Lethal Role of Interferon in Lymphocytic Choriomeningitis Virus-induced Encephalitis

By CHARLES J. PFAU, 1, ION GRESSER 2 AND KATHERINE D. HUNT 1

1 Department of Biology, Rensselaer Polytechnic Institute, Troy, New York 12181, U.S.A. and
2 Laboratory of Viral Oncology, Institut de Recherches Scientifiques sur le Cancer, PB8, 94802 Villejuif, France

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SUMMARY

After intracerebral inoculation of adult C3H mice, the ‘docile’ strain of lymphocytic choriomeningitis (LCM) virus multiplied to high titre in several visceral organs. Although the virus content of lung, liver, spleen and brain was high, these mice did not die but became long-term carriers of the virus. Injection of mice with the same dose of the ‘aggressive’ strain of LCM virus resulted in much lower virus titres in these organs; nevertheless, 100% of the mice died within 7 to 9 days. The results presented here show that mice infected with the ‘aggressive’ virus do not die if treated with anti-interferon globulin. Under these conditions the titres of ‘aggressive’ virus were as high in the different organs as in mice infected with the ‘docile’ virus. These results are consistent with the hypothesis that inhibition of LCM virus multiplication in various organs by interferon results in a lethal disease. The possible mechanisms underlying this seemingly paradoxical phenomenon are discussed.

It is generally held that interferons play an important role in recovery from acute primary virus infections. One way of demonstrating this is by use of antibody to interferon. Neutralization of virus-induced interferon results in rapid and extensive virus multiplication and early onset of disease in a number of murine virus infections (Gresser et al., 1976a, b). However, the studies presented here using anti-interferon globulin show that in lymphocytic choriomeningitis (LCM) virus infection of adult mice, interferon does not protect the mice but is somehow responsible for the lethal outcome of virus infection.

Two strains of LCM virus were used in these studies. Adult mice infected intracerebrally with the ‘aggressive’ strain usually died with a convulsive central nervous system (CNS) disease 7 to 9 days after infection. Most mice infected intracerebrally with the ‘docile’ strain did not die (Jacobson & Pfau, 1980) but developed a life-long (> 9 months; C. J. Pfau, unpublished observations) persistent infection. Paradoxically, the ‘docile’ virus multiplied to much higher titres in the brain and in the visceral organs than the ‘aggressive’ virus (Pfau et al., 1982b). Interferon seemed to play a pivotal role in influencing both virus multiplication and the evolution of the disease. Thus, mice injected with the ‘docile’ virus developed CNS disease when injected at appropriate times with inducers of endogenous interferon (Jacobson et al., 1981), while mice infected with an ‘aggressive’ strain were spared by injection with anti-interferon globulin (Saron et al., 1982a). In the latter experiments the titres of virus in the blood were increased 100- to 1000-fold. The following experiment was designed to determine the role of endogenous interferon in the evolution of disease induced by the ‘aggressive’ strain, and specifically to examine whether administration of anti-interferon globulin to mice injected with the ‘aggressive’ strain would result in a pattern of disease observed in mice injected with the ‘docile’ strain.

C3H mice were injected intracerebrally with 300 p.f.u. of either the ‘aggressive’ or ‘docile’ LCM virus strains (Pfau et al., 1982a). Thirty-four mice were infected with the ‘aggressive’ virus and divided into three groups. The first group (10 mice) served as the untreated controls, while
After blood was taken from the ophthalmic venous plexus the lungs (a), liver (b), spleen (c), brain (d) and kidneys (e) were aseptically removed daily from one infected mouse in each of the following groups: aggressive virus + anti-interferon globulin (○); aggressive virus + normal sheep globulin (□); aggressive virus alone (○); docile virus alone (■). Pooled sera (f) were treated as described in the text; the organs were washed, weighed, ground with sterile sand, and resuspended in tissue culture media containing 10% foetal bovine serum. Samples were centrifuged at low-speed and the supernatants stored at −70°C until titrated by plaque assay (Jacobson et al., 1979).

The second and third groups (12 mice each) received either anti-interferon globulin or normal sheep globulin. A fourth group (10 mice) served as the 'docile' virus controls. For the first 5 days after infection, blood, brain and visceral organs were removed daily from one mouse in each of the four groups. On the third day after infection and 2.5 h after injection of either anti-interferon globulin or normal sheep globulin, two mice were exsanguinated from each of these two groups. The sera within each group were pooled, dialysed to pH 2, redialysed to pH 7 and assayed for interferon (Jacobson et al., 1981). Interferon was not detected (<16 International Units, IU) in mice infected with 'aggressive' virus and treated with sheep anti-interferon globulin. Fig. 1 shows that the titres of 'aggressive' virus in antibody-treated mice were comparable to those in untreated mice infected with 'docile' virus. Under these circumstances the ordinarily lethal 'aggressive' virus infection was benign, again comparable to the outcome of infection of mice with the 'docile' virus (Table 1). On the other hand, the serum interferon titre was high (1024 IU/ml) in mice infected with 'aggressive' virus and treated with normal sheep globulin. These mice died and the virus titres in most visceral organs were low (Table 1, Fig. 1).

