoneal fluid. Alternatively, however, there may be mechanisms by which viruses cause the activation of NK cells that are independent of interferon induction. Evidence of this stems from work in our laboratory in which we have found that HSV virions inactivated by UV light or by heat are no longer capable of inducing measurable titers of interferon but do activate the NK cell system in the peritoneal cavity [9]. Again, the possibility that low quantities of interferon not detected in the fluids were capable of interferon induction could not be eliminated. Yet another explanation may be that the late interferon in SM/J is still capable of inducing NK cell activity early enough to be measured in our system.

In conclusion, our data on SM/J mice supported our previous conclusion [6] that interferon is relevant in genetically controlled primary resistance of mice against HSV infection, whereas NK cells may be of less relevance. We have also shown that mouse macrophages in which HSV replicates in vitro and presumably in vivo in the peritoneal cavity can be efficiently protected in an in vitro system by interferon from infection by HSV (J. Brücher and H. Kirchner, unpubl. data). Thus, the role of endogenously produced interferon in genetically controlled resistance against HSV may be caused by its direct effect on the targets of viral replication and not by the activation of the NK cell system.

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