IMMUNE RESPONSE OF THE LONG-TAILED FIELD MOUSE (APODEMUS SYLVATICUS) TO TICK-BORNE ENCEPHALITIS VIRUS INFECTION

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Abstract. The immune response following infection with a virulent strain of Central European encephalitis (CEE) virus in a natural host, long-tailed field mouse (Apodemus sylvaticus L.) and white laboratory-bred ICR mouse, was compared. Viraemia was demonstrated in ICR mice after intra-peritoneal infection with a dose of 10^4 LD_{50}/0.5 ml. The virus titres were high in the spleen and, particularly, in the brain. In A. sylvaticus the virus was detected in the blood and spleen, but not in the brain. CEE virus multiplied in peritoneal macrophages from ICR mice, but not from A. sylvaticus. The infection induced a strong interferon response in both hosts. The natural killer (NK) cell activity increased twice as high in A. sylvaticus compared to ICR mice. The neutralization antibodies appeared sooner in A. sylvaticus and reached higher titres in the early phases of infection.

The immune response of laboratory animals to experimental infection with CEE virus has been studied by many researchers. They have described interferon induction (Hofmann et al. 1971), the role of macrophages (Khozinsky et al. 1985), activation of natural killers (Vargin and Semenov 1986), production of neutralizing antibodies (Radda et al. 1968, Hofmann et al. 1978), as well as individual mechanisms of specific cellular immunity including cytotoxic T lymphocyte activity (Gajdošová et al. 1980, Khozinsky and Semenov 1980). The role of these mechanisms in the immune response or immunopathology following a CEE virus infection has been studied extensively in inbred lines of susceptible laboratory mice. Less information has been obtained about the immunity of natural hosts, such as voles or field mice. Papers concerning natural hosts mostly deal with the development of viraemia and specific antibodies (Mormsteinová and Albrecht 1957, Heigl and von Zeipel 1966, Kožuch et al. 1981).

The aim of the present study was to describe the immune response of a natural host, the long-tailed field mouse (Apodemus sylvaticus L.), to CEE virus infection and to compare it with the response of a susceptible laboratory-bred mouse. This comparison enabled us to compare the activity of individual immune mechanisms in two hosts markedly differing from one another in their susceptibility to infection.

MATERIALS AND METHODS

Virus. The 62 strain of CEE virus (western subtype) was isolated from Ixodes ricinus (L.) and passaged three times through the brain of suckling mice. The virus was fully virulent for adult mice after extraneural infection.

Cell cultures. The porcine kidney cell line PS was used. Cells were cultured in L-15 medium containing 3 % newborn calf serum.

Animals. Outbred ICR mice (Velaz Praha) weighing 13-20 g and male and female A. sylvaticus weighing 10-20 g, caught in CEE-free localities, were used. The animals were infected intraperitoneally with a dose of 10^4 mouse i. p. LD_{50} in 0.5 ml of L-15 medium. Examination of organs and evaluation of
immune responses were carried out in three animals at defined intervals after infection and mean values were calculated.

**Virus titration.** The virus titres in 10% suspensions prepared from spleen and brain, in blood serum, and in the medium from macrophage cultures were determined using a plaque assay in PS cells with a methylcellulose overlay (Madrid and Porterfield 1969).

**Interferon titration.** The serum interferon titres were determined by the method of inhibition of the cytopathic effect of encephalomyocarditis virus in L 929 cells (Dahl and Degrie 1972).

**NK test.** The activity of NK cells was evaluated in a suspension of splenocytes using a test based on the release of $^{51}$Cr from target YAC-1 cells (Welsh 1978). Briefly, $10^6$ YAC-1 cells were incubated with $50 \mu$Ci of $\text{Na}_2^{51}\text{CrO}_4$ (Academy of Sciences, Dresden, FRG) in M 199 medium at 37°C for 1 h. The labelled target cells in the M 199 medium supplemented with foetal bovine serum were pipetted into microtiter plates (Dynatech, V bottom), $10^4$ cells/well. Into each well, $10^6$ splenocytes were added and plates were centrifuged at 200 g for 5 min and incubated at 37°C and 5% CO$_2$ for 5 h. The plate was then centrifuged and the medium harvested and measured in a Gammaautomat Tesla counter. The percentage of specific release of $^{51}$Cr was calculated using the following formula:

$$\% \text{ of specific release of } ^{51}\text{Cr} = \frac{\text{test cpm} - \text{spont. cpm}}{\text{total cpm} - \text{spont. cpm}} \times 100,$$

where spontaneous cpm = release of $^{51}$Cr from target cells into the culture medium, and total cpm = release of $^{51}$Cr after lysis of target cells by Triton X-100 solution.

