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INTERACTION OF TICK/BORNE ENCEPHALITIS VIRUS WITH MOUSE PERITONEAL MACROPHAGES. THE EFFECT OF ANTIVIRAL ANTIBODY AND LECTIN

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Summary. - The interaction between tick-borne encephalitis (TBE) virus strain Hypr and mouse peritoneal macrophages was followed *in vitro*. Macrophages from juvenile mice (8-11 days old) were more permissive for virus infection than macrophages from adults (25-30 days old). Anti-TBE antibody in the subneutralizing dose increased the number of infected macrophages as well as virus release into the culture medium. Concanavalin A (con A), which bound to the virus as well as to the surface of macrophages, increased the uptake of the virus, but it neither enhanced the number of infected cells nor increased the virus release into medium. Antibody and lectin can modify the interaction between TBE virus and macrophages. Nevertheless, the Fc receptor-mediated endocytosis seems to be a necessary prerequisite for enhancing the effect of a ligand.

Key words: macrophages; tick-borne encephalitis virus; antibody dependent enhancement

Introduction

Macrophages are professional phagocytes which play a major role in defending the organism against foreign agents including viruses. However, in some viral infections macrophages can replicate the virus and serve as the source of infection in tissues and organs. This function can be enhanced by the action of the antiviral antibody. The phenomenon called antibody-dependent enhancement (ADE) involves the Fc receptor (Peiris *et al.*, 1981) or complement receptor (Cardosa *et al.*, 1983) of macrophages acting in combination with antiviral antibody; it has been demonstrated with members of *Flaviviridae* family as well as with other viral families (as reviewed by Porterfield 1985 and Brinton 1986). ADE phenomenon of TBE virus infected mouse macrophage-like cell line P388D1 was described by Phillpotts *et al.* (1985).

The ADE in TBE virus infection has been studied only in macrophage-like cells different from the primary macrophage cultures and remote from the conditions *in vivo*. Thus the aim of the present work was to demonstrate ADE in the culture of mouse peritoneal macrophages in relation to the donor's age. The possibility to enhance the infectivity of TBE virus for macrophages by an other ligand, for example by a lectin, was also investigated.

Materials and Methods

Mice. Outbred mice ICR (Velaz, Prague) of age 8–11 days (juvenile) and 25–30 days (adult) were used.

Virus. Prototype TBE strain Hypr was prepared as 10 % brain suspension in phosphate buffered saline (PBS) with 10 % newborn calf serum.

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

Peritoneal macrophages. Nonstimulated macrophages were obtained by peritoneal washing with TC medium 199; the cells were incubated in Leighton tubes containing coverslips in TC medium 199 supplemented with 10 % foetal calf serum. The tubes were seeded with 5×10^5 cells per tube. After incubation overnight at 37^0C the coverslips with adherent cells were washed in PBS and transferred into tubes containing medium.

Establishing of the experiments. Peritoneal cells adhering to the coverslips were infected with TBE virus at the multiplicity of 0.5 PFU per cell. Mouse anti-TBE serum (inactivated at 56°C for 30 min., neutralization titre 256) diluted 1:1000 and con A (Laboratory for research, production and control of lectin preparations, Charles University, Prague) in a concentration of 20 μg/ml were added to some cultures simultaneously with the virus. At intervals 0, 24, 48 and 72 hr post-infection (p.i.), the supernatant fluids were harvested to estimate the virus titre. Infected macrophages were demonstrated by indirect immunofluorescence (IF) method, counted at each abovementioned interval and the mean value was calculated.

Virus assay. TBE virus was assayed by the plaque method in PS cells using the technique of De Madrid and Porterfield (1969).

Indirect immunofluorescence assay (IFA). Adherent cells were fixed with acetone and stained with hyperimmune mouse anti-TBE serum (1:20) and with swine anti-mouse Ig conjugate (SWAM/FITC, Institute of Sera and Vaccines, Prague) diluted 1:10 and counterstained with Evan's blue (0.02 %).

Preparation of conA/FITC. Con A was conjugated with fluorescein isothiocyanate (FITC, Polysciences Inc., U.S.A.) in the presence of dimethylformamide and ethylenglycol. Free molecules of FITC were separated from the conjugate con A/FITC using gel filtration on Bio Gel P-6.

Surface immunofluorecsence. Adherent peritoneal cells on coverslips were incubated with con A/FITC diluted in cold PBS 1:10 for 45 min at 4^0C. After washing in cold PBS the cells were immediately examined in microscope Jena Lumar (Zeiss) and photographed.

