Neutralization of Interferon Produced Early during Rabies Virus Infection in Mice

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SUMMARY

Inoculation of mice (strain C3H/He) with a purified preparation of fixed rabies virus led to the production of interferon with two different peaks of activity detectable in the plasma: an early peak 24 h after inoculation followed by another peak on the 7th day after infection. The level of splenic 2–5A synthetase was enhanced in parallel with the pattern of interferon activity. Neutralization of the first peak of interferon activity by anti-mouse α/β interferon globulin blocked the induction of splenic 2–5A synthetase and modified the development of disease. Infected mice given anti-interferon globulin had a significantly shorter morbidity period than normally infected mice. These results suggest that interferon produced early after virus inoculation plays a role in the onset of rabies disease.

The production of interferon during rabies virus infection has been reported by several investigators (Stewart & Sulkin, 1966; Wiktor et al., 1972; Marcovistz et al., 1984a). Inoculation of mice with a mouse brain homogenate containing rabies virus (challenge virus strain: CVS) results in the production of interferon with two different peaks of activity detectable in the plasma: an early peak 24 h after inoculation followed by another peak during the terminal phase of the infection. The specificity of the first peak of interferon activity has been controversial, since it is also detectable in control mice injected with a brain homogenate from a normal uninfected mouse (Marcovistz et al., 1984a). The second peak of interferon activity, however, may be due to the presence of high levels of interferon produced in the brain in response to rabies virus replication (Marcovistz et al., 1984a, b). Interferon produced during rabies virus infection is stable at pH 2 and is neutralized by antibodies against mouse α/β interferon. This interferon is active in infected mice as judged by the induction of pppA(2'p5'A)n synthetase (2–5A synthetase) and poly(rI).poly(rC)–Sepharose-bound protein kinase in different organs. Here, we show that the first peak of interferon is also detectable in mice injected with a purified preparation of fixed rabies virus from infected baby hamster kidney (BHK-21) cells. Rabies virus is therefore capable of inducing interferon at the site of the virus inoculation, before its migration to the central nervous system. This early peak of interferon production seems to play a role in the development of infection, since mice inoculated with rabies virus and injected with anti-mouse α/β interferon globulin showed a shorter morbidity period than the corresponding control infected mice. Previously, antibodies against mouse α/β interferon have been used to demonstrate the role of interferon in the pathogenesis of several acute virus diseases in mice (Fauconnier, 1970; Gresser et al., 1976a, b, 1979, 1983; Rivière et al., 1977; Guillon & Tsiang, 1980; Saron et al., 1982; Pfau et al., 1983). However, this is the first report which provides evidence to indicate that such antibodies actually neutralize interferon in the circulation. The induction of 2–5A synthetase is inhibited in rabies virus-infected mice treated with anti-mouse α/β interferon globulin.

The CVS fixed strain of rabies virus, cultured on BHK-21 cells and purified by centrifugation in a sucrose gradient (Arita & Atanasiu, 1980), was used in these experiments. Two-month-old
mice were inoculated in the footpad. The infectivity of virus in brain extracts was titrated in mice as described by Kaplan & Koprowski (1973). Antibody production against rabies virus was titrated by the fluorescent focus reduction test (Sureau et al., 1982). Mouse interferon activity was measured by inhibition of the cytopathic effect of vesicular stomatitis virus (VSV) on mouse L-929 cells (Ito & Montagnier, 1977). The effect of interferon produced during rabies virus infection was assessed by measuring the level of 2-5A synthetase in the spleen (Marcovitz et al., 1984a). Anti-mouse α/β interferon globulin was obtained from a sheep immunized with mouse α/β interferon at a specific activity of 2.5 × 10^8 NIH units/mg protein (Galabru et al., 1985).

Inoculation of mice with a purified preparation of fixed rabies virus led to the production of two peaks of interferon activity (Fig. 1a). The early peak was detectable 24 h after virus inoculation whereas the second peak reached its maximum value at the time of paralysis and death of animals. In order to investigate the role of the early production of interferon in the pathogenesis of rabies virus infection, we treated mice with anti-mouse α/β interferon globulin at 1, 10 and 40 h after virus inoculation. In such mice, the circulating interferon activity only became detectable on the 4th day of infection and increased until death (Fig. 1a). Thus, infected mice which were injected with anti-interferon globulin, showed only one peak of interferon activity which started 2 days earlier than the corresponding second peak of interferon in control infected mice. This was probably because the development of rabies disease was accelerated by neutralizing the first peak of interferon (see below).

