Protection of Newborn Mice Against Herpes Simplex Virus Infection by Prenatal and Postnatal Transmission of Antibody

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SUMMARY

Pre- and postnatally acquired protection against herpes simplex virus type 2 (HSV-2) infection mediated by maternal antibody was investigated in the newborn mouse. Newborn mice, 2 days old, were inoculated with HSV-2 intraperitoneally after maternal immunization with live or inactivated virus. The survival rates improved in proportion to the maternal neutralizing antibody titres. Ninety-three percent of animals delivered by Caesarean section from immune mothers and suckled by non-immune mothers survived viral infection, whereas 79% of control animals survived. The same was true with animals born to non-immune mothers and nursed by immune mothers. In foetal sera and milk of immunized mice, anti-HSV activity was associated primarily with antibody of the IgG class as measured by enzyme-linked immunosorbent assay (ELISA). In addition, oral administration of antibody conferred protection on newborn mice. These studies indicate that maternal IgG, acquired not only postnatally but also prenatally, plays an important role in protecting newborn mice against HSV infection.

INTRODUCTION

Herpes simplex virus (HSV) causes fatal, disseminated infection in newborn humans (Nahmias et al., 1971; Hanshow, 1973; Whitley et al., 1980) and mice (Johnson, 1964; Hirsch et al., 1970; Kern et al., 1973). Concerning the influence of maternally derived antibody in protection against human neonatal HSV infection, several reports have revealed conflicting estimations of its importance (Nahmias et al., 1971; Whitley et al., 1980; Yeager et al., 1980). In animal studies, mice born to immunized mothers were somewhat resistant to HSV infection (Berry & Slavin, 1943; Amstey & Kobos, 1976).

Maternal antibody is acquired transplacentally before birth in humans. However, newborn mice acquire maternal antibody principally after birth by intestinal absorption from colostrum or milk, and a small amount of antibody is transmitted from mother to foetus prenatally (Guyer et al., 1976). Therefore, the protective mechanism against murine neonatal HSV infection mediated by maternal antibody should be analysed in an attempt to distinguish prenatal immunity from postnatal immunity. Little is known about the effectiveness in mice of prenatal immunity against postnatal infection by HSV. Moreover, there has been little direct evidence that maternally derived antibody actually mediates protection.

In the present study, we demonstrate that foetal mice acquire protective levels of maternal antibodies in utero. The effectiveness of prenatal immunity in mice indicates that this species can provide a model for human prenatal immunity. In addition, regarding postnatal transmission of resistance, we detected anti-HSV IgG antibody in immune milk by enzyme-linked immunosorbent assay (ELISA), and confirmed the protective capacity of maternally derived antibody by oral administration of antibody.

METHODS

Viruses. The KOS strain of HSV type 1 (HSV-1) and the YS-4 strain of HSV type 2 (HSV-2) were used. The YS-4 strain, an isolate from a recurrent facial lesion of an infant (Mori et al., 1973), was used for inoculation of newborn
mice. The viruses were propagated in Vero cells maintained in Eagle's minimal essential medium supplemented with 2% calf serum. Virus infectivity was assayed on Vero cells as p.f.u./ml.

**Mouse immunization.** Female and male 6-week-old C3H/N mice were obtained from the Laboratory of Animal Experiments, Kyushu University. Female mice were immunized by injecting four doses of infectious HSV-1 or HSV-2 into a footpad at intervals of 7 days, 1 × 10^6 p.f.u. in 0.05 ml three times and then 1 × 10^6 p.f.u. in 0.05 ml. None of these animals died after the immunization. The same virus suspension exposed to u.v. light (2.0 × 10^6 J/m^2) was used for immunization with inactivated virus. There was no residual virus infectivity after this treatment and this preparation was injected in the same way as above.

**Breeding and fostering.** One week after the last injection, the female mice were mated. The morning on which the vaginal plug was seen was denoted day 0 of pregnancy. Newborn mice were usually delivered between day 18.5 and day 19.0 of pregnancy. To assess the relative effect of milk and prenatal immunity, some litters were delivered by Caesarean section on day 18.5 of pregnancy and placed with foster mothers. Others were allowed to suckle on their natural mothers.

