The Role of Antibody and Host Cells in the Resistance of Mice Against Infection by Coxsackie B-3 Virus

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SUMMARY

Peritoneal exudate cells transferred from adult to suckling BALB/c mice before infection of the recipients with Coxsackie B-3 virus protected them against the lethal effects of the virus. Undiluted antibody alone also prevented lethal virus infections in suckling mice but diluted antibody conferred protection only when transferred together with syngeneic adult peritoneal exudate cells. Impairment of macrophage function by intravenous injection of silica increased the susceptibility to virus infection of adult mice. Peritoneal exudate cells inactivated the virus in vitro, and this property may be related to their protective effect in vivo. It is suggested that antibody and host cells collaborate to provide effective resistance against the spread of Coxsackie virus.

INTRODUCTION

Several parenteral virus infections are lethal in newborn mice but not in mice a few weeks old. Evidence suggesting that maturation of peritoneal exudate cells, and of comparable cells elsewhere in the body, is a contributory factor in the development of age-dependent resistance to herpes simplex virus and yellow fever virus has already been published (Hirsch, Zisman & Allison, 1970; Stevens & Cook, 1971; Zisman, Wheelock & Allison, 1971).

Coxsackie B-3 (CoxB-3) virus displays a prominent age-dependent lethality after parenteral or intracerebral inoculation. As mice age the susceptibility of the brain steadily decreases and obvious signs of CoxB-3 infection diminish. In 15-day-old mice the inoculation produces neither signs nor histological lesions affecting the CNS. In contrast, when mice are inoculated at the age of 12 days with CoxB-3 virus severe myocarditis is observed. However, as mice reach adulthood less virus multiplication in the heart and fewer lesions are observed (Grodums & Dempster, 1959). Investigations reported in the accompanying paper (Rager-Zisman & Allison, 1973) suggest that antibody plays a major role in the resistance of adult mice. The experiments now reported were designed to analyse the parts played by antibody and host cells in protection of susceptible target organs against infection with CoxB-3 virus.

METHODS

Mice. Inbred CBA and BALB/c mice from the breeding colony of the Clinical Research Centre were used.

Virus. Coxsackie B-3 virus (Nancy strain, CoxB-3) was used as described in the preceding paper.

Silica. Quartz dust (average particle size 5 μm), obtained from Dowson and Dobson Ltd.,
was sterilized in hot air and suspended in isotonic saline solution at a concentration of 100 mg/ml. Immediately before use silica particles were dispersed by brief exposure to ultrasonic vibration.

**Peritoneal exudate cells.** Unstimulated peritoneal exudate cells were obtained from adult mice by washing abdominal cavities with tissue culture medium. Stimulated adult peritoneal exudate cells were prepared by inoculating mice intraperitoneally (i.p.) with 2 ml of 10% proteose-peptone solution (Difco Laboratories, 60 to 80 ml/kg) 72 h before removing cells as described above.

**Peritoneal exudate cell cultures.** Unstimulated and proteose-peptone (p.p.) stimulated peritoneal exudate cells were pooled and distributed into tissue culture tubes at a concentration of $2 \times 10^6$/ml. The culture medium was L-15 with foetal calf serum (FCS) and cells were incubated at 37 °C in a stationary position. Twenty-four h later, when cells were well spread, cultures were infected with virus in fresh medium.

Other details varied according to requirements of individual experiments and are described in the text.

**Other procedures.** Methods of virus titration, interferon assay, preparation of antisera and histology are described by Rager-Zisman et al. (1973).

**RESULTS**

**Development of age-dependent resistance**

BALB/c mice of different ages were inoculated intraperitoneally (i.p.) or intracerebrally (i.c.) with $10^6$ p.f.u. of virus. All mice were susceptible to the virus on the first day after birth. Resistance to i.c. inoculation of the virus developed within the first week of life. A more gradual decrease in susceptibility occurs following i.p. inoculation with no mortality at 28 days (Fig. 1).

Fig. 2 illustrates the effects of different virus doses on the development of resistance against this virus infection. As mice age, more virus is required to produce a fatal infection. At the age of 28 days, mice were completely resistant to the highest virus inoculum given in these experiments.
Resistance against CoxB-3 virus infection

Fig. 2. Effects of different virus doses on the development of resistance against i.p. inoculation of CoxB-3 virus. Groups of BALB/c mice at different ages were inoculated i.p. with 0.1 ml containing varying doses of virus. Each group consisted of 8 to 10 mice. [ ], $10^3$; [ ], $10^4$; [ ], $10^5$

Fig. 3. Effects of pretreatment with silica on mortality of adult mice following i.p. infection with $10^5$ p.f.u. CoxB-3 virus. [••••], CoxB-3 virus (control); [•••], silica 2 h before CoxB-3 virus; — — —, silica only. Each group consisted of 20 CBA mice at 3 weeks old.