In accord with the production of interferon, the level of splenic 2-5A synthetase was enhanced in infected mice. Two peaks of enhancement were observed in control infected mice on days 2 and 7 (Fig. 1b). On the other hand, the level of 2-5A synthetase remained constant for the first 3 days and thereafter increased in infected mice injected with the antibodies (Fig. 1b). These results indicate that interferon produced during rabies virus infection is active at least for the induction of 2-5A synthetase. The lack of enhancement of 2-5A synthetase (during the first
Table 1. Effect of neutralization of the first peak of interferon activity on the development of rabies virus infection in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of onset of paralysis†</th>
<th>Day of death†</th>
<th>Antibody activity (IU)‡</th>
<th>Virus infectivity (log₁₀ LD₅₀/ml)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.0 ± 0</td>
<td>7.4 ± 0.5</td>
<td>13</td>
<td>6.0, 6.2, 7.6</td>
</tr>
<tr>
<td>Control IgG</td>
<td>5.2 ± 0.4</td>
<td>7.6 ± 0.5</td>
<td>13</td>
<td>6.8, 7.0, 7.5</td>
</tr>
<tr>
<td>Anti-IFN IgG</td>
<td>4.6 ± 1.1</td>
<td>5.8 ± 1.3</td>
<td>13</td>
<td>5.8, 7.5, 6.3</td>
</tr>
</tbody>
</table>

* Three groups of five male mice were infected with rabies virus as described in the legend to Fig. 1. The control IgG and anti-IFN (interferon) IgG groups received three injections intravenously (1, 10 and 40 h) of sheep control immunoglobulin and sheep anti-mouse α/β interferon globulin, respectively (as in the legend to Fig. 1). Mice were examined daily and separated as soon as the first signs of paralysis became apparent. Blood and brain were collected from mice with general prostration.

† Mean ± standard deviation.
‡ Average activity from three mice.
§ Virus infectivity from three mice.
||, Difference between mice injected with sheep control immunoglobulin and untreated mice; P₂, difference between mice injected with sheep anti-mouse α/β interferon globulin and untreated mice.

3 days) in mice injected with anti-interferon globulin demonstrates the neutralization of interferon. In these experiments, the quantity of anti-interferon globulin given to infected mice was just sufficient to neutralize the first peak of interferon. In an attempt to neutralize the second peak of interferon, infected mice were treated with the antibody on days 3, 4 and 5 after virus inoculation. However, in these latter experiments, only 20% of the circulating interferon activity was neutralized (data not shown). It should be noted that very high levels of interferon are produced during the replication of rabies virus in the brain (Marcovizt et al., 1984a).

The pathogenesis of rabies virus infection in mice inoculated with the CVS strain (1 LD₅₀) is characterized by paralysis of the posterior limbs on day 5. This infection is lethal for all mice by day 7 or 8. Virus becomes detectable in the brain by immunofluorescence on day 4. Interferon of type α/β and antibodies specific against rabies virus are produced during the disease (Fig. 1 and Table 1). Neutralization of the first peak of interferon activity accelerated the development of the disease. Infected mice which received anti-interferon globulin had a morbidity period which was significantly (P < 0.02) less than that for the control infected mice. On the other hand, the activity of antibody against rabies virus in the plasma and infectivity of virus in the brain were similar for both groups (Table 1). These results indicate that interferon produced early during the course of the disease may play a role in the onset of the infection. This effect probably occurs soon after virus inoculation and during the extraneural phase of the disease. The replication of virus started on day 4 after inoculation when the first peak of interferon activity had passed. When infected mice were treated with anti-interferon globulin, then high virus infectivities were found on days 4 and 5. In these mice, therefore, virus replication started at least by day 3. It should also be noted that enhancement of plasma interferon activity during the period of virus replication in the brain was different in the two experimental groups (Fig. 1a). Interferon activity was detectable 2 days earlier in mice treated with anti-interferon globulin.

All these experiments were done with C3H/He mice. Among other strains of mice tested, AKR mice showed a higher sensitivity to rabies virus infection than strains C3H/He, DBA/2 or BALB/c. Interestingly, only AKR mice failed to produce the first peak of interferon activity. The kinetics of the production of interferon activity and the development of the infection were similar in C57BL/6, DBA/2, BALB/c and C3H/He mice.

Rabies virus-infected mice die in the presence of very high levels of interferon activity in the circulation (the second peak) and the brain (Marcovizt et al., 1984a). This interferon seems to be ineffective in inhibiting replication of virus since infected mice do not survive. It might be that interferon is produced in insufficient amounts or is produced too late in the infection to exert an efficient antiviral effect in the brain. Furthermore, it is possible that the protective
action of interferon is inhibited during rabies virus infection. It should be emphasized also that the development of rabies disease depends on many interacting factors such as the host immune response (Guillon & Tsiang, 1980; Smith et al., 1982). Thus, the first peak of interferon activity may control the onset of rabies infection whereas the effect of the second peak of interferon activity might be masked by the immunopathological response of infected mice.

REFERENCES


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