**Oral feeding of antibody to newborn mice.** Human IgG having a neutralizing titre of 1:256 was prepared from pooled human plasma by the method of Kistler & Nitschmann (1962) and further purified by DEAE-cellulose column chromatography. The protein concentration was 137 mg/ml. This human IgG was kindly provided by the Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan. Newborn mice, born to and suckled by non-immune natural mothers, were given oral supplements of human IgG at 24 h and 4 h before infection. Polyethylene tubing (6.4 mm diam.) attached to a 250 µl glass syringe was inserted into the lower oesophagus, and 25 µl of human IgG was injected into the stomach. Control animals were given phosphate-buffered saline (PBS, pH 7.2) orally.

**Challenge of newborn mice with virus.** Litter size was maintained at six to eight animals per mother. At 2 days of age, mice were inoculated intraperitoneally (i.p.) with 0.05 ml of virus suspension containing 1 × 10^7 p.f.u. of HSV-2. This dose corresponds to 25 LD_50_ determined by the Reed–Muench method. The animals were observed daily for signs of illness or death for 28 days after inoculation. No deaths occurred after that time.

**Collection of serum and milk.** At the time of challenge of the newborns, some of the mother mice were bled by cardiac puncture under ether anaesthetia. In a separate experiment, foetal mice were bled on day 18.5 of pregnancy, and paired sera of mothers and newborns were obtained 2 days after birth. Because of the small amount available, foetal and neonatal sera obtained by decapitation were pooled within each litter. One vol. of pooled milk curd taken from the stomachs of the newborns was mixed with three vol. of PBS (pH 7.2). The suspension was centrifuged at 100 g for 10 min. After the lipid supernatant layer was discarded, the aqueous layer was centrifuged at 130000 g for 120 min. The resultant supernatant was regarded as a fourfold dilution of the original milk.

**ELISA.** The assays were carried out by a modification of the method described by Voller et al. (1978). Microtitre plates (Microelisa, Dynatech Laboratories, Alexandria, Va., U.S.A.) were sensitized with 1 µg of purified HSV-2 antigen (Gilman & Docherty, 1977) in 100 µl of coating buffer (pH 9-6) per well overnight at 4 °C. The plates were washed three times with PBS (pH 7-2) containing 0.05% Tween 20 (PBS–Tween). The wells were filled with 100 µl quantities of samples diluted in PBS–Tween containing 0.5% bovine serum albumin (PBS–Tween–BSA). The plates were incubated at 37 °C for 2 h and washed. Peroxidase-labelled goat antiserum to mouse IgG, IgA or IgM (heavy chain-specific, Cappel Laboratories, Cochranville, Pa., U.S.A.) diluted to 1:500 in 100 µl PBS–Tween–BSA was added to each well. After incubation at 37 °C for 2 h, the plates were washed and 100 µl of substrate containing o-phenylenediamine (1 mg/ml) and 0.03% H_2O_2_ in PBS (pH 6-4) was added to each well. The reaction was stopped after 30 min at room temperature by adding 50 µl 14 M HCl. The absorbance (A) was read at 490 nm using a colorimeter (Microelisa minireader, Dynatech Laboratories). The ELISA titre was expressed as the reciprocal of the highest dilution giving an A value of more than 0.30, calculated by subtracting the A of the non-immune control sample from the A of the immune sample.

**Neutralization test.** Titres of neutralizing antibody to HSV-2 were determined by the plaque-reduction method in 96-well tissue culture plates (Falcon Plastics) as previously described (Nagafuchi et al., 1979), and expressed as the reciprocal of the highest dilution causing 80% plaque reduction.

**Statistics.** The P values were calculated by Fisher's exact test.

**RESULTS**

**Protection of newborn mice by maternal immunization**

Table 1 shows the relationship between the maternal antibody titres and the survival rates of 2-day-old mice infected with HSV-2. Maternal immunization with inactivated virus as well as live virus was protective against infection with HSV-2. Heterotypic virus was also effective as an immunogen. The survival rates for newborn mice were found to correlate with the levels of...
Protection of newborn mice against HSV

Table 1. Protection of newborn mice against infection with HSV-2 by maternal immunization, and neutralizing activities in maternal sera

