Experimental Infection of Inbred Mice with Herpes Simplex Virus
IV. Comparison of Interferon Production and Natural Killer Cell Activity in Susceptible and Resistant Adult Mice

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With 2 Figures

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Summary

Inbred mouse strains differ in susceptibility to infection with herpes simplex virus type 1 or type 2 (HSV-1, HSV-2). In this study interferon production was tested in the peritoneal exudate of mice after intraperitoneal (i.p.) injection of HSV-1 or HSV-2. In HSV-resistant mice (C57BL/6, C3H/HeJ) high titers of interferon were already present 2 to 4 hours after injection. In comparison, less resistant mice (DBA/2, AKR) lacked this early response. There was no correlation between interferon titers and resistance at post-infection times later than twelve hours. At twelve hours, however, high titers of HSV were detected in the peritoneum of DBA/2 mice and significantly lower titers in C57BL/6 mice. In a comparative analysis of eight different inbred mouse strains, again early (2 to 4 hours) interferon production was correlated to resistance. In assays of HSV-stimulated early (24 hours) NK cell responses not only the good interferon producer strains but also one of the less resistant low interferon producers (BALB/c) showed significant cytotoxic activities. Conversely, SJL mice that are very low in HSV-induced NK cell activity are resistant and show high early interferon responses at the local site.

Introduction

Resistance of mice against primary infection with HSV is genetically determined (10) and associated with bone marrow dependent cell functions (12). The relevant defense mechanisms involved in resistance have not yet been identified. Recent data, however, from the two laboratories participating in the present study have suggested that both interferon and NK cell functions may play a role in resistance in the model used (2, 16). Since in the initial investigations of
the two laboratories different virus preparations and mouse strains were used only limited comparisons were possible. We have now performed a collaborative study in which we utilized the same viruses and mouse strains. The aim of the study was to attempt to correlate HSV-1 and HSV-2 induced interferon and NK cell responses to resistance in a variety of different inbred mouse strains. The presumed resistance functions were determined at the site of infection in the peritoneal cavity. Such tests probably reflect more closely the decisive events in antiviral defense than for example measuring NK cell activity in central lymphoid organs such as the spleen or interferon levels in the serum.

**Materials and Methods**

**Viruses**

HSV-1 (WAL) and HSV-2 strain 72 were prepared as previously described (1, 16). They will be referred to as HSV-1 and HSV-2, respectively.

**Mice**

AKR/J, C3H/HeJ, C57BL/6J, DBA/1J, DBA/2J, SJL/J and SWR/J were obtained from Jackson Laboratories (Bar Harbour, ME, U.S.A.). BALB/c/A Bom mice were purchased from G1. Bomholtgard Ltd. (Ry Denmark). Eight to ten week old mice were delivered to both laboratories. All experiments were performed during the subsequent six weeks.

**Experimental Design**

Groups of mice were injected i.p. with HSV. At various times thereafter 5—6 mice were killed and the peritoneal exudate cells (PEC) were tested for NK cell activity after pooling. The peritoneal cavity was rinsed extensively for maximal cell yields. Five or more additional mice were tested for the interferon contents of the cell-free peritoneal wash-out. The peritoneal fluid for interferon testing was usually harvested within 30 minutes after i.p. injection of 1 ml depending on the size of the experimental groups.

**Assay for NK Activity**

A 5 hours ⁵¹Cr-release assay was performed as detailed in reference 1. PEC from HSV infected mice served as effector cells, YAC-1 lymphoma cells as targets. PEC from normal mice, which do not exhibit cytotoxic activities higher than culture medium alone (see reference 1) served as low controls in the tests. Data for mock virus preparations have been omitted from the results. In our hands they do not induce any NK cell activities (1).

**Interferon Assay**

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 (16).

**Virus Titration**

Virus titers in the peritoneal fluid were determined by plaque titration on RITA cells (origin: monkey kidney).

**Results**

**LD⁵₀ for HSV-1 and HSV-2**

Data with respect to the LD⁵₀ for HSV-1 and HSV-2 in the different mouse strains studied have already been published (2, 9, 10). The LD⁵₀ obtained with the HSV preparations used in the present investigation were in the same range.