Acta virol. **36**: 589–591, 1992

IMMUNOELECTRON MICROSCOPY OF RABBIT HAEMORRHAGIC DISEASE VIRUS USING MONOCLONAL ANTIBODIES

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Received February 27, 1992

Summary. – Five monoclonal antibodies (MoAbs) to rabbit haemorrhagic disease virus (RHDV), prepared and tested in ELISA, immunoperoxidase (IP) and immunofluorescence (IF) test previously, reacted specifically in immunoelectron microscopy (IEM), too. No differences in binding of individual MoAbs with full or empty RHDV particles were found by IEM.

Key words: rabbit haemorrhagic disease virus; monoclonal antibodies; immunoelectron microscopy

The first outbreak of viral haemorrhagic disease of rabbits was recorded in China in 1984 (Liu *et al.*, 1984). A similar fatal infectious disease affecting domestic rabbits was reported from Czechoslovakia in 1987 (Šeševičková *et al.*, 1988) and, by the end of 1988, the infection spread throughout the country. Electron microscopy was used to identify the causal agent of infection (Šmíd *et al.*, 1989). In negatively stained electron micrographs, RHDV virions lacked envelope, had a diameter of 31.5 – 33.0 nm and their structure corresponded to the general morphology of caliciviruses (Valíček *et al.*, 1990).

Five clones of hybridomas, producing MoAbs to RHDV, were prepared and tested (Rodák *et al.*, 1990). Some of the MoAbs reacted with the dominant RHDV proteins in Western blot analysis, while others did not. However, all MoAbs reacted highly specifically when RHDV infected cells were examined by ELISA, IP and IF tests. The aim of our experiments was to examine the specificity of the MoAbs in IEM.

RHDV was purified from organ homogenates of rabbits dying after experimental infection with the CAPM V-351 strain of RHDV isolated in Czechoslovakia (Šmíd *et al.*, 1989). The homogenates were extracted with chloroform and ultracentrifuged onto a CsCl cushion (Rodák *et al.*, 1990).

Five MoAbs denoted E9, C3, F2, E7/D10 and E7/C3 were tested by IEM. Twofold dilution series of MoAbs, ranging from 1:100 to 1:800, were prepared. 100 μl of each dilution was incubated with an equal volume of a diluted virus suspension at 37 °C for 60 min. Some of the samples were kept at 4 °C overnight before the examination by IEM. After incubation, a drop of the antigen-MoAb mixture was transferred onto a formvar-carbon coated grid. A short adsorption

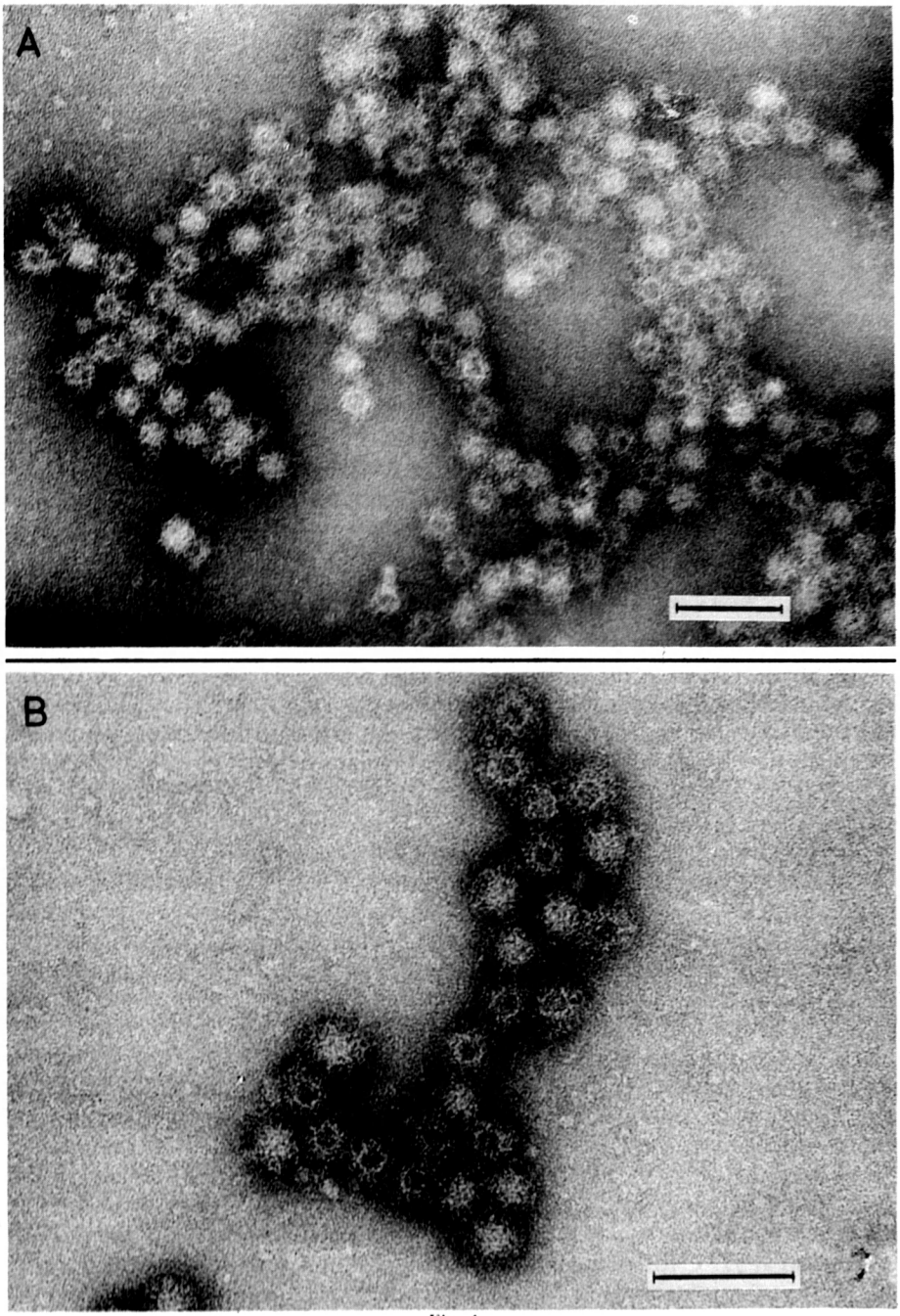

Fig. 1

period was followed by sucking off the fluid and negative staining with 2 % ammonium molybdate. The samples were examined by the electron microscope Tesla BS 500 at 90 kV.

Specific immune complexes were demonstrated by IEM after incubation with any of the five MoAbs tested (Fig. 1). No difference in binding of individual MoAbs to specific types of virus particles were found. All immune complexes formed contained both full and empty virus particles.

All five MoAbs, reacting specifically with RHDV in IEM did so in ELISA, IP and IF tests too (Rodák *et al.*, 1990) and therefore they can be used for the identification of RHDV by IEM. The results of IEM have confirmed our assumption that two MoAbs (E9 and C3), non-reactive with major RHDV proteins in Western blot analysis, reacted with antigenic structures damaged during the treatment of the virus for polyacrylamide gel electrophoresis under reducing conditions (Rodák *et al.*, 1990).

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Fig. 1
Immune complexes formed after incubation of RHDV with the MoAbs MoAb E9 diluted 1:600 (A), MoAb F2 diluted 1:100 (B). Bars: 100 nm.