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Role of interferon in the resistance of C3H/HeJ mice to infection with herpes simplex virus

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Summary

C3H/HeJ mice known to be defective in their responses to bacterial lipopolysaccharides, are more resistant to infection with herpes simplex virus (HSV) than the closely related strain C3HeB/FeJ. The increased resistance is reflected in higher early local interferon titers after HSV infection. However, NK cell activation by HSV is not correlated with resistance, since the NK cell response of C3H/HeJ mice was significantly lower than that of the control strain.

C3H mice; LPS gene; interferon; HSV infection; NK cells

Lopez first showed that resistance of mice to infection with herpes simplex virus type 1 (HSV) is under genetic control [9], which was subsequently confirmed by our laboratory [8]. These studies were performed with a variety of inbred mouse strains, including for example AKR, A/J, C57BL/6 and DBA/2. Two closely related substrains of C3H, i.e. C3H/HeJ and C3HeB/FeJ, are known to basically differ in one property, i.e. the sensitivity to bacterial lipopolysaccharide (LPS) and this difference has been found to be caused by a mutation in a single gene [11–13]. We have reported on differences of about 100-fold in the resistance of the two substrains to HSV infection [7]. Thus, the LPS-insensitive strain C3H/HeJ is 100 times more resistant to intraperitoneal (i.p.) challenge with HSV. The reasons for these differences were not understood at the time of this report.

In previous studies we have investigated a number of parameters of natural defense after infection with HSV. These have included the role of macrophages, of natural killer (NK) cells and of the locally produced interferon [2–4,14,15]. The following

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studies were undertaken to see whether any of these parameters were relevant to the difference between C3H/HeJ and C3HeB/FeJ mice.

Male mice of the two strains C3H/HeJ and C3HeB/FeJ were obtained from Jackson laboratories (Bar Harbour, Maine, USA) at the age of 6 weeks. They were used in the experiments within the subsequent 4 weeks since preliminary testing had revealed that the differences in susceptibility to HSV can be most reproducibly shown at this age. In one experiment, lethality to HSV type 1 strain WAL (subsequently referred to as HSV) was analyzed, whereas in the other experiments HSV was injected intraperitoneally (i.p.) at a dose of 10^6 PFU and at various times thereafter three parameters were assayed: viral titers, titers of interferon and activity of NK cells. For the latter determination the peritoneal cells were washed out with buffered saline, whereas small amounts of peritoneal wash-fluid were recovered for the measurements of interferon and virus titers. All methods have been described in a previous paper of our laboratory [2].

In initial experiments (and in control experiments throughout the study) spleen cells of the two substrains of mice were tested to ensure the defective LPS response of C3H/HeJ mice. The cells did not proliferate in response to LPS, and as in the observations of Ascher et al. [1], they were also incapable of LPS-induced interferon production (data not shown).

We have already shown that C3H/HeJ mice are more resistant to the lethal outcome of i.p. infection with HSV than the closely related strain C3HeB/FeJ [7]. Thus, from Table 1 it can be clearly seen that C3H/HeJ mice were about 100 times more resistant to i.p. challenge with HSV than C3HeB/FeJ mice.

Next the NK activity of the peritoneal cell population 24 h after injection of HSV into mice of the two substrains was assayed. A set of three experiments in which the injection of LPS or *C. parvum* was compared is shown in Table 2. As can be seen, peritoneal cells of C3H/HeJ mice did not react to injection of LPS with activation of NK cells. They showed NK cell activity after injection of *C. parvum*, albeit to a lower degree than peritoneal cells of C3HeB/FeJ mice. Gangemi et al. [5] have studied NK cell activity of spleen cells of LPS-injected C3H/HeJ mice and have also observed a defective response whereas the responses to *C. parvum* were normal. These authors have, however, not studied NK cell activity of peritoneal cells. In Table 2 the results of

TABLE 1

Mortality of C3H/HeJ and C3HeB/FeJ mice after intraperitoneal injection of HSV-1 (WAL)

Virus dose (log_{10} PFU)	% mortality[a]	
	C3H/HeJ	C3HeB/FeJ
6	70	-
5	25	100
4	0	100
3	-	25

[a] 20 mice per group.

TABLE 2

Activation of NK cells in the peritoneal cell population of C3H/HeJ and C3HeB/FeJ mice after injection of different materials

Material injected	Number of individual experiments[a]	NK cell activity[b]	
		C3H/HeJ	C3HeB/FeJ
HSV (10^5 PF)	7	13 ± 2	26 ± 4
LPS (100 μg)	3	1 ± 1	7 ± 1
C. parvum (700 μg)	2	8 ± 3	11 ± 2
Saline	7	1 ± 1	1 ± 1

[a] With three mice in each.

