

PURIFICATION OF TICK-BORNE ENCEPHALITIS VIRUS BY AFFINITY CHROMATOGRAPHY USING MONOCLONAL ANTIBODY

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Summary. — The application of a simple technique for purification of tick-borne encephalitis (TBE) virus is described. TBE virus was grown in chick embryo cell (CE) cultures and the virus was concentrated by differential centrifugation. Final purification was made by the filtration through Sepharose column to which monoclonal antibodies to TBE virus had been bound. The method was effective in eliminating avian retroviruses.

Key words: tick-borne encephalitis virus; monoclonal antibodies; affinity chromatography

The purification of TBE virus is of basic necessity for any further characterization of the virus. In addition to the variety of procedures for the purification of TBE virus previously described (Slonim and Štěpánek, 1956; Kowac *et al.*, 1961, 1962; Sokol *et al.*, 1961; Slávik *et al.*, 1967; Heinz and Kunz, 1977; Heinz, 1981; Blaškovič and Slávik, 1981), we have used a simple technique for the purification of the Skalica strain (Grešíková *et al.*, 1976). This strain is considered to be a spontaneous variant belonging to the TBE complex with a lowered virulence for adult white mice after subcutaneous inoculation (Rajčáni and Grešíková, 1982); it was thermolabile and in adult mice caused threshold viraemia only (Grešíková and Sekeyová, 1980).

The purpose of this study was the purification of the Skalica strain belonging to the TBE complex by affinity chromatography using monoclonal antibodies.

The Skalica strain of TBE virus (Grešíková *et al.*, 1976) was grown in CE cells, 24 hr post-infection (p.i.) the culture fluid was harvested and centrifugated at 3,000 rev/min for 10 min.

Monoclonal antibodies were prepared as described (Novák *et al.*, 1983; Poláková and Russ, 1983). Ascitic fluids containing the monoclonal antibody were adjusted to pH 7.0, and the immunoglobulins were precipitated with ammonium sulphate (at 50% saturation). The precipitate was collected by centrifugation and redissolved in PBS. About 10 mg of immunoglobulins were bound to 1 gr CNBr-Sepharose (Pharmacia, Sweden).

Purification procedures. The virus was concentrated by differential centrifugation. The final suspension of the pellet was resuspended in borate buffer at pH 9.0 and clarified at 3,000 rev/min for 10 min. Crude Skalica virus concentrated by differential centrifugation was loaded onto the Sepharose-antibody S-16 column. The column was washed with phosphate buffered saline; the virus was eluted with glycine buffer pH 3.4 and 2.4. The diluted fractions were examined for the haemagglutination activities (Clarke and Casals, 1958) and for their reactivity in RIA.

Haemagglutination (HA) tests. The HA titres were determined according to the method of Clarke and Casals (1958).

Solid phase radioimmunoassay (RIA) of individual fractions was performed as described (Poláková and Russ, 1983).

We have developed a technique suitable for the purification of TBE virus. The virus was grown in CE cells, concentrated by differential centrifugation and fractionated on Sepharose column to which monoclonal antibody to

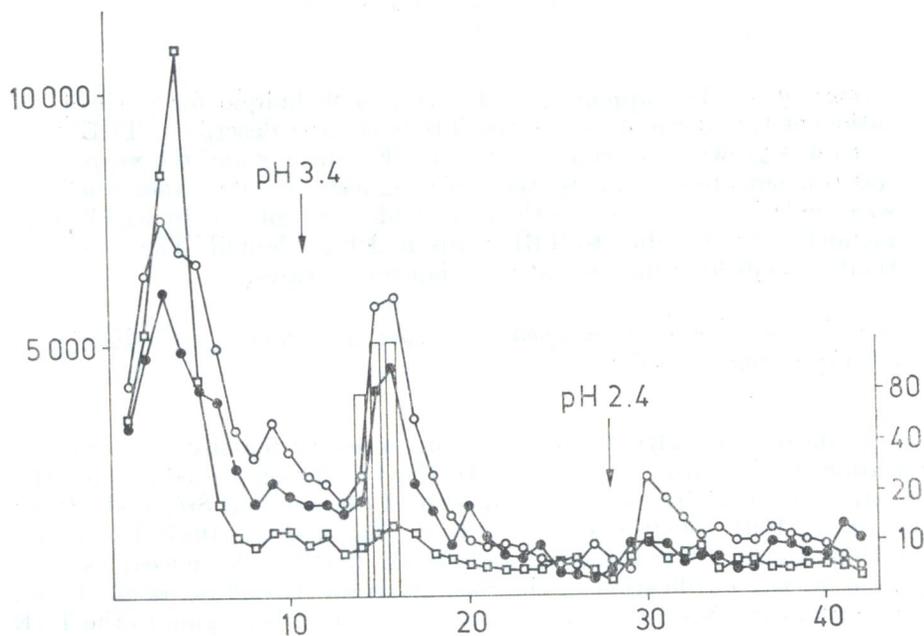


Fig. 1.

Affinity chromatography of the Skalica strain using monoclonal antibodies

Fractions were analysed in solid phase RIA with: mouse antiserum against Skalica strain of TBE (○), hybridoma antibody against TBE S-16 (●), and with hybridoma antibody IF9 against p/9 protein of avian retrovirus (□).

Abscissa: fraction number; left ordinate: ^{125}I — rabbit anti-mouse $\text{F}(\text{ab}')_2$ bound (counts/min); right ordinate: HI titres (empty columns).

TBE virus was allowed to bound. It follows from Fig. 1 that highly purified TBE virus can be obtained by affinity chromatography. The relative content of avian retrovirus was reduced after a single step of affinity chromatography about 1,200 times. The final preparation, i.e. the purified virus (released with glycine buffer pH 3.4) was assayed for its haemagglutination activity. The following titres of TBE haemagglutinin purified by affinity chromatography using monoclonal antibodies were obtained: 14th sample 1 : 40, 15th and 16th samples 1 : 80.

As follows from the results of our experiments, the Skalica strain of TBE virus can be purified by absorption to S-16 monoclonal antibody. Of importance is the finding that the purification of the Skalica strain by monoclonal antibodies is also effective in eliminating contamination by avian retroviruses. Finally, this method can be used with advantage for the preparation of a vaccine to TBE and/or for the preparation of TBE virus antigens.

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