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Identification of a cellular receptor for fish rhabdoviruses

M Béarzotti, A Lamoureux, S Chilmonczyk, M Brémont *

INRA, laboratoire de virologie et immunologie moléculaires, Jouy-en-Josas, 78350 France

The first event during the viral replication process is the recognition of a specific target (receptor) on the cell surface. To date a number of cellular receptors have been identified for various virus families (for a review see Haywood, 1994). In an attempt to identify a cellular receptor for the viral hemorrhagic septicemia virus (VHSV), a rainbow trout rhabdovirus, we developed a strategy previously used in our laboratory to identify the cellular receptor for a porcine coronavirus (Delmas *et al*, 1992). This strategy is based on the generation of receptor-directed monoclonal antibodies that are able to block infection by the virus in a permissive cell line.

To obtain monoclonal antibodies (MAbs) against the VHSV receptor, hybridomas were prepared from a mouse immunized with rainbow trout gonad (RTG) cells. The RTG cell line is highly susceptible to the VHSV. The mouse was immunized 3 times every 2 weeks with intact RTG cells. The final boost consisted of 200 μg of purified cell membranes. Three days after the final boost, immunized mouse spleen lymphocytes were fused with SP20 myeloma cells and hybridomas were produced. To select the MAbs potentially directed against the VHSV receptor, the RTG cells were preincubated with hybridoma supernatants before infection with VHSV. Three days later the cells were fixed and stained with violet crystal, and the inhibition of the cytopathic effect (CPE) was evaluated. Three out of 3 000 MAbs, which were able to protect cell monolayer against VHSV infection, were selected and were used to produce mouse ascitic fluids. One of these, MAb M45, was studied further.

An inhibition of the CPE and a reduction in plaque number was observed when the cells were preincubated with a dilution of M45 before being infected with VHSV (until the dilution reached 1:50 000). To test whether MAb M45 was species-specific, we used a VHSV-sensitive carp cell line, epithelioma papulosum cyprini (EPC). No CPE inhibition was observed for this carp-derived cell line, indicating that MAb M45 was specific for the Salmonidae-derived cell lines.

The specificity of the blocking activity of M45 to VHSV was also studied. RTG cells were incubated with M45 and then infected with several other fish rhabdoviruses including infectious hematopoietic necrosis virus

* Correspondence and reprints

(IHNV) and with unrelated viruses such as a birnavirus (IPNV), a herpesvirus (OMV) and an iridovirus (EHNV). The results allowed us to conclude that the M45 blocking activity was specific to fish rhabdoviruses.

To ensure that M45 was directed against a cellular membrane component, live RTG cells were incubated with MAb M45 and stained with an FITC goat anti-mouse immunoglobulin and were examined under a UV light microscope or stained using an immunogold-labelling method and examined by electron microscopy. In both cases, MAb M45 recognized a membrane component.

To characterize the VHSV cellular receptor recognized by M45, we tried to immunoprecipitate ^{35}S-radiolabelled RTG cells using various detergent conditions. None of the employed conditions gave a positive result. These negative results are probably due to the peculiar detergent sensitivity of the receptor. To overcome this problem, we constructed a RTG-derived cDNA expression library. This library was constructed in a pCDNA1 eucaryotic expression vector (Seed and Aruffo, 1987) and transfected in the EPC cell line. The objective was to select, by immunoscreening with the M45 MAb, a transfected cell expressing the VHSV receptor.

In summary, we selected 3 MAbs potentially directed against the VHSV cellular receptor. This receptor seems to be highly detergent-sensitive and cannot, as yet, be characterized by immunoprecipitation or Western-blot analysis. We undertook the cloning of the gene encoding the receptor using an approach based on the expression cloning of an RTG-derived cDNA library in a non-immuno-recognized cell line.

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