Experimental Infection of Inbred Mice with Herpes Simplex Virus. III. Comparison between Newborn and Adult C57BL/6 Mice

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SUMMARY

We have previously shown that adult C57BL/6 mice are relatively resistant to intraperitoneal (i.p.) infection with herpes simplex virus type 1 (HSV-1). Here we show that newborn mice of the strain C57BL/6 are highly susceptible to i.p. infection with HSV-1. Newborn C57BL/6 mice, in contrast to adult mice, did not develop natural killer cell activity in the peritoneal cavity 24 h after i.p. injection of HSV-1, and showed only minimal titres of interferon in the peritoneal fluid after 4 h. After 24 h the peritoneal fluid of newborn mice contained high amounts of interferon and high titres of HSV-1. In contrast, the virus titres in the peritoneal cavity of adult mice were significantly lower. It is suggested that the early titres of interferon at the infection site that are observed in adult, resistant C57BL/6 mice but not in susceptible, newborn mice play a decisive role in resistance.

INTRODUCTION

It has long been established that newborn mice are susceptible to intraperitoneal (i.p.) infection with herpes simplex virus (HSV), whereas adult mice are resistant (Andervont, 1929). These studies were usually done with outbred mice. More recently, the studies of Lopez (1975) and of our laboratory (Kirchner et al., 1978) have shown that adult mice of certain inbred strains are highly susceptible to i.p. infection with HSV, whereas other strains are relatively resistant. The reasons for resistance, and particularly the relevant defence mechanisms, have not been defined. However, previous studies in this laboratory have suggested that there was a correlation between resistance and interferon production (Zawatzky et al., 1981). Resistant strains such as C57BL/6 produced high titres of serum interferon in response to HSV, whereas moderate titres were produced in susceptible mouse strains. Armerding & Rossiter (1981) have analysed activation of natural killer (NK) cells by HSV. In this study, a similar, although not complete correlation was observed. This was also suggested in a preliminary report from our laboratory (Kirchner et al., 1980).

In the present work, we have applied a simplified technique that allowed us to measure virus titres and titres of interferon simultaneously from a small sample of tissue fluid from the peritoneal cavity of infected mice. We have used this technique to test interferon production, NK cell activation, and virus replication in a comparison between newborn and adult C57BL/6 mice.

METHODS

Mice. C57BL/6J BOM mice were obtained from Bomholtgard (Ry, Denmark) and were bred in our own animal colonies to obtain newborn mice. Usually, the latter were used for the experiments during the first week of life. Preliminary testing had revealed that the parameters we were testing did not differ on different days during the first week of life. Subsequently, we
have used mice at different days during the first 8 days of life and have uniformly called them newborn mice.

Virus. In all experiments HSV-1 (WAL) was used and prepared as described previously (Zawatzky et al., 1982).

Experimental protocol. In the experiments of this study mice were injected i.p. with HSV-1 and at various times thereafter a small amount (0.5 ml for newborn mice and 1 ml for adult mice) of RPMI 1640 supplemented with 2.5% foetal bovine serum was injected. The abdomen was gently massaged for a few minutes, and then the peritoneal cavity was opened. The fluid was recovered by using a Pasteur pipette and freed of cells by centrifugation.

Experimental test systems. In different experiments of this study three parameters were determined, including titres of HSV-1, activity of NK cells and titres of interferon. These assays have been described in detail in previous communications from our laboratory (Kirchner et al., 1978; Schröder et al., 1981; Zawatzky et al., 1981; Engler et al., 1981). In experiments in which virus titres and interferon titres were determined in the same samples, these were divided and half were treated for 2 days at pH 2 to inactivate the virus before the interferon determination. Virus titres in the peritoneal fluid were determined using RITA cells of monkey kidney origin and a standard plaque assay (Kirchner et al., 1976). For testing NK cell activity, a 4 h 51Cr-release assay was utilized with mycoplasma-free YAC-1 lymphoma cells as targets (Engler et al., 1981). Interferon titrations were performed exactly as described by Beck et al. (1980) using a one-step assay, L cells, and vesicular stomatitis virus. The titres were expressed as international units (IU) per ml by using an international standard (NIH-standard G-002-904511).