**Anti-CEE antibody titration.** Specific antibodies to CEE virus were titrated using the plaque-reduction neutralization test in PS cells. Diluted sera were incubated with 100 plaque-forming units (PFU) of CEE virus at 37°C for 1 h. The highest dilution which reduced the number of plaques by 50% represented serum titre.

**Virus interaction with peritoneal macrophages in vitro.** Non-stimulated macrophages were obtained by washing the peritoneum with M 199 medium, and then cultured in Leighton tubes with cover slips in M 199 medium with 10% foetal bovine serum ($3 \times 10^5$ cells/tube). After incubation at 37°C for 24 h, the adhered cells were infected with CEE virus at multiplicity of infection (MOI) = 0.5 PFU/cell. The virus titre in the culture medium was determined at 0, 24, 48 and 72 h post infection, and the infected macrophages were detected by indirect immunofluorescence. Three cultures were evaluated at each interval and mean values were calculated.

**Indirect immunofluorescence.** The cells adhering to the cover slips were fixed with acetone and stained with hyperimmune mouse anti-CEE serum and swine anti-mouse conjugate (SwaM/FITC, USOL Praha) and Evans' blue (0.02%).

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**Fig. 1.** CEE virus titres in organs of ICR mice. Virus titre in serum (▲), spleen (■), and brain (●).
RESULTS

The titres of CEE virus in the spleen, brain and blood of ICR mice and *A. sylvaticus* were compared (Figs. 1 and 2). The level of viraemia reached approximately the same values in both species in the first two days after infection. The decrease in virus titre in the blood of ICR mice occurred sooner than in *A. sylvaticus*. Virus titres in one spleen were approximately 1 log higher in ICR mice compared with *A. sylvaticus*. The main difference between the two species was in virus replication in the brain. Whereas the virus titres were high in the brain of white mice, no virus could be detected in the brains of *A. sylvaticus*.

In vitro studies of CEE virus interaction with peritoneal macrophages revealed that the virus multiplied in a low percentage of macrophages from ICR mice (Table 1) and was released into the culture medium, whereas the virus titres in *A. sylvaticus* macrophages decreased according to the thermoinactivation curve of the virus in the culture medium (Fig. 3).

![Graph](image)

**Fig. 2.** CEE virus titres in organs of *A. sylvaticus*. Virus titre in serum (▲), spleen (■), and brain (●).

**Table 1.** Mean number of cells positive in the immunofluorescence test

<table>
<thead>
<tr>
<th>Days p.i.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
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<tbody>
<tr>
<td>Macrophages from ICR mice</td>
<td>—</td>
<td>6</td>
<td>22</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Macrophages from <em>A. sylvaticus</em> field mice</td>
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The serum interferon titres reached the maximum value on day 1 p.i. in ICR mice and on day 2 in *A. sylvaticus* (Fig. 4). Interferon was not demonstrated in the serum of *A. sylvaticus* at later intervals, whereas in the serum of ICR mice, the titre increased to 80 units on day 7 p.i.

**Fig. 3.** CEE virus interaction with peritoneal macrophages. Virus titre in macrophage culture from ICR mice (▲), *A. sylvaticus* (■), and virus thermostability in culture medium (○).

**Fig. 4.** Serum interferon titres after infection of ICR mice (▲) and *A. sylvaticus* (○) with CEE virus.
Changes in the NK activity after CEE virus infection were found to be markedly different in the two species (Fig. 5). Maximum activity was found on day 3 p.i. in ICR mice and on day 5 p.i. in *A. sylvaticus*. The values obtained in *A. sylvaticus* field mice

Fig. 5. Changes in NK activity of splenocytes from ICR mice (○) and *A. sylvaticus* (○) after CEE virus infection. + Endogenous NK activity of non-infected *A. sylvaticus*, ++ endogenous NK activity of non-infected ICR mice.

Fig. 6. Production of neutralizing antibodies in ICR mice (○) and *A. sylvaticus* (■) infected with CEE virus.
were twice as high as those in ICR mice. A similar difference was found in the values of non-induced NK activity.

Neutralizing antibodies were detected in the serum of *A. sylvaticus* sooner and reached higher titres than in ICR mice, in the early phases of infection (Fig. 6).

