Natural Killer Cellular Cytotoxicity Against Herpes Simplex Virus-Infected Cells in Igh-1-Disparate Mice

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Susceptibility to Herpes simplex virus type 1 (HSV-1)-induced stromal keratitis (HSK) in the mouse has previously been linked to the Igh-1 locus. The role of natural killer cells (NK) in resistance to viral infections is controversial. The authors studied the influence of the Igh-1 locus on in vitro murine NK activity against HSV-1 infected cell lines. The HSV-1 infected targets were lysed better than uninfected cells by murine splenic lymphocytes. Strain had no influence on virus-augmented cell lysis. Spleen cells from naive HSK-susceptible CAL-20 (Igh-1\(^d\)) and BALB/c (Igh-1\(^a\)) mice lysed YAC-1 targets better than HSK-resistant C.B-17 (Igh-1\(^b\)) mice. The reverse was seen 24 hours after in vivo infection intraperitoneally with HSV-1. In contrast, CAL-20 splenocytes lysed PUS-1R targets better than BALB/c and C.B-17 splenocytes 24 hours after intraperitoneal (IP) infection. No significant differences were detected in interferon (IFN) levels after IP challenge with HSV-1 among the Igh-1 congenics. The data show that differences in NK activity were determined by both the Igh-1 genotype and the uninfected target cell. Susceptibility to HSK in these Igh-1-disparate congenics thus cannot be explained simply by differences in NK activity against HSV-1-infected targets. Invest Ophthalmol Vis Sci 31:2224-2229, 1990

Susceptibility to Herpes simplex virus type 1 (HSV-1)-induced stromal keratitis (HSK) in the mouse has previously been linked to the Igh-1 locus on chromosome 12. Both A/J (Igh-1\(^e\)) and CAL-20 (Igh-1\(^d\)) mice develop severe stromal keratitis after HSV-1 challenge, but C.B-17 (Igh-1\(^b\)) mice are resistant. BALB/c (Igh-1\(^a\)) mice display intermediate susceptibility to stromal keratitis.

Recent studies suggested a role for natural killer (NK) cells in the initial protection against certain viral infections. Investigators in several laboratories found that murine and human NK cells preferentially lyse target cells infected with HSV-1 over uninfected targets. Genetic experiments suggest that at least three different interacting genes govern NK activity in the mouse, in which an H-2D region gene and two non-H-2-linked genes are involved.

We studied in vitro murine NK activity of Igh-1-disparate congenics against HSV-1-infected targets to define the immunologic basis of susceptibility to HSK. We also examined the effect of in vivo infection on NK activity and searched for strain-dependent differences in early interferon (IFN) production.

Materials and Methods

Animals

C57BL/6J (Igh-1\(^b\)), C57BL/6J-beige (beige), CAL-20, BALB/c, and C.B-17 mice, 6–8 weeks of age, were obtained from Jackson Laboratories (Bar Harbor, ME) and housed in microisolators in our animal facility. All studies conformed with the Declaration of Helsinki and The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) and the ARVO Resolution on the Use of Animals in Research.

Virus

The HSV-1 KOS strain, originally obtained from Dr. David Knipe (Harvard Medical School, Boston, MA), was grown and titered on Vero cell monolayers (CCL 81; American Type Culture Collection (ATCC), Rockville, MD) in our laboratory.

Target Cell Line

The YAC-1 cells (TIB 160; ATCC), an A/Sn T-lymphoma, and PUS-1R cells (TIB 61; ATCC), a BALB/c monocytic tumor, were grown in RPMI-1640 supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 \(\mu\)g/ml), and amphotericin B (250 \(\mu\)g/ml).
Cytotoxicity Assays

The mice were killed by cervical dislocation. Effector cells from mouse spleens were prepared by teasing spleens apart between two frosted-glass slides. The resulting cell suspension was layered on 7 ml of Lympholyte-M (Cedarlane, Hornby, Ontario) and centrifuged at 1800 rpm for 20 min. Lymphocytes were harvested from the interface, washed twice in Hank’s balanced salt solution and diluted to 4 × 10^7 cells/ml in RPMI-1640 medium containing 10% FCS, penicillin (100 units/ml), streptomycin (100 μg/ml), and 10 mM HEPES. The HSV-infected target cells were prepared by incubating YAC-1 or PU5-1R cells with HSV-1 at a multiplicity of infection of 5.0 plaque-forming units (PFU) per cell for 2 hr at 37°C in 5% CO₂. Infected and uninfected target cells were labeled with 200 μCi of 51 Cr (as NaCrO₄; New England Nuclear, Boston, MA). Target cells were washed three times in culture media and diluted to 4 × 10^5 cells/ml. Titrated numbers of effector cells and 2 × 10^4 51Cr-labeled targets were mixed in 96-well flat-bottomed microtiter plates (FALCON; Becton Dickinson, Lincoln Park, NJ) at effector-to-target ratios of 200, 100, and 50. Samples were plated in six replicates. After 12 hr of incubation at 37°C in 5% CO₂, 100 μl of supernatant was harvested from each well, and radioactivity was counted in an LKB-Wallac (Turku, Finland) 1272 gamma counter. Spontaneous release was less than 30% of total release. Specific release was calculated as