RESULTS

Mortality of newborn C57BL/6 mice after i.p. infection with HSV-1

Experiments were performed to test the virulence of HSV-1 after i.p. infection of newborn mice. The LD50 of newborn C57BL/6 mice after i.p. infection with HSV-1 (WAL) was 2 × 101 p.f.u., whereas it was at least a 1000-fold higher in adult C57BL/6 mice as described previously (Kirchner et al., 1978).

Testing of NK cell activity and of interferon induction in newborn mice

Newborn or adult C57BL/6 mice were injected i.p. with different doses of HSV-1. Peritoneal fluid was taken at 4 h to determine the interferon level, and from parallel groups of mice peritoneal exudate cells (PEC) were recovered at 24 h for testing of NK cell activity against YAC-1 target cells. As can be seen in Table 1, newborn mice, in contrast to adult mice, showed very low interferon production and no NK cell activation after injection of HSV-1.

Kinetics of NK cell activation

We have shown the absence of an NK cell response in newborn mice at 24 h. The kinetics of NK cell activity in adult and 2-week-old mice after i.p. injection of 104 p.f.u. HSV-1 is shown in Table 2, showing that young mice are able to develop NK cells but only later than adults.

Comparison of virus titres and of interferon titres in adult and newborn C57BL/6 mice

HSV-1 (106 p.f.u.) was injected into adult or newborn C57BL/6 mice and, after 4 h and 24 h, virus titres and interferon titres were determined in the peritoneal fluid. A representative experiment is shown in Fig. 1. As can be seen, no virus could be detected in either newborn or adult mice 4 h after infection. At 24 h, titres of HSV-1 in the peritoneal fluid of newborn mice
HSV infection in newborn and adult C57BL/6 mice

Fig. 1. Kinetics of HSV-1-induced interferon (IFN) production (a) and HSV-1 replication (b) in the peritoneal fluid of adult (○) and newborn (●) C57BL/6 mice after injection of $10^6$ p.f.u. HSV-1.

Table 1. NK cell activation and induction of interferon in the peritoneal exudate of newborn and adult C57BL/6 mice after infection with HSV-1 *

<table>
<thead>
<tr>
<th>Age of mice (weeks)</th>
<th>Virus dose (p.f.u./animal)</th>
<th>Interferon titre† (IU/ml)</th>
<th>NK cell activity‡ (% specific lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>$2 \times 10^6$</td>
<td>1280</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^5$</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^4$</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>$2 \times 10^6$</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^5$</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

* Values represent means of three experiments with eight mice in each group.
† At 4 h after injection.
‡ At 24 h after injection.

Table 2. Kinetics of NK cell activity in peritoneal exudate cells of HSV-1-infected C57BL/6 mice at different ages *

<table>
<thead>
<tr>
<th>Age of mice (weeks)</th>
<th>Time of testing (h)</th>
<th>NK cell activity (% specific lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td></td>
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<td>20</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>34</td>
</tr>
</tbody>
</table>

* Values represent means of three experiments with eight mice in each group (infecting dose, $10^5$ p.f.u./animal).

were significantly higher than in adult mice. When interferon titres were determined (Fig. 1), high levels were observed in the peritoneal fluid of adult mice 4 h after injection of HSV-1, whereas levels in newborn mice were at the borderline of detection. At 24 h, interferon titres were higher in young than in adult mice.