<table>
<thead>
<tr>
<th>Maternal immunization</th>
<th>No. of survivors/ no. tested*</th>
<th>Protection (%)</th>
<th>Maternal neutralizing antibody titre to HSV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-immune</td>
<td>0/32</td>
<td>0</td>
<td>&lt;8, &lt;8, &lt;8, &lt;8</td>
</tr>
<tr>
<td>Inactivated HSV-1</td>
<td>8/32</td>
<td>25</td>
<td>&lt;8, 8, 8, 8</td>
</tr>
<tr>
<td>Inactivated HSV-2</td>
<td>14/32</td>
<td>44</td>
<td>8, 16, 16, 16</td>
</tr>
<tr>
<td>Live HSV-1</td>
<td>15/28</td>
<td>54</td>
<td>16, 16, 16, 16</td>
</tr>
<tr>
<td>Live HSV-2</td>
<td>32/32</td>
<td>100</td>
<td>64, 128, 128, 128</td>
</tr>
</tbody>
</table>

* Two-day-old mice were inoculated i.p. with $1 \times 10^2$ p.f.u./0.05 ml of HSV-2.

Table 2. Prenatal and postnatal acquisition of resistance to infection by HSV-2 in newborn mice*

<table>
<thead>
<tr>
<th>Natural mother</th>
<th>Foster mother</th>
<th>No. of survivors/ no. tested†</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-immune</td>
<td>Non-immune</td>
<td>2/30</td>
<td>7</td>
</tr>
<tr>
<td>Immune‡</td>
<td>Non-immune</td>
<td>28/30</td>
<td>93</td>
</tr>
<tr>
<td>Non-immune</td>
<td>Immune‡</td>
<td>28/30</td>
<td>93</td>
</tr>
</tbody>
</table>

* Newborn mice were delivered by Caesarean section and nursed by foster mothers. Control animals were allowed to suckle on their natural mothers.
† The mice were infected i.p. with $1 \times 10^2$ p.f.u./0.05 ml of HSV-2 on day 2 after birth.
‡ Immunized with live HSV-2.

Table 3. Immunoglobulin class-specific ELISA titres and neutralizing antibody titres to HSV-2 in serum and milk samples of immunized mice*

<table>
<thead>
<tr>
<th>Sample</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>Neutralizing antibody titre†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum‡</td>
<td>1783</td>
<td>&lt;16</td>
<td>32</td>
<td>111</td>
</tr>
<tr>
<td>Foetal serum§</td>
<td>724</td>
<td>&lt;16</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Milk‡</td>
<td>194</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>8</td>
</tr>
<tr>
<td>Newborn serum‡</td>
<td>1176</td>
<td>&lt;16</td>
<td>16</td>
<td>74</td>
</tr>
</tbody>
</table>

* Immunized with live HSV-2.
† Expressed as geometric mean titres of four to six samples.
‡ Samples were obtained on day 2 after birth.
§ Samples were obtained on day 18.5 of pregnancy.

neutralizing antibodies in maternal sera. When mother mice had neutralizing antibodies with high titres ($\geq 1:64$), newborn mice were completely protected.

Time of acquisition of resistance

Newborn mice were delivered by Caesarean section on day 18.5 of pregnancy and suckled by foster mothers to determine whether resistance was acquired prenataally or postnatally (Table 2). The day following Caesarean section was considered as day 0 after birth. In animals delivered from immune natural mothers and nursed by non-immune foster mothers, the survival rate was 93%, whereas it was 7% in controls ($P < 0.001$). Similarly, 93% of newborn mice survived if they had been delivered by Caesarean section from non-immune natural mothers and nursed by immune foster mothers. The results show that resistance to murine neonatal HSV infection had been acquired not only postnatally but also prenatally.

Antibody titre in serum and milk of immunized mice

In serum samples of immunized mice, anti-HSV activity was associated primarily with antibody of the IgG class, although some weak activity was exhibited by IgM. A considerable amount of antibody was also found in foetal serum (Table 3). In immune milk, anti-HSV IgG antibody was demonstrated by ELISA, whereas neither IgA nor IgM could be detected. The
Table 4. Protection of newborn mice against infection with HSV-2 by oral administration of antibody

<table>
<thead>
<tr>
<th>Oral supplement*</th>
<th>No. of survivors/no. tested† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>2/48 4</td>
</tr>
<tr>
<td>Human IgG</td>
<td>30/48 63</td>
</tr>
</tbody>
</table>

* Newborn mice were given oral supplements of 25 μl of human IgG or PBS at 24 h and 4 h before infection. † The mice were infected i.p. with $1 \times 10^2$ p.f.u./0.05 ml of HSV-2 on day 2 after birth.

amount of anti-HSV IgG was about 10 times less in milk than in maternal serum. The neutralizing antibody titre of mice born to non-immune mothers and nursed by immune mothers for 2 days after birth was 1:64.