[b] Expressed as percent specific ^{51}Cr release (± S.E.M.).

seven experiments with HSV are also summarized. HSV-induced NK cell activity was significantly higher in (the less resistant) C3HeB/FeJ mice than in C3H/HeJ mice. The same finding was obtained when NK cell activity was tested 1, 2 or 3 days after infection (Fig. 1).

In a further set of experiments, mice of the two substrains were injected with HSV and at different times thereafter mice were killed and samples of peritoneal fluid were removed. They were tested both for the titers of HSV and for their interferon contents (Figs. 2 and 3). Interferon titers 1 and 4 h after infection were significantly higher in C3H/HeJ mice. Conversely, viral titers were lower in the peritoneal wash fluid of C3H/HeJ mice at all times tested.

Thus the data obtained in the two closely related substrains of C3H are in agreement with our previous studies using a variety of inbred mouse strains [4]. In these studies we also found a close correlation between the magnitude of the early, local interferon response towards virus infection and resistance. Conversely, there was also

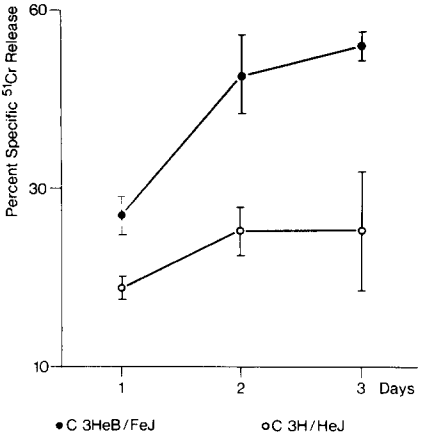

Fig. 1. NK cell activity in the peritoneal cell population at different times after infection with HSV (mean values of 3 experiments, bars indicate ± 1 S.E.M).

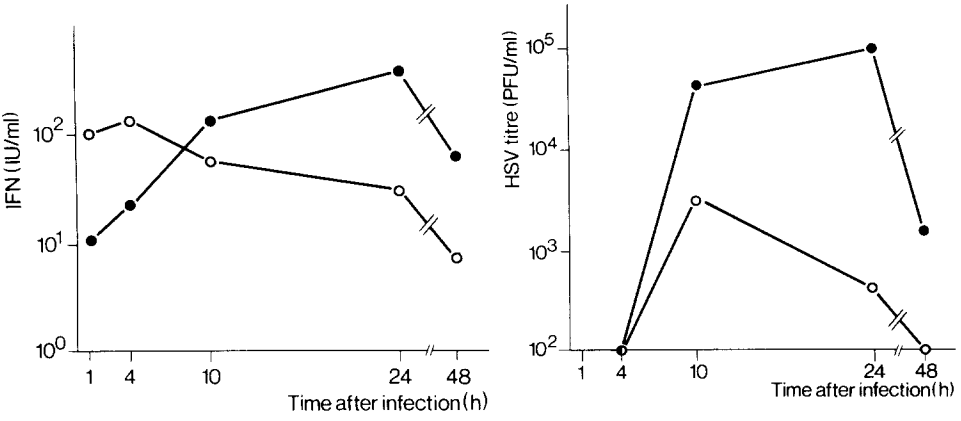

Fig. 2. Interferon titers in the peritoneal wash fluid of C3H/HeJ (○) and C3HeB/FeJ (●) mice at different times after infection with HSV.

Fig. 3. Titers of HSV in the peritoneal fluid of C3H/HeJ (○) and C3HeB/FeJ (●) mice at different times after infection.

no stringent correlation between the degree of NK cell activation and resistance.

Ascher et al. have already shown that interferon production in response to LPS in cultures of C3H/HeJ spleen cells is defective – which is explained by their general nonresponsiveness to all known effects of LPS [1]. This defect is determined by a single gene linked to Mup-1 on chromosome 4 of the mouse [13]. Our previous and current findings suggest that this gene may influence the resistance against viral infection and that the LPS-locus is also involved in the control of virally induced early interferon production. Segregation analysis of F_2 on backcross progeny derived from the two mouse strains is required to establish this point unequivocally.

It has to be recalled that at least two other defects have been observed in C3H/HeJ mice. One is a subnormal incidence of colony-forming B cells [6] and the other is a defect of developing cytotoxic macrophages in response to a variety of in vivo or in vitro activation stimuli [10]. It remains to be determined if there is an association between this latter defect and the increased interferon production towards HSV documented here.

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