Effects of silica on CoxB-3 infection in adult mice

CBA mice at 3 weeks old were inoculated i.p. with 50 mg of silica followed 2 h later by $10^5$ p.f.u. of CoxB-3 virus, using the same route. A second group of mice received only an i.p. inoculation of virus, and a third was inoculated i.p. with 50 mg of silica only. The results are presented in Fig. 3. In the group pretreated with silica, mice began to die on the fifth day after infection. By the sixth day the mortality rate was 50% with a total mortality of 70% by day 15. The mortality of mice infected with virus only was 25%. The difference in mortality between the silica-pretreated and virus-only groups was significant at the 5% level. No deaths occurred in mice receiving silica only. All animals surviving for 22 days lived for the 6 months of observation.
**Transfer of peritoneal exudate cells**

BALB/c mice, 48 h old, received an i.p. inoculation of $10^7$ proteose-peptone-stimulated syngeneic adult peritoneal exudate cells in 0.1 ml medium. Five h later one group of mice was inoculated i.p. with $10^3$ p.f.u. of CoxB-3 virus and another group of mice received the same virus inoculum subcutaneously (s.c.). Control groups were inoculated i.p. or s.c. with virus only. The transfer of stimulated syngeneic peritoneal exudate cells before i.p. inoculation of virus conferred 89% protection on susceptible suckling mice, whereas only 0 to 11% of the mice not receiving cells survived following i.p. or s.c. virus inoculation. Transfer of stimulated peritoneal exudate cells did not protect mice against s.c. challenge although mortality was somewhat delayed (Fig. 4).

On histopathological examination, meningitis, encephalitis, skeletal muscle necrosis and brown fat necrosis were observed in the non-protected mice on day 4 following virus inoculation. These pathological changes became progressively more severe, and these animals subsequently died. In the protected suckling mice, only slight involvement of the same organs could be detected on the same day, which later disappeared.

**In vitro inactivation of CoxB-3 virus in the presence of adult mouse peritoneal exudate cells**

Cultures of p.p.-stimulated adult-mouse peritoneal exudate cells were infected with $10^5$ p.f.u. of CoxB-3 virus. After adsorption for 1 h at 37 °C the cells were washed three times with warm saline to remove residual unadsorbed virus and 1 ml of growth medium (L-15 + 3% FCS) was added to each tube. At intervals thereafter, samples were frozen and thawed three times and assayed for total virus infectivity in Vero cell monolayers. As illustrated in Fig. 5, infectivity decreased rapidly in cultures of stimulated cells; by 3 days infective virus could not be recovered from these cultures. The rate of thermal inactivation of CoxB-3 virus was measured during incubation at 37 °C in growth medium or in growth medium with frozen and thawed cells. In these cases the rate of inactivation was slower than in peritoneal exudate cell cultures. No interferon was detected in the supernatant fluids of the infected cells.
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Fig. 5. Infection of mouse peritoneal exudate cell (p.e.c.) cultures with 10⁶ p.f.u. CoxB-3 virus. Each point represents an average virus infectivity for two tubes. ■—■, thermal inactivation of CoxB-3 virus at 37 °C in growth medium; • - - - •, CoxB-3 virus in cultures of stimulated p.e.c.

Infective centre assays

The ability of single adult mouse peritoneal exudate cells to take up and inactivate CoxB-3 virus was measured by a modified infective centre assay. Suspensions of stimulated and unstimulated mouse peritoneal exudate cells were infected with CoxB-3 virus at an input multiplicity of 10 p.f.u./cell. Adsorption was allowed to occur for 1 h. To remove non-adsorbed or extracellular virus, infected cell suspensions were washed once with saline, treated with antibody, followed by a further wash in saline. Later, 100 virus-infected cells were plated on monolayer of indicator Vero cells. Plaques of virus were counted after incubation for 3 days at 37 °C. Suspensions of Vero cells infected with the same inoculum of CoxB-3 virus were used as a positive control and to determine the efficiency of plating under these experimental conditions. Virus titres measured in the supernatant fluids of the first wash (saline) and third (saline after antibody treatment) were used to assess the amount of virus that had been taken up by the peritoneal exudate cells. The input multiplicity of virus was 1 × 10⁷ p.f.u./ml. Only 1 × 10⁶ p.f.u./ml of virus was recovered from the first wash and no virus could be recovered in the third wash. Thus, most of the virus was taken up by the cells. The numbers of infective centres obtained after incubation of 100 virus-infected cells were 23 in the unstimulated exudate cells and 8.5 for stimulated cells. Both counts were much less than the 60 obtained after infection of Vero cells. Differences were statistically significant (P < 0.001).