Protection of newborn mice by orally administered antibody

Since the gastrointestinal tract is known to be one of the routes for transmission of maternal antibody to mice, an oral feeding experiment was performed to assess the protective effect of maternally derived antibody against neonatal HSV infection. Human IgG was used in this experiment, because pooled human antisera contain anti-HSV antibody and are easier to obtain. As shown in Table 4, there was a significant increase in the survival rate of animals fed human IgG as compared with controls (63% versus 4%; $P < 0.001$), and deaths occurred later in animals fed human IgG (days 6 to 13) than in control animals (days 5 to 10). The neutralizing antibody titre of pooled sera of newborn mice fed 25 μl of human IgG 24 h and 4 h before bleeding was 1:16.

DISCUSSION

Studies by Nahmias et al. (1971) and Whitley et al. (1980) revealed that there was no difference in the frequency of disease caused by HSV between human neonates with transplacentally acquired antibody and those without antibody. However, Yeager et al. (1980) pointed out that neonates with high titres of neutralizing antibody have a more favourable prognosis than neonates with low antibody titres. Therefore, further information on clinical and experimental studies are needed regarding the protective capacity of maternal antibody.

We analysed pre- and postnatally acquired protection against HSV-2 infection mediated by maternal antibody in the newborn mouse model. Maternal immunization with inactivated virus conferred resistance to HSV on newborn mice in proportion to the mothers' antibody level, suggesting that the animals were not actively immunized in utero. Berry & Slavin (1943) showed that 2-week-old mice acquired resistance to HSV through milk after birth. We found, by using Caesarean section and foster nursing, that prenatally acquired immunity was enough to protect newborn mice against postnatal HSV infection. A low level of antibody, relative to maternal serum, was detected in foetal serum, but its antibody level seems to be sufficient for protection. Therefore, it is possible that prenatally transmitted antibody alone, which is principally IgG, can protect newborn mice against HSV infection in the early period of postnatal life. The effectiveness of prenatal immunity in mice may provide a useful model for human prenatal immunity.

With regard to the postnatal transmission of antibody, we demonstrated anti-HSV IgG in immune milk, although neither IgA nor IgM could be detected. The quantity of IgG was about 10 times less in milk than in maternal serum, whereas it was slightly less in neonatal serum than in maternal serum. In the mouse, IgG in milk is selectively absorbed from intestinal epithelial cells by IgG Fc receptors (Guyer et al., 1976). It has been suggested that some lymphoid cells in milk are capable of transferring cell-mediated immunity and reacting with HSV antigen (Head & Beer, 1979; Kohl et al., 1982). These cells appear to reach neonatal tissues by penetrating the mucosa.

In the experiments reported here, however, the maternal antibody level correlated with the outcome of murine neonatal HSV infection. In addition, oral administration of antibody was
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carried out to distinguish the action of antibody from the effect of lymphoid cells in milk. When human IgG having a high titre to HSV was given orally to newborn mice, the animals showed improved survival rates. The serum-neutralizing antibody titre of antibody-treated mice was 1:16, whereas that of mice suckled by immune mothers was 1:64. The difference of the antibody amount may be the reason why 63% of antibody-treated mice survived viral infection although 93% of neonates suckled by immune mothers survived infection with HSV. These results indicate that maternally derived IgG, acquired prenatally and postnatally, plays an important role in protecting newborn mice against HSV infection.

Our data do not indicate the precise mechanism of the protection. Neutralization of virus may occur to some degree. Antibody given shortly after infection has been shown to confer partial protection on newborn mice (Luyet et al., 1975; Baron et al., 1976; Georgiades et al., 1982). Neonatal mice were protected against lethal HSV infection by a combination of human leukocytes and antibody (Kohl & Loo, 1982). Using adult mice, we and others have described antibody-mediated protection against HSV infection in which mechanisms other than neutralization may be involved (Oakes & Lausch, 1981; Hayashida et al., 1982). However, it is still not known whether antibody exerts its action on virus-infected cells in cooperation with cellular elements in this model.

In the case of clinical disease, exposure of a neonate to a member of the hospital staff or family with oral herpes lesions occasionally results in disseminated neonatal HSV infection (Schreiner et al., 1979). Our findings suggest that a neonate born to a seronegative mother would be highly susceptible to HSV, and that a safe and effective vaccine is desired for seronegative pregnant women.

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REFERENCES


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