When the experiment was terminated at 42 days post-infection, all mice had virus in their blood. The five mice infected with 'aggressive' virus and treated with antibody (Table 1) had titres of virus ranging from 0.6 × 10³ to 2.8 × 10³ p.f.u./ml. The four mice infected with 'docile' virus (Table 1) had titres ranging from 2.3 × 10⁵ to 9.4 × 10⁵ p.f.u./ml. As there is no great difference in the growth rates of these two virus strains in tissue culture (Jacobson, 1980) the explanation for the 300-fold difference in titres in vivo may be that the aggressive virus induces a continuous production of interferon (Saron et al., 1982b).

We had previously observed that intracerebral inoculation of very high concentrations of 'aggressive' virus mimicked the behaviour of 'docile' virus, and did not induce fatal CNS disease. On the other hand, intracerebral inoculation of very low concentrations of 'docile' virus
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Table 1. Effect of anti-interferon globulin on the evolution of disease in mice infected with the 'aggressive' (A) and 'docile' (D) strains of LCM virus*

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Treatment</th>
<th>No. of dead mice/ Mean no. of days till death (range):</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Untreated control</td>
<td>5/5 8.4 (8-9)</td>
</tr>
<tr>
<td>A</td>
<td>Normal globulin</td>
<td>5/5 8.7 (8-9)</td>
</tr>
<tr>
<td>A</td>
<td>Anti-interferon globulin</td>
<td>0/5 -</td>
</tr>
<tr>
<td>D</td>
<td>Untreated control</td>
<td>1/5 19.0</td>
</tr>
</tbody>
</table>

* Three-week-old female C3HeB/FcJ mice (Jackson Laboratories, Bar Harbor, Me., U.S.A.) were injected with virus and observed daily. Beginning on the fifth day after infection the mice were spun by the tail to hasten convulsive death. Mice received interferon antibody 0.5 h before, as well as 72 h after infection with aggressive virus. Each intraperitoneal injection consisted of 0.1 ml of a 1:3 dilution of globulin taken from sheep immunized with mouse interferon (Gresser et al., 1976a). This globulin had been extensively absorbed on mouse C-243 cells as well as on a pool of mouse thymocytes and splenocytes. Its neutralizing titre was 1:400000 against 8 IU of C-243 cell mouse interferon. The same treatment schedule was followed for the group receiving normal (absorbed) sheep globulin.

† Number of dead mice does not include deliberately sacrificed animals.
‡ The experiment was terminated 42 days after infection.

resulted in low virus titres and was lethal (Pfau et al., 1982b). Thus, infection with the 'docile' virus can under some circumstances result in death. There appears to be, therefore, an inverse relationship between the rapidity and the extent of virus multiplication in the brain and visceral organs and death. As death in LCM virus disease is attributed to the development of virus-specific thymus-derived cytotoxic lymphocytes (Cole et al., 1972), we may formulate the following question. What is the relationship between high virus titres (induced by 'docile' virus or 'aggressive' virus in mice treated with anti-interferon globulin) and the failure of virus-specific T lymphocytes to induce a lethal disease? We know that the 'docile' virus induces a virus-specific T lymphocyte response as well as the 'aggressive' virus (Pfau et al., 1982a). Moreover, when mice were given fully functional virus-specific T cells by adoptive transfer shortly after infection with 'docile' virus at a time when their own T cell response to the infection had not reached detectable levels, they died with convulsions 5 to 6 days later (Pfau et al., 1982b). We propose that high titres of virus in multiple target organs may cause dispersion of the pool of cytotoxic T lymphocytes, thus preventing a focused (lethal) T lymphocyte concentration in the brain.

Whatever the mechanism, our results show that when endogenous interferon induced by 'aggressive' virus is neutralized by anti-interferon globulin, the virus multiplies to high titres as in mice infected with the 'docile' virus, and the mice now survive infection. Interferon thus appears to be associated in some manner with lethality in LCM virus-infected adult mice. It has previously been shown that endogenous interferon induced by LCM virus was also directly responsible for a lethal syndrome in suckling mice (Riviere et al., 1977) and the development of glomerulonephritis in adult mice surviving neonatal infection (Gresser et al., 1978). The relevance of all these studies on LCM virus infection in mice to human disease has yet to be determined, but it is possible that under some circumstances in man interferon may exacerbate rather than alleviate disease.

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