Inhibition by anti-interferon serum of lymphocytic choriomeningitis virus disease in suckling mice*

(liver necrosis/interferon/interferon antibodies)

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ABSTRACT Inoculation of newborn mice with lymphocytic choriomeningitis (LCM) virus resulted in decreased weight gain, liver cell necrosis, and death. Injection of potent sheep immunoglobulin against mouse interferon markedly inhibited these manifestations of LCM virus disease despite the fact that these treated mice had 100-fold more LCM virus in their serum. We conclude that interferon induced by LCM virus is responsible in large part for the syndrome of growth inhibition, liver cell necrosis, and death observed in LCM virus-infected suckling mice.

Daily inoculation of newborn mice with potent interferon preparations results in inhibition of growth, liver cell degeneration, and death in the second week of life (1). Similar clinical and pathologic findings are observed when newborn mice are inoculated with lymphocytic choriomeningitis (LCM) virus (2–6). Two hypotheses may be suggested to explain this similarity of syndromes: (i) interferon was lethal for suckling mice because it activated a latent virus (such as LCM virus), and (ii) some of the manifestations of LCM virus disease in suckling mice are due to interferon induced by the virus. We found no evidence to support hypothesis (i) because neither LCM virus nor mouse hepatitis virus or any other infectious agent was recovered from the liver and kidneys of interferon-treated suckling mice (1). The results of experiments reported herein support hypothesis (ii) in that administration of potent immunoglobulin against mouse interferon inhibited the decreased growth, liver cell necrosis, and death observed in LCM virus-infected suckling mice.

MATERIALS AND METHODS

Mice. Pathogen-free Swiss mice were obtained from the Centre de Sélection des Animaux de Laboratoire d’Orléans (CNRS) (experiment 1) and IFFA-CREDO (experiment 2).

Virus. LCM virus strain GIPV 76001 of the Pasteur Institute was titered by intracerebral inoculation of 3-week-old Swiss mice. The titer of LCM virus was 10⁹ median lethal doses (LD₅₀) per g of brain homogenate.

Sera. We have previously described (7) the immunization procedures used to obtain potent sheep antiserum to mouse interferon as well as the techniques for partial purification of normal sheep globulin and immunoglobulin and for the assay of the different serum globulin fractions for interferon-neutralizing activity. In the experiments described here, the sheep anti-interferon globulin was diluted 1:2 or 1:3 in phosphate-buffered saline to give a titer of 8 × 10⁻⁵ or 5.3 × 10⁻⁵.

The absence of LCM virus antibody in the sheep anti-interferon globulin was demonstrated by indirect immunofluorescence (8) and by neutralization tests in mice. As previously described (7), the serum globulin fractions of three other sheep were used as controls: (i) normal sheep serum.

\[\text{Abbreviations: LCM virus, lymphocytic choriomeningitis virus; LD₅₀, median lethal dose.} \]

* This article is no. 3 of the series "Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum." Article no. 2 is ref. 12.

FIG. 1. Growth of suckling mice infected at birth with LCM virus and injected with anti-mouse-interferon globulin. X, uninfected untreated; A, infected untreated; O, infected and treated with normal sheep globulin; D, infected and treated with serum globulin from sheep no. 4; ⊘, infected and treated with anti-human-interferon globulin; , infected and treated with anti-mouse-interferon globulin.
globulin, (i) the globulin fraction from a sheep (no. 4) partially immunized to mouse interferon (7) (the mouse-interferon-neutralizing titer was 2.5 × 10⁻⁴), and (ii) serum globulin from a sheep (lovar) immunized with human leucocyte interferon. The human-interferon-neutralizing titer was 3 × 10⁻⁵ and its mouse-interferon-neutralizing titer was 1 × 10⁻¹.

Interferon. Mouse interferon was assayed by inhibition of cytopathic effect on mouse L cells infected with vesicular stomatitis virus (9). One of our mouse interferon units equals 4 mouse interferon reference units.

Histology. Tissues were fixed in Bouin’s solution and stained with hematoxylin and eosin.

Experimental Plan. Newborn mice of all litters were randomized at birth and 10 mice (5 male, 5 female) were placed with each mother. Newborn mice were inoculated subcutaneously in the interscapular region with 0.05 ml of anti-interferon globulin or control globulin fractions; 6 hr later they were given 10⁵ LD₅₀ of LCM virus subcutaneously. Three days later, globulin-treated mice were reinjected subcutaneously with 0.05 ml of globulin. The mice were weighed daily. At given intervals, they were sacrificed for histologic examination of organs, and the amount of virus and interferon in the serum was determined.

RESULTS

Presence of Interferon in Serum of Suckling Mice Injected at Birth with LCM Virus. Although for some time a subject of controversy, it seems likely that LCM virus can induce interferon in adult mice (10). Our results to be reported in detail elsewhere (Y. Rivière and M. T. Bandu, unpublished data) show that the serum interferon titers on the third and fourth days of life of suckling mice infected at birth with 10⁶ LD₅₀ of LCM virus averaged 1:200 and 1:320, respectively. [Interferon was not detected in the serum before day 3 or after day 4.] The serum virus inhibitory factor met the accepted criteria for classification as an interferon (11) and was neutralized in vitro tests by the antibody to mouse interferon used in the experiments to be described.