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\text{% Specific release} = \left( \frac{\text{expt. cpm} - \text{spont. cpm}}{\text{total cpm} - \text{spont. cpm}} \right) \times 100
\]

The data were expressed as lytic units (LU), defined as the number of effector cells required for 10% specific chromium release. The LUs were calculated using an exponential-fit equation as previously described.¹³

IFN Assay

Serum was collected from male mice 4, 8, and 24 hr after intraperitoneal infection with 1 × 10⁷ PFU of HSV-1 KOS strain. These samples were tested for antiviral activity against encephalomyocarditis virus challenged-L929 cell monolayers in a microtest assay (LEE BioMolecular, San Diego, CA). Results were evaluated relative to an international IFN standard (G-002-904-511) and normalized to National Institutes of Health reference units (IU/ml).

Statistical Analysis

The significance of the results was assessed by analysis of variance. The significance of differences between specific pairs of means was then assessed by Fisher’s least significant difference test.

Results

Naive Mice as Sources of Effector Cells

The spontaneous cytolytic activity of splenic lymphocytes from naive Igh-1-disparate congenics was compared using both HSV-1-infected and uninfected YAC-1 targets. This cell line is sensitive to the cytolytic activity of NK cells in mice. Preliminary experiments confirmed previous observations that while cytotoxicity against uninfected targets reached a plateau after 4-6 hr of incubation, killing against infected targets reached a plateau after 6-12 hr;¹⁰ subsequent experiments were done at 12 hr. Values presented in Figure 1 are the means of eight experi-
ments ± the standard error of the mean. As in previous reports, murine splenic lymphocytes spontaneously lyse HSV-1-infected targets better than uninfected targets ($P < 0.05$). The CAL-20 and BALB/c mice had significantly higher NK lytic activity against either infected or uninfected YAC-1 targets than did C.B-17 mice ($P < 0.05$). There was no significant difference in NK-mediated lysis between CAL-20 and BALB/c mice. When the magnitude of the difference between the lysis of infected and uninfected cells [NK (HSV-YAC) − NK (YAC)], which we will refer to as virus-enhanced or virus-directed lysis, were compared among the three strains, no significant differences were found.

HSV-1-Infected Mice as Sources of Effector Cells

Groups of five CAL-20 and C.B-17 mice were infected with $1 \times 10^7$ PFU of HSV-1 KOS strain intraperitoneally at 6, 12, and 24 hr before death in five experiments. Splenic lymphocytes were then tested for their ability to lyse HSV-1-infected and uninfected YAC-1 cell targets. In vivo HSV-1 infection induced a rapid increase in NK activity in both strains of mice during the first 24 hr after infection as shown in Figure 2. Splenic lymphocytes from infected C.B-17 mice had significantly higher NK activity against HSV-1-infected targets than did CAL-20 splenocytes 24 hr after intraperitoneal infection with HSV-1 ($P < 0.05$). This contrasts with the results reported above for spleen cells from naive mice where CAL-20 splenocytes had significantly higher NK cytolitic activity than did C.B-17 splenocytes ($P < 0.05$). No significant difference in the magnitude of virus-enhanced cytotoxicity between the two strains was seen.

Cytotoxicity Against PU5-1R Targets

We tested the ability of splenic lymphocytes from Igh-1-disparate congenics to mediate cytotoxicity against PU5-1R cell targets in three experiments. This cell line has been shown previously to be lysed by NK cells.10 Groups of five mice were infected intraperitoneally with $1 \times 10^7$ PFU of HSV-1 KOS strain and were killed 24 hr after infection. As shown in Figure 3, uninfected mice showed no
significant lysis against either HSV-1-infected or uninfected PU5-1R cell targets. Twenty-four hours after intraperitoneal infection, CAL-20 splenic lymphocytes showed significantly greater cytotoxic activity against both HSV-1-infected and uninfected targets than did splenocytes from BALB/c and C.B-17 mice ($P < 0.05$). There was no difference in cytotoxic activity between BALB/c and C.B-17 splenic lymphocytes. Killing of infected targets was significantly increased compared with uninfected targets in all three strains tested ($P < 0.05$). There was no difference in the magnitude of virus-augmented lysis among the three strains.

**IFN Response to HSV-1 Challenge**

The kinetics of early IFN production during the first 24 hr after intraperitoneal infection with HSV-1 was examined in C57BL/6J+, C57BL/6J-bg (beige), C.AL-20, BALB/c, and C.B-17 mice (Fig. 4). Mice with the C57BL/6J background had significantly higher levels of IFN than mice of the BALB/c background within 4 hr after infection ($P < 0.05$). Time-course dynamic studies disclosed no difference in IFN production among any of the murine strains after 4 hr of infection. Although CAL-20 and C.B-17 mice had higher mean values of serum IFN than BALB/c mice, this difference was not significant. There was no significant difference in early IFN titers between the beige and the C57BL/6J+ mice.