Antibody transfers

The importance of serum antibodies in the resistance of young mice against CoxB-3 virus was ascertained by examining the ability of transferred antibody to protect CoxB-3-infected suckling mice against the development of a fatal infection. Groups of 6 to 12 BALB/c mice 48 h old were inoculated i.p. with 1000 p.f.u. of CoxB-3 virus. Three h later 0.1 ml of various dilutions of anti-virus serum were inoculated i.p. into the infected animals. Results
summarized in Fig. 6 demonstrate that only relatively high doses of transferred antiserum passively protected suckling mice against infection with CoxB-3 virus. These concentrations of antibody in recipients were, however, about the same as found in intact infected mice.

Transfers of peritoneal exudate cells and antibody

The possible co-operation between cells in peritoneal exudates and serum antibodies in the protection of virus-infected suckling mice was examined in a series of experiments. BALB/c mice 48 h old were inoculated i.p. with 1000 p.f.u. of CoxB-3 virus; 3 h later they received i.p. inoculations of syngeneic adult peritoneal exudate cells and diluted antiviral serum. (The titre of antiserum was 1/640 against 100 p.f.u. of virus.) The experiments summarized in Table 1 show that the ability of serum antibodies to protect virus-infected suckling mice is increased when it is transferred together with adult peritoneal exudate cells. Only one-hundredth of the concentration of antibody required to protect mice when administered alone, was required for complete protection when inoculated into suckling mice together with stimulated adult peritoneal exudate cells. Peritoneal exudate cells alone conferred some protection, but this was incomplete in these experiments, in contrast to the response of mice (Fig. 4) which received cells before virus. Fig. 7 shows the mortality of suckling mice infected with CoxB-3 virus, in some cases followed by inoculation of stimulated adult peritoneal exudate cells with or without antibody.

Although all animals receiving peritoneal exudate cells died after infection with CoxB-3
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Table 1. Effects of passive protection by diluted antiserum with and without syngeneic normal adult peritoneal exudate cells (p.e.c.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Unstimulated p.e.c.</td>
<td>0</td>
</tr>
<tr>
<td>Stimulated p.e.c.</td>
<td>0</td>
</tr>
<tr>
<td>Antibody</td>
<td>0</td>
</tr>
<tr>
<td>Unstimulated p.e.c.+antibody</td>
<td>46</td>
</tr>
<tr>
<td>Stimulated p.e.c.+antibody</td>
<td>100</td>
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Groups of 6 to 12 BALB/c mice at 48 h old were inoculated with 1000 p.f.u. of CoxB-3 virus and 3 h later given antiserum (10⁻³ dilution in 0.1 ml), p.e.c. (8 x 10⁶ in 0.1 ml culture medium), or both.

Fig. 7. Protection of BALB/c syngeneic suckling mice infected i.p. with 1000 p.f.u. CoxB-3 virus following transfers of peritoneal exudate cells (p.e.c.) and antibody. ○—○, CoxB-3 virus only (control); ●—●, virus followed by 8 x 10⁶ stimulated p.e.c.; ■—■, virus followed by 8 x 10⁶ proteose-peptone stimulated macrophages and 0.4 ml of anti CoxB-3 serum. Groups consisted of 6 to 10 suckling mice at 48 to 72 h old.

virus, their death was delayed markedly in comparison with animals receiving virus only. The infection of suckling mice with CoxB-3 virus was fatal. Death was preceded by paralysis, especially of the hind quarters, and involvement of the brain. Macroscopically, white patchy areas appeared in the brain at about the same time as limb paralysis appeared; these changes were followed by haemorrhages and necrosis. At this time, high infectivities of virus were recovered from the brain (~ 10⁻⁷ p.f.u./brain).

Histological examination of the brain revealed severe meningitis and encephalitis with mononuclear infiltration, consisting predominantly of macrophages. Severe pathological changes were observed also in the muscles of the hind quarters. Similar histological changes, occurring later, were seen in infected mice which had received peritoneal exudate cells. Virus-infected mice which were protected following inoculation
of stimulated peritoneal exudate cells and antibody showed only transient mild histological changes in brain, muscle and heart.