Effects of Anti-Mouse-Interferon Globulin on Evolution of LCM Virus Disease in Suckling Mice. Newborn mice were distributed into the following groups: uninfected and untreated; virus infected and untreated; virus infected and treated with (i) normal sheep serum globulin, (ii) sheep anti-human-interferon globulin, (iii) the globulin fraction of sheep no. 4 incompletely immunized to mouse interferon, or (iv) sheep anti-mouse-interferon globulin.

Growth. No difference in weight gain was observed among the different groups of mice for the first 7–8 days of life. After day 8, the daily weight gain in virus-infected mice treated with the various control serum globulins or left untreated was considerably less than in virus-infected mice inoculated with anti-mouse-interferon globulin (Fig. 1). In experiment 1 (Fig. 1) there was a difference in weight gain between uninfected mice and infected mice treated with anti-mouse-interferon globulin.

Table 1. Mortality in suckling mice infected at birth with LCM virus and injected with anti-interferon (IF) or other globulins

<table>
<thead>
<tr>
<th>Virus</th>
<th>Treatment</th>
<th>Total mice</th>
<th>No. mice dead* (%)</th>
<th>Mean day of death ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>10</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>LCM</td>
<td>Normal sheep globulin</td>
<td>10</td>
<td>6 (60)</td>
<td>11.8 ± 3.3 (9–18)</td>
</tr>
<tr>
<td>LCM</td>
<td>Anti-human IF globulin</td>
<td>10</td>
<td>7 (70)</td>
<td>12.9 ± 4.5 (8–21)</td>
</tr>
<tr>
<td>LCM</td>
<td>Anti-mouse IF globulin</td>
<td>19</td>
<td>15 (79)</td>
<td>10.4 ± 3.7 (8–19)</td>
</tr>
<tr>
<td>LCM</td>
<td>Anti-mouse IF globulin</td>
<td>30</td>
<td>8 (27)</td>
<td>12.6 ± 2.8 (10–17)</td>
</tr>
</tbody>
</table>

* Mice dying in the 2 days after virus inoculation are not included.

Table 2. Frequency of liver lesions in suckling mice infected at birth with LCM virus and injected with various globulins

<table>
<thead>
<tr>
<th>Virus</th>
<th>Treatment</th>
<th>Frequency, * by day after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>0/8* NE NE 0/3 NE NE NE NE</td>
</tr>
<tr>
<td>LCM</td>
<td>None</td>
<td>NE NE NE 5/5 NE NE NE NE</td>
</tr>
<tr>
<td>LCM</td>
<td>Sheep no. 4 globulin</td>
<td>NE NE 4/4 NE NE NE NE</td>
</tr>
<tr>
<td>LCM</td>
<td>Normal sheep globulin</td>
<td>0/8 2/3 3/3 4/4 3/5 2/5 0/3</td>
</tr>
<tr>
<td>LCM</td>
<td>Anti-mouse-interferon globulin</td>
<td>0/8 0/3 0/3 0/5 0/5 0/5 0/3</td>
</tr>
</tbody>
</table>

* Number of mice with liver lesions/number of mice examined. NE = not examined.
globulin whereas in experiment 2 there was no difference between these groups for the first 15 days of life.

Mortality. Considered together, the mortality of LCM virus-infected mice untreated or injected with the various control sheep serum globulin was 28/39 mice (72%) and 28/90 mice (31%), respectively, in experiments 1 and 2 (Table 1). In contrast, the mortality in infected mice inoculated with sheep anti-mouse-interferon globulin in the same experiments was 8/30 mice (27%) and 4/40 mice (10%), respectively.

Histologic examination of organs. Suckling mice in the different groups were sacrificed at intervals and histologic sections of the central nervous system, thymus, spleen, intestine, kidney, and liver were examined. Lesions were observed only in the livers of LCM virus-infected mice. The sequential appearance of these lesions may be summarized as follows. On day 5 after virus infection there were foci of steatosis at the periphery of the liver lobules and below the capsule; on day 8 (when mice began to die) there was necrosis of liver cells, most marked below the capsule but also involving most of the liver parenchyma in some instances; on day 12, numerous fibroblasts were observed in necrotic areas which showed giant cell formation and calcium deposits toward day 16. After day 20, lesions were less frequently observed either because mice with extensive lesions died or because of regression of lesions. After day 36 the livers of surviving mice appeared normal. It should be emphasized that the development and nature of the liver lesions observed in LCM virus-infected suckling mice appeared indistinguishable from those previously reported for interferon-treated suckling mice (1).

