Antibody-mediated Enhancement of Rabies Virus Infection in a Mouse Macrophage Cell Line (P388D1)

By A. A. King,* J. J. Sands and J. S. Porterfield 1
Central Veterinary Laboratory, New Haw, Weybridge, Surrey and 1Sir William Dunn School of Pathology, University of Oxford, U.K.

(Accepted 22 February 1984)

SUMMARY

The suggestion that antibodies might enhance rabies virus infection of macrophages through opsonization of immune complexes was tested in vitro by adaptation of the rapid fluorescent focus inhibition technique for the examination of a macrophage cell line (P388D1). Some enhancement of rabies virus infection was shown. The relationship between such enhancement with the 'early death' phenomenon and its occurrence in vivo is discussed.

The precise way in which rabies vaccine confers protection against disease, and the pathogenesis of rabies virus infection are not fully understood. There are occasional failures even when vaccines of established potency have have used. In both monkeys and mice immunized with rabies vaccine and subsequently challenged with rabies virus, some die sooner than non-vaccinated controls given the same challenge (Sikes et al., 1971; Blancou et al., 1980). The mechanism underlying this 'early death' phenomenon is obscure, but it appears to be mediated by antibody, rather than by cell-mediated immune processes (Prabhakar & Nathanson, 1981). It has been suggested (Porterfield, 1981) that antibodies might enhance rabies virus infection of macrophages through their opsonization of immune complexes in a manner analogous to that which has been shown to occur with dengue, West Nile and several other viruses (Halstead & O'Rourke, 1977; Peiris & Porterfield, 1979, 1981). An example of antibody-mediated enhancement of viral infection in vivo occurs with feline infectious peritonitis virus (FIPV). When non-immune kittens received normal or FIPV immune feline serum intravenously followed 6 h later by intraperitoneal challenge with FIPV, the antibody-sensitized animals developed clinical signs more rapidly and died earlier than did control animals (Weiss & Scott, 1981).

We have studied the interaction between rabies virus strains and cells of the mouse macrophage cell line P388D1 (Koren et al., 1975), in the presence and absence of a number of different rabies virus antisera. Rabies virus does not induce lytic plaques in these cells, but we have been able to adapt the well-established rapid fluorescent focus inhibition test for rabies virus-neutralizing antibodies (Smith et al., 1973) by substituting P388D1 cells for the BHK21 cells normally used in this assay. Neutralization curves produced by incubating a fixed dose of rabies virus with serial dilutions of rabies virus antisera revealed a biphasic response; high serum concentrations neutralized infectivity, but on dilution the number of foci increased above the level produced by virus controls without antibody, falling again to reach control levels on further dilution (Fig. 1).

This type of response was also seen when serum from a person vaccinated with human diploid cell vaccine (HDCV) was tested with five different rabies virus strains (CVS, ERA, Pitman-Moore, Flury LEP and HEP 675). No such biphasic response was seen when similar tests were carried out in BHK21 cells.

The magnitude of enhancement (two- to fourfold) is substantially less than that found in the same cell line with viruses of the Togaviridae and Bunyaviridae families, where 30- to 100-fold increases in plaque counts are regularly observed (Peiris & Porterfield, 1981). Table 1 shows the
Fig. 1. Twenty-five µl of a suspension containing 100 TCID$_{50}$ of Flury HEP 675 rabies virus was mixed with 25 µl of serial 0.5 log$_{10}$ dilutions of International Standard rabies virus antiserum (initially 10 International Units/ml) in individual wells of a Costar 96-well microtitre plate. After 90 min incubation at 35 °C, 25 µl of Leibovitz L15 medium with 3% heat-inactivated (56 °C for 30 min) foetal calf serum containing 7.5 x 10$^3$ cells were added to each well, and the plates were re-incubated for 5.5 h. An overlay containing the same medium but including carboxymethylcellulose at a final concentration of 0.75% was then added, using 80 µl/well. After incubation at 35 °C for 4 days, the plates were fixed in 25% formalin for 15 min, washed in phosphate-buffered saline, and stained with fluorescein isothiocyanate-conjugated anti-rabies globulin. Fluorescent foci were counted using a Leitz SM Lux microscope illuminated by a 50 W mercury vapour lamp and employing filter system H2, BG38 and GG 455 edge filters and K530 barrier filters. Mean counts are plotted, based on eight replicates per serum dilution; bars represent standard deviations; ---, control.

degree of enhancement seen with a variety of different rabies antisera, together with details of neutralizing antibody titres as measured by conventional neutralization assays in BHK21 cells or mice.

In a separate experiment it was shown that CVS virus replicated in, and infective virus was released from, the P388D1 cells in the presence or absence of antiserum. Interestingly, more virus was released in the presence of antiserum at a dilution of 10$^{-4}$ than was released in its absence (data not shown).

Although these tests were carried out in vitro with a continuous line of macrophage which may differ in a number of respects from resident and elicited macrophages, our results support the
Table 1. Ability of various rabies antisera to enhance the titres of rabies virus strain HEP 675 in P388D1 cells

<table>
<thead>
<tr>
<th>Species in which antiserum was prepared</th>
<th>Vaccine used</th>
<th>Serum neutralization titre*</th>
<th>Dilution showing peak enhancement</th>
<th>Enhancement ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>CVS</td>
<td>NT</td>
<td>10⁻³.⁵</td>
<td>2.4</td>
</tr>
<tr>
<td>Man</td>
<td>Human diploid cell</td>
<td>10⁻².⁹ (H)</td>
<td>10⁻⁴</td>
<td>4.1</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Unknown</td>
<td>10⁻².⁰ (M)</td>
<td>10⁻⁴</td>
<td>3.4</td>
</tr>
<tr>
<td>Mouse</td>
<td>Duck embryo vaccine</td>
<td>10⁻².⁸ (M)</td>
<td>10⁻⁴</td>
<td>3.3</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>Flury HEP 675</td>
<td>10⁻².⁹ (H)</td>
<td>10⁻⁴</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Determined in BHK21 cells (H) or in mice (M). NT, Not tested.
† Number of foci at peak serum dilution/number of foci in the absence of antibody.

hypothesis that macrophages are not only capable of supporting the replication of rabies virus, but may give enhanced yields of rabies virus in the presence of rabies virus antiserum. If this can occur in vitro, it may also occur in vivo. Thus, the interaction between virus and antiviral antibody does not always result in the neutralization of viral infectivity, but may result in consequences which are detrimental to the host.

The authors would like to thank Dr G. S. Turner of the Blood Products Laboratories for the gift of antisera used in this work.

REFERENCES


(Received 5 September 1983)