DISCUSSION

The development of age-dependent resistance of mice to infection by CoxB-3 virus follows a similar course to that reported for many other host-virus systems (Johnson, 1964; Hirsch et al. 1970; Zisman et al. 1971). Although no single mechanism can account for this resistance, evidence is accumulating that two factors are of major importance. One is circulating antibody, and experiments described in this paper show that relatively high levels of anti-virus antibody can passively protect suckling mice from lethal CoxB-3 infections. However, it is improbable that young mice can produce sufficient antibody soon enough to prevent the virus from spreading to and multiplying in highly susceptible target organs such as skeletal muscle, pancreas and brain.

We therefore examined the role of host cells that may take up and inactivate virus and thereby prevent its spread from primary sites of multiplication to the blood and then to target organs. Of special interest in this regard are macrophages, which provide a barrier between infected tissue spaces such as the peritoneal cavity, subcutaneous tissues or lung and the blood. Several observations suggest that macrophages play an important role in limiting the spread of systemic virus infections. Injections of silica particles, which are selectively taken up by macrophages and impair their function (Allison, Harington & Birbeck, 1966) increase the mortality of adult mice infected with herpes simplex virus (Zisman, Hirsch & Allison, 1970) and yellow fever virus (Zisman et al. 1971). In the present study this has also been observed in CoxB-3 virus infections. The ability of yellow fever virus and other group B togaviruses, as well as murine hepatitis virus, to provide lethal infections in genetically susceptible mice is paralleled by the capacity of cultured peritoneal macrophages from the mice to support multiplication of these viruses (Bang & Warwick, 1960; Goodman & Koprowski, 1962). In all these cases, parenteral inoculations of virus into suckling mice produce lethal infections, even in strains of mice which are resistant as adults. This suggests that maturation of macrophage function during the first few weeks of postnatal life, with an accompanying increase in their capacity to inactivate viruses, makes a substantial contribution to age-dependent resistance.

Experiments reported previously demonstrated that peritoneal exudate cells from adult mice have a greater capacity to inactivate herpes simplex virus than have the cells recovered from suckling mice (Hirsch et al. 1970). In the present study peritoneal exudate cells from adult mice, especially after stimulation with proteose-peptone, were found again to inactivate CoxB-3 virus, as shown by both infectivity and infective centre assays. This virus can therefore be included among the many that fail to undergo a complete cycle of replication in adult mouse macrophages.

The ability of cells in peritoneal exudates of adult mice to limit virus spread in vivo was confirmed by experiments in which these cells were transferred to susceptible suckling mice before the latter were infected with herpes simplex virus (Hirsch et al. 1970) or with CoxB-3 virus, as now shown. However, this protection was precarious, and if the cell transfers were delayed until after virus inoculation, protection was incomplete. However, the simultaneous administration of very small amounts of antiviral serum, which by itself had no protective effect, in the presence of adult peritoneal exudate cells conferred high resistance against the virus. This suggests that small amounts of antibody and host cells co-operate in virus inactivation.
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These results should be considered in relation to other recent reports of the mechanism of virus neutralization. Under several conditions antisera, particularly those obtained early in the course of virus infections, neutralize virus poorly. However, they sensitize the virus to neutralization by antiglobulin (Hampar et al. 1968) or by addition of complement components (Daniels et al. 1970). Neutralization appears to follow the piling up of components on the surface of the virus particle. Similarly in vivo, early antibody may be unable to neutralize virus, thereby preventing its multiplication in susceptible cells, such as the myocardium in the case of CoxB-3. However, macrophages have receptors for IgM, IgG and complement (Huber & Fudenberg, 1968) and virus combined with antibody in the form of potentially infective complexes may be taken up by macrophages and inactivated before it has a chance to infect highly susceptible target cells. In this way there is collaboration between antibody and non-immunocompetent host cells such as macrophages, and possibly also the A cells recently identified in peritoneal exudates (Allison, 1972). The host cells participating in this collaboration are immature in newborn mice but achieve functional maturity during the first few weeks of life.

Intraperitoneal inoculation of CoxB-3 virus in suckling mice produces a lethal infection preceded by paralysis of the hind limbs. Histological examination revealed severe pathological changes in the brain and skeletal muscles. High infectivities of virus (1 × 10^7/ml) were recovered from the brains of infected mice. It therefore seems that the brain is an important target organ for CoxB-3 virus infection of suckling mice. In contrast, little involvement of the brain was observed in immunosuppressed adult mice (Rager-Zisman & Allison, 1973), suggesting that some maturation process other than acquired immunity may make the adult brain relatively insusceptible to Coxsackievirus infection. In contrast, the results for immunosuppressed animals show that the myocardium and pancreas of the adult mouse remain highly susceptible to infection.

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REFERENCES


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