As shown in Table 2 and Fig. 2, liver lesions were observed only in LCM virus-infected suckling mice untreated or treated with the various control sheep serum globulins. Liver lesions were not observed in uninfected mice or in LCM virus-infected mice injected with anti-interferon globulin (even in mice in the latter group that died).

Determination of Serum Virus and Interferon Levels in LCM Virus-Infected Mice Inoculated with Normal Sheep Globulin or Sheep Anti-Mouse-Interferon Globulin. In newborn mice inoculated at birth with LCM virus and injected with normal sheep serum globulin (experiment 2 in Fig. 1 and Table 1), the virus titer in two pools of serum was $10^{4.5}$ and $10^{4.7}$ LD$_{50}$/ml on the third day of life, and interferon was present in the serum at this time (Table 3). In contrast, in infected mice treated with anti-mouse-interferon globulin, the serum virus titer was $10^{6.6}$ LD$_{50}$/ml and interferon was not detected in the serum. Subsequently interferon was not detected in the serum of infected mice in either group and the virus titers in both groups were similar.

DISCUSSION

Infection of newborn mice with LCM virus was characterized by viremia and interferonemia on days 3 and 4, decreased weight gain after day 7, extensive liver cell degeneration, and a significant degree of mortality (usually between days 8 and 15). Our results indicate that interferon itself is in large part responsible for this syndrome and may be summarized as follows.
Table 3. Serum virus and interferon levels in LCM virus-infected mice inoculated with normal sheep globulin or anti-mouse-interferon globulin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum titer*</th>
<th>Day after inoculation with LCM virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Normal sheep globulin</td>
<td>Virus†</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>Sheep anti-mouse-interferon globulin</td>
<td>Interferon‡</td>
<td>1:68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:74</td>
</tr>
<tr>
<td></td>
<td>Virus†</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.6</td>
</tr>
</tbody>
</table>

* Pools of serum from three to five suckling mice were assayed for virus and interferon.
† Expressed as log_{10}/ml.
‡ Titer per 0.2 ml of serum.

1. Sheep anti-mouse-interferon globulin markedly decreased the manifestations of disease in infected mice: the mice gained weight normally or showed a slight decrease in weight gain, their livers appeared normal on histologic examination, and their mortality was decreased.

2. Infected mice had significant levels of serum interferon on day 3 whereas interferon was not detected at this time in the serum of infected mice injected with anti-mouse-interferon globulin. It should be emphasized that a 100-fold greater amount of LCM virus was detected in the serum of mice injected with anti-mouse-interferon globulin than in control infected mice.

3. Inhibition of LCM virus disease was observed only in mice injected with anti-mouse-interferon globulin. No effect was observed in infected mice injected with normal sheep serum globulin, with serum globulin from a sheep having a low titer of anti-mouse-interferon neutralizing antibody, or with sheep anti-human-leukocyte-interferon globulin.

4. As previously reported (1), daily administration of potent mouse-interferon preparations to newborn mice also results in decreased weight gain, a pattern of liver cell degeneration indistinguishable from that induced by LCM virus, and significant mortality.

Our results clearly show that interferon is an important factor in the evolution of LCM virus disease. We do not mean to imply, however, that all the signs of disease in suckling mice infected with LCM virus at birth are due to interferon. For example, blepharitis occurred in LCM virus-infected mice (4) treated with anti-mouse-interferon globulin and deaths did occur (although liver lesions were not observed in these mice).

In previous studies, treatment of newborn or adult mice with anti-mouse-interferon globulin markedly enhanced the evolution of diseases induced by several different viruses, attesting to the importance of interferon in the resistance of the host to virus infection (7, 12). Likewise, the therapeutic value of exogenous interferon in virus-infected animals is well established (13, 14). It may therefore seem a paradox to inhibit a viral disease by using an anti-interferon antiserum to block the action of interferon. We and others, however, have repeatedly emphasized the multiple effects of interferon on cells (15–19). It seems likely that under some circumstances some of these effects, such as inhibition of cell division (15, 20, 21), may prove injurious to the host. Thus, the syndrome of decreased growth, liver cell necrosis, and death is only observed in suckling mice treated with interferon or injected with LCM virus; it is not seen in weanling or adult mice. Even in the adult animal it seems likely that some of the common manifestations of virus diseases such as transient immune depression may be due to the interferon (22–33) induced by the virus rather than to a viral cytoytic effect. It may be that other lesions considered heretofore to be due to cell destruction by the virus may be due in part to host substances (such as interferon) induced by the infectious agent. We may speculate that even the embryotoxic effects ascribed to rubella virus may be related to interferon induced either in the mother or in the embryo itself. Some effects of interferon may only become apparent later in life. For example, interferon treatment of suckling mice, even when stopped prior to extensive liver necrosis and death (1), can lead to a progressive lethal glomerulonephritis in adult mice (34).

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From our previous work (7) on the effect of anti-mouse-interferon globulin in viral diseases of mice, it is likely that interferon was in fact produced in these mice but that it was neutralized in the tissues by the anti-interferon globulin.