Results

Resident macrophages from juvenile ICR mice supported TBE virus replication to a limited extent. Viral antigen was found in small percentage of cells. Production of virus particles as well as the number of infected macrophages

Table 1. Anti-TBE antibody dependent enhancement of TBE virus replication in macrophages

Group	Days post-infection		
	1	2	3
Without antibody	3*	11	3
With antibody	7	242	15

* Average numbers of IFA positive cells per slide

increased in the presence of anti-TBE antibody within 48 hr p. i. (Table 1 and Fig. 1). Viral antigen-positive cells had always the morphology of macrophages.

The effect of antibody on infection of macrophages was compared with action of con A which binds to the surface of living macrophages (Fig. 2) and also to the viral envelope glycoprotein E (Grubhoffer *et al.*, 1989). Both ligands decreased extracellular virus titre at 24 hr p.i., but at 48 hr the virus titre was higher in cultures with anti-TBE antibody in comparison with controls. The virus titre in cultures with con A further decreased, so that the difference between the extracellular virus titre in presence of antibody and the presence of con A at 48 hr p. i. was 1.7 $\log_{10}$ (Fig. 3). But at 72 hr p. i. the virus titres

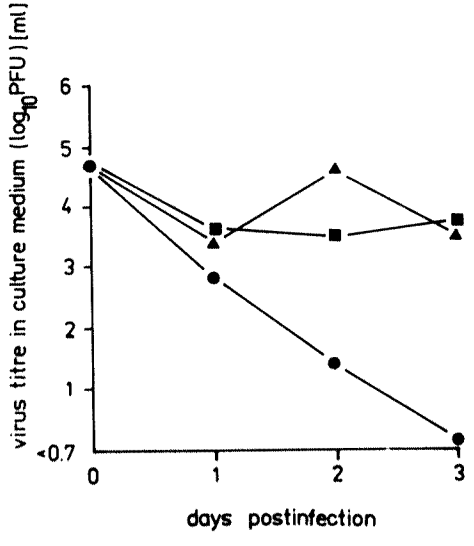

Fig. 1
Interaction of macrophages from juvenile mice with TBE virus *in vitro:* Effect of anti-TBE antibody
Extracellular virus titre in the culture without antibody (■), with antibody (▲) and thermal inactivation of the virus in culture medium (●).

Fig. 2
Surface immunofluorescence of Con A/ FITC on mouse peritoneal macrophages (800 x)

became nearly identical in all groups.

In cultures of macrophages from adult mice the virus titre decreased gradually but not so rapidly as in the cell free system. Presence of antiviral antibody or con A caused further decrease of the extracellular virus titres. The reason of this phenomenon was probably enhanced uptake of the virus by macrophages. The enhancing effect of the antibody on virus infectivity was not observed (Fig. 4).

Considering that Con A stimulates macrophage activity, further experiments included combination of both ligands. Virus titres in the cultures which contained both antibody and lectin, determined at 48 hr p. i., varied between the values ascertained in cultures with separate ligands. The number of infected macrophages in particular cultures correlated with the results of virus infectivity assay (Table 2).

Discussion

The different susceptibility of macrophages to TBE virus reflects in some extent the susceptibility of adult and juvenile mice to i.p. infection with this virus. Infection is always lethal, but has shorter incubation period in the case of juvenile mice (Slonim *et al.*, 1966). Correlation of age-dependent resistance to

Table 2. Combined effect of anti-TBE antibody and Con A on TBE virus infection of macrophages from juvenile mice. Results at 48 hr post-infection

Group	Extracellular virus titre (PFU/ml)	Number of IFA positive cells per slide
Control	4.4×10^2	2
Antibody	3.5×10^3	17
Con A	2.0×10^1	-
Antibody + Con A	1.0×10^3	13

virus infection with the resistance of macrophages has been observed with several viruses (Gallili *et al.*, 1967; Mintz *et al.*, 1980). The resistance of juvenile mice increased in some cases by adoptive transfer of macrophages from adult animals (Hirsch *et al.*, 1970).

In the present work macrophages from juvenile and adult mice were used to study the immunological enhancement of virus infectivity. While the ADE phenomenon has been proved in resident macrophages from juvenile mice, the infection of macrophages from adult mice in the presence of antiviral antibody was characterized by increased uptake of the virus by macrophages (decrease of the extracellular virus titre at 24 hr p.i.), but the replication of the virus was not enhanced and virus titres in culture media moved under the control level during the whole period of the experiment. This data confirm the difficulties with the approximation to the *in vivo* conditions of results obtained in macrophage cell lines. It has been proved, that the outcome of the interaction between flaviviruses and macrophages depends not only on the age of cell donors, but also on the inbred mouse line used and the activation stage of macrophages (Cardosa *et al.*, 1985). Relatively low increase of virus titre (about 1 $\log_{10}$) in cultures with antiviral antibody consists with the results of Phillpotts *et al.* (1985), who in a macrophage cell line P388D1 infected with TBE virus in the presence of hyperimmune rabbit anti-TBE serum observed ninefold enhancement as compared to the virus control with pre-immune rabbit serum. Similar values of enhancement have been reported in other flaviviruses (Halstead *et al.*, 1980).