**Discussion**

The role of NK cells in resistance to HSV-1 infection is controversial. Depletion of NK cell activity with $^{89}$Sr or with antibody to asialo GM1 enhances susceptibility to HSV-1 encephalitis in mice.14 Correlations have been made between genetically high NK cell activity and resistance to HSV-1.15 Confounding the understanding of natural resistance to HSV-1 has been the antiviral role of IFN. Mice with genetically high NK cell activity, such as the C57BL/6J strain, generally also have high levels of HSV-1-induced IFN.16 Thus the genetics of NK activity directed against virus-infected cells may be linked with the genetics of IFN production. These investigators found that resistance to viral infection could be correlated with high IFN titers produced during the first 2–4 hr after viral challenge. It is known that SJL mice, which have a defective NK system, produce high titers of IFN in response to HSV-1, and these mice are relatively resistant to this virus.17 However, SJL mice are less resistant than C57BL/6 mice which show a high early IFN response and a high NK response. The situation is therefore complex in regard to the relative contributions of IFN and NK cells in resistance of mice against viral infections, and it is possible that activation of NK cells by IFN is of greater importance. Others reported a significant correlation between resistance to the lethal effects of murine cytomegalovirus infection and the degree of IFN-dependent augmentation of NK activity in ten mouse strains tested.18 Although the structural genes for IFN have been localized to chromosome 4,19-21 the genes which regulate IFN production in response to HSV have not yet been identified. There are data to suggest that at least three genes are involved in regulation of IFN production, one of which is X-linked.22

Previous reports indicate that the effectors which lyse YAC-1 and PU5-1R targets are NK cells.10 Our studies demonstrate that the Igh-1 locus influences the ability of murine splenic lymphocytes to mediate spontaneous cytotoxic activity against these targets. This raises the possibility that the Igh-1 locus exerts its influence through the expression of receptors on the NK cell responsible for recognizing target-cell structures. Interestingly, one report indicated that NK cells express products on the NK-cell receptor that are closely linked to Igh-1.23
Because little is known about the target structures recognized by NK cells, it is presently impossible to determine whether viral determinants alone or in combination with some structure on the uninfected cell are recognized by effector cells. Although HSV-1-infected targets were generally lysed better than uninfected targets, no difference in the magnitude of virus-specific or virus-augmented lysis was seen among the different Igh-1 congenics in our experiments.

Other investigators showed that a nonspecific mechanism involving IFN produced by the effector cells and self-activation after interacting with a virus-infected cell can account for the increased cytotoxicity seen against HSV-infected YAC-1 and PUS-1R cells. Based on these results, it is unlikely that the Igh-1 locus plays a role in regulating cytotoxicity directed against viral determinants on HSV-1-infected cells.

Although differences in NK activity among the Igh-1-disparate congenics were observed in our study, the level of NK activity did not correlate with resistance to HSK. Preliminary in vivo studies in our laboratory indicated that C57BL/6J mice with the beige mutation (bg/bg), known to have NK cells able to bind to target cells but unable to lyse them, were just as resistant as normal C57BL/6J (+/+) mice to HSK (manuscript in preparation). Our studies on early IFN production confirmed previous reports that mice with the C57BL/6J background produced higher levels of IFN than mice with a BALB/c background. Based on these results, it is tempting to attribute resistance to HSK in mice solely to high titers of IFN produced early in response to infection rather than to differences in NK cell lytic activity. No significant differences in early IFN levels, however, were found among the Igh-1-disparate congenics in our study. It is therefore unlikely that differences in levels of early IFN production or NK activity alone can account for the different susceptibilities to HSK seen in these mice nor can the Igh-1 gene locus be considered a possible candidate to be one of the still unidentified IFN regulatory genes.

Our experiments confirm that HSV-1 infection of NK targets induces increased cell lysis of these targets, but this is apparently due to a nonspecific and Igh-1-independent mechanism. The lysis of tumor cell targets by NK cells appears to be regulated by the Igh-1 gene locus, but NK activity did not correlate with susceptibility to HSK. No significant differences in early IFN titers were found among the Igh-1-disparate congenics. Together, our data show that the different susceptibilities of the Igh-1-disparate congenic mice to HSK cannot be explained by differences in NK activity against HSV-1 infected cells.

Key words: Herpes simplex virus type 1, natural killer cells, Igh-1, interferon, keratitis

References


18. Bancroft GJ, Shalam GR, and Chalmer JE: Genetic influences
Correction

In the article "Expression of Collagenolytic/Gelatinolytic Metalloproteinases by Normal Cornea," by M. Elizabeth Fini and Marie T. Girard, which appeared in the September 1990 issue of Investigative Ophthalmology and Visual Science, Figure 2 (page 1783) was incorrect. The corrected figure is shown here. The publisher regrets any inconvenience this error may have caused.