Thus, our data show that adult C57BL/6 mice produce high titres of interferon early after injection of HSV-1 and have only low virus titres at 24 h, whereas newborn mice produce no interferon immediately upon infection, but have high virus titres at 24 h, as well as detectable interferon titres at this time.
Table 3. *Age dependency of NK cell activity and interferon production in the peritoneal cavity of HSV-1-infected C57BL/6 mice*

<table>
<thead>
<tr>
<th>Age of mice (days)</th>
<th>NK cell activity (% specific lysis)</th>
<th>Interferon titre (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>20</td>
</tr>
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<td>18</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>27</td>
<td>250</td>
</tr>
</tbody>
</table>

* NK cell activity was tested 24 h after infection with $10^5$ p.f.u./animal; interferon titres were determined 4 h after infection with $10^6$ p.f.u./animal. Mean values of five animals for each test are shown.

Comparison of young C57BL/6 mice at different ages

In agreement with the previous data of Kohl & Loo (1980) we have found that C57BL/6 mice became resistant to HSV-1 infection at about 20 days of life (data not shown). We have therefore compared NK cell activation and interferon titres at various times between day 7 and day 24 (Table 3). As can be seen, both responses were low or absent at 7 and 15 days, but became positive between 18 and 24 days. Thus, a correlation was seen between the development of resistance and the two types of response we have measured.

DISCUSSION

In this paper we have shown that newborn C57BL/6 mice are highly susceptible to i.p. infection with HSV-1. This confirms an observation of Lopez reported in the discussion at an International Meeting (see Kirchner et al., 1980) and a recent paper by Kohl & Loo (1980). Furthermore, our data have shown that injection of HSV-1 in newborn mice, in contrast to adult mice, does not induce NK cell activity in the cell population of the peritoneal exudate at 24 h. Previously, we have expressed the view that NK cell activation is probably an indirect sequel of interferon induction and that it is therefore important to study interferon production in the peritoneal exudate cell population (Engler et al., 1981). Here, we have documented that early interferon production and NK cell activation is very low or absent in newborn C57BL/6 mice.

It is generally accepted that NK cell activity in mice is age-dependent and that there is no spontaneous 'uninduced' NK cell activity detectable during the first weeks of life. Our data have shown that the 'pre-NK cells' are present in newborns, since high NK cell activity may be measured 72 h after virus injection. Interferon is known to be an inducer of NK cell activity (Gidlund et al., 1978). However, the early interferon response is defective in newborn mice and, thus, early NK cell activation is also defective. Later on, interferon is produced and NK cells are activated.

Lopez (1978) has shown that resistance of adult mice to HSV is caused by active defence mechanisms which are bone marrow-dependent. It has also been documented by our laboratory that resistance of C57BL/6 mice against HSV infection may be broken by immunosuppressive measures (Kirchner et al., 1978). That resistance is probably independent of mature T cells was documented in experiments in which we have shown that homozygous nude mice are not more susceptible to HSV infection than their heterozygous littermates (Zawatzky et al., 1979).

It remains to be determined which of the various defence mechanisms against primary virus infections that exist in the body play the decisive role against the experimental infection of the mouse with HSV. A previous paper of this series has established that there was a correlation between high interferon titres and resistance (Zawatzky et al., 1981). Mouse strains resistant to HSV produced high titres of serum interferon upon injection of HSV,
whereas low titres were produced by susceptible mice. Gresser et al. (1976) have shown that resistance of mice against HSV can be broken by injection of anti-interferon serum, an observation which has subsequently been confirmed in our laboratory (Zawatzky et al., 1982).

In the present paper, we have used a simplified method of obtaining tissue fluid directly from the infection site, i.e. the peritoneal cavity. From these samples we have at various times determined titres of interferon and of HSV. Our data have suggested that it is the early interferon which plays a decisive role in resistance. This interferon obviously is produced in response to the infecting (input) virus. It is found only in resistant adult mice and not in susceptible newborn mice. At 24 h, virus titres in newborn mice are significantly higher than in adult mice. At this time interferon titres in newborns were even higher than in adult mice.

One of the major objections against a decisive role of interferon in natural resistance against virus infections has been based on situations similar to the one we have described in newborn mice. In many studies, interferon was detected at the time of virus replication and had no protective effect, since the fatal outcome of the infection was already determined, However, we suggest that the early interferon may play a significant role.

REFERENCES


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