Immunological enhancement of flavivirus infection has been described for cells bearing Fc receptor and complement receptor (Peiris and Portefield 1979; Halstead *et al.*, 1980; Cardosa *et al.*, 1983). Opsonized virus particles bound firmly to Fc or complement receptors on the surface of macrophages. The viral

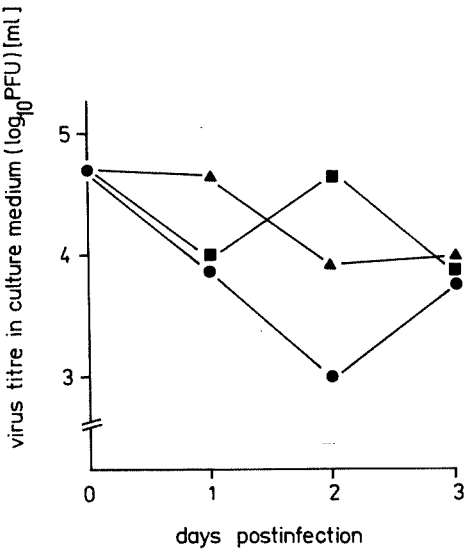

Fig. 3
Interaction of macrophages from juvenile mice with TBE virus *in vitro:* Effect of anti-TBE antibody and Con A
Extracellular virus titre in the culture without a ligand (▲), with anti-TBE antibody (■), with Con A (●).

internalization pathway into macrophages was identical both in the presence and absence of antiviral antibody. Much RNA escaped from internalized virons into cytoplasm from endosomal prelysosomal vacuoles (Gollins and Porterfield 1985).

One of the aims of the present study was to ascertain, if the mechanism of enhancement consists in simple increase of virus attachment to macrophage surface by means of a ligand. Con A has been used, which binds to both TBE virus and the macrophage enhancing the ingestion of red blood cells by macrophages (Goldman and Cooper 1975). This lectin increased the virus uptake by macrophages, but the virus did not replicate in such cells.

Ragarding the stimulatory effect of Con A on macrophages (Edelson and Cohn 1974), the combined effect of both antibody and lectin was studied. Provided that Con A could induce the enhancement of the resistance of macrophages to virus infection as it had been described for Japanese encephalitis virus *in vivo* (Kelkar *et al.,* 1987), the enhancing effect of antibody on virus titres would remain on the level of cultures with Con A only.

The results provided evidence in favour for the competitive effect of both ligands. The increased virus adsorption as described for Japanese encephalitis virus and Con A-treated PS cells probably played a role in this process (Kelkar 1985). But TBE virus adsorbed in this way could not replicate in macrophages.

Differences in the efficiency of phagocytosis of erythrocytes opsonized with a lectin from *Botrylloides leachii* or with an antibody have been observed by Coombe *et al.* (1986). In spite of the fact, that the uptake of erythrocytes opsonized by both ligands was nearly the same, greater phagocytosis was mediated

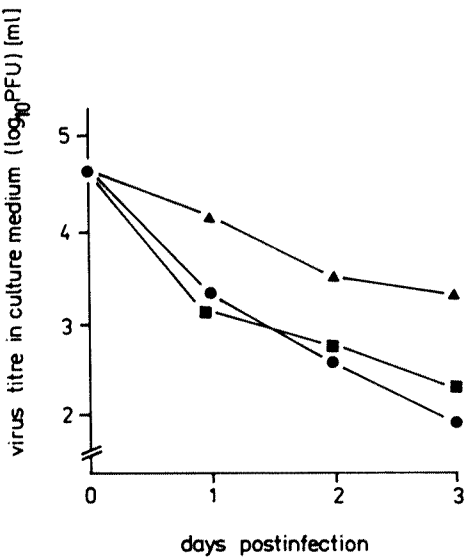

Fig. 4
Interaction of macrophages from ad
mice with TBE virus *in vitro:* Effect
anti-TBE antibody and Con A
Extracellular virus titre in the cultu
without a ligand (▲), with anti-TBE ar
body (■), with Con A (●).

by antibody. It could be explained by different level of signals for the inducti
of phagocytosis or by different phagocytosis mechanism.

Our results confirmed that the interaction of TBE virus with mouse macr
phages could be modified by both antibody and lectin. The binding on the
receptor seemed to be necessary for the enhancing effect of a ligand on t
virus infectivity.

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