**DISCUSSION**

The results obtained show that, although ICR mice and *A. sylvaticus* did not differ markedly in the CEE virus titre in blood and spleen, only the ICR mice contained the virus in their brains at the time interval under study. Virus titre in serum seems to be a true reflection of the viraemic titre, since the majority of the virus was found in the plasma (Mášková 1967). Replication of CEE virus in the brain is undoubtedly associated with the death of white mice of encephalitis at later stages of infection. CEE virus infection of field mice is asymptomatic and the virus in brain is not detected outside the viraemic phase (Erněk et al. 1963). The fact that CEE virus crossed the blood-brain barrier in ICR mice but not in *A. sylvaticus* needs a further investigation. Solution of this problem could be achieved through proving the replication of the virus in vascular endothelial cells. The differences found in the pathogenesis of CEE virus infection cannot be explained by differences in the ages of animals used, since both experimental groups consisted of adult and subadult animals. Similar results were obtained by Mášková et al. (1965), who compared the course of CEE virus infection in the bank vole (*Clethrionomys glareolus* Schr.) and in the white mouse.

The results of our studies of CEE virus interaction with peritoneal macrophages confirmed the conclusions of Mims (1964) that the resistance of macrophages to virus infection reflects the resistance of the whole organism. The macrophages from ICR mice supported virus replication to some extent, and can be presumed to contribute to virus spread in the animal. In contrast the macrophages from *A. sylvaticus* did not support virus replication and showed no evidence of phagocytosis and subsequent inactivation of the virus. According to Olson et al. (1975), the ability of a virus to survive in macrophages may relate to virulence. The defensive role of macrophages *in vivo* is ascribed to their role in antibody-dependent cellular cytotoxicity (Khozinsky et al. 1985). The role of cells of the mononuclear phagocytic system as a source of replicating virus has been described for dengue virus (Halstead et al. 1973). The phenomenon of enhancement of macrophage infection by an antiviral antibody has been demonstrated for several arboviruses including CEE virus (Phillpotts et al. 1985).

Interferon production was comparable in both ICR mice and *A. sylvaticus*. The interferon of *A. sylvaticus* was titrated using heterologous mouse cells L 929 and it is probable that the interferon titre would be still higher in a homologous system. In ICR mice, a decrease corresponding to the state of hyporeactivity was observed after the first peak (Baron et al. 1970), and was followed by another increase, probably connected with virus replication in target organs. This second increase was not observed in *A. sylvaticus* probably because of limited virus replication. These results suggest that the interferon mechanism does not influence the susceptibility or resistance to CEE virus in this host. Similar results were obtained by Hofmann et al. (1971) in *Mus musculus* and Swiss albino mice after CEE virus infection.

Activation of NK cells following CEE virus infection may be accounted for by the production of interferon or other interleukins (Vargin and Semenov 1986). The role of NK cells as a defence mechanism in viral infections has not been fully evaluated.
(Welsh 1986). The increased NK cell activity in A. sylvaticus field mice compared to ICR mice may reflect an increased resistance of the former species to CEE virus infection.

Neutralizing antibodies were produced in both species, but they were demonstrated sooner in A. sylvaticus and attained titres up to eight times higher in the respective time intervals. Though it is difficult to explain this difference on the basis of the data on virus replication in the organs, a somewhat longer viraemia in A. sylvaticus may play a role, as was described by von Zeipel and Heigl (1966) in four wild species of rodents experimentally infected with CEE virus.

There is another component of cellular immune mechanisms, represented by virus specific cytotoxic T lymphocytes, which plays an important role in CEE virus infection. The cytotoxic activity of these cells against infected target cells reaches a maximum on day 5 p.i. and is detectable one month after infection (Kopecký, unpublished observations). Due to the fact that no suitable histocompatible target cells were available for A. sylvaticus field mice, the cytotoxic T lymphocyte testing was not included in this study.

The significance of the immune system for natural resistance to CEE virus infection in A. sylvaticus is indicated by the increased virus replication in the brain of immunosuppressed animals. After application of cyclophosphamide, the original inapparent infection became a lethal encephalitis in the treated animals (Mayer 1972).

Among the immune mechanisms tested in the two hosts, the greatest difference was observed in the activity of macrophages and NK cells and, to a certain extent, in the dynamics of neutralizing antibody production. For the time being, the participation of individual mechanisms in the general resistance to infection cannot be ascertained. This question can be answered only by selective suppression of individual components of the immune system.

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