Production of a Monoclonal Antibody against an Epitope on HeLa Cells that Is the Functional Poliovirus Binding Site

By P. NOBIS,* R. ZIBIRRE, G. MEYER, J. KÜHNE, G. WARNECKE AND G. KOCH

Abteilung Molekularbiologie, Universität Hamburg, Grindelallee 117, D-2000 Hamburg 13, F.R.G.

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SUMMARY

Cell lines of primate origin carry receptors on their plasma membrane which are responsible for the specific binding of poliovirus. This paper describes the isolation and characterization of a monoclonal antibody reacting with the plasma membrane of HeLa cells. The antibody (D171) was selected for its protection of HeLa cells against the cytopathic effect of poliovirus type 1. This protection was found to extend to all three viral serotypes, while the replication of five other viruses in HeLa cells was not affected. The 125I-labelled purified antibody did not react with cell lines derived from pig, dog or rodents but bound specifically to all lines of human or primate origin. Immunoglobulin or Fab fragments of D171 prevented the binding of 35S-labelled poliovirus to HeLa cells. Conversely, nearly all binding sites of 125I-labelled D171 immunoglobulins or Fab fragments could be blocked after preincubation of HeLa cells with poliovirus. These results indicate that D171 recognizes the poliovirus receptor site on different susceptible cells and that practically all D171 binding sites are involved in the specific attachment of poliovirus to the plasma membrane. To determine whether the epitope recognized by D171 could be separated from the receptor for poliovirus, human–mouse cell hybrids were prepared and analysed. In all 40 clones tested, the susceptibility to poliovirus correlated with the binding of D171.

INTRODUCTION

The initial event in the interaction of virions with the host cell is the attachment of the virus to receptors on the plasma membrane. The presence of specific cellular receptors is to some extent a determinant of the species and/or tissue tropism and thus the pathogenesis of certain viruses (Holland, 1961). The polioviruses provide an example of this type of specific virus–host cell interaction. Normally, polioviruses infect only primates, and can replicate in all cells of primate origin growing in cell culture (for review, see Crowell et al., 1981).

During recent years research on poliovirus has focused more on the features of the virus than of the host cell (Koch & Koch, 1985). The viral genome has been sequenced (Kitamura et al., 1981) and epitopes on the viral capsid involved in neutralization have been identified (Minor et al., 1983; Wychowski et al., 1983; Emini et al., 1983). In comparison to the extensive amount of knowledge concerning the molecular biology of the virus, relatively little is known about its receptor on the plasma membrane of susceptible cells. Polioviruses of each of the three serotypes bind to the same receptor on cells of primate origin, but other members of the picornavirus family do not compete for this receptor (Crowell & Siak, 1978). The expression of the receptor for poliovirus in human–mouse hybrid cell lines was shown to be dependent on the presence of human chromosome 19 (Miller et al., 1974). Isolated plasma membrane components can still specifically bind poliovirus (Guttman & Baltimore, 1977; Krah & Crowell, 1982); however, further attempts to isolate, purify and characterize the receptor were unsuccessful. Based on the
results of studies on receptor blockage and inactivation it was suggested that the receptor is a
glycoprotein (Crowell & Landau, 1983).

We chose to study the plasma membrane receptor for poliovirus by preparing and utilizing a
monoclonal antibody against this receptor. After immunization of mice with HeLa cells, hybridi-

oms were prepared and selected for their ability to protect HeLa cells from the c.p.e. of
poliovirus. This approach was used by Campbell & Cords (1983) to isolate monoclonal
antibodies that inhibit attachment of group B coxsackieviruses. We obtained one hybridoma
(designated D171) which showed good protection against poliovirus after re-cloning. The
culture fluid of this hybridoma prevented the infection of several susceptible cell lines by
polioviruses of type 1, 2 and 3 but not by five other viruses. It could be demonstrated that this
protecting activity of the hybridoma supernatant was due to the blockage of poliovirus binding
to its receptor. The characteristics of D171 correspond to the properties of two monoclonal
antibodies previously described by Minor et al. (1984). These two antibodies were selected for
their ability to block the binding of radioactively labelled poliovirus types 1, 2 and 3 to Hep2c
cells. These antibodies were found to bind to all poliovirus-susceptible cells and to prevent
poliovirus growth in these cells. These features seem to be identical to the properties of the
antibody described here. We further characterized the specificity of the antibody produced by
hybridoma D171 after the purification of the immunoglobulin from ascites fluid.

To exclude any non-specific lectin-like blockage of the membrane binding sites and to
determine whether or not the antibody D171 specifically recognizes the cellular receptor for
poliovirus the following experiments were carried out: competition of the immunoglobulin or its
Fab fragments with poliovirus for cellular binding sites; competition of poliovirus preparations
with radiolabelled Fab fragments or undigested antibody D171 for binding to cells; analysis of
antibody D171 binding to poliovirus-susceptible and -non-susceptible cell lines; determination
of antibody D171 binding to human–mouse hybrid cell lines which were characterized for their
susceptibility to poliovirus. The results of the present study indicate that poliovirus and the
antibody D171 bind to the same site on the plasma membrane of permissive cells.

**METHODS**

*Viruses and cells.* HeLa S3 cells were propagated as suspension culture in Joklik’s modified Eagle’s medium
supplemented with 10% newborn calf serum. All other cells were grown as monolayers in Dulbecco
and Vogt’s modified Eagle’s medium (DME/M) supplemented with 10% heat-inactivated foetal calf
serum (FCS).

The growth and purification of Mahoney type 1 poliovirus was performed as described (Schärl & Koch, 1984).
Viral infectivity was determined by plaque assay according to the procedure described previously (Koch et al.,
1966). For binding assays poliovirus was labelled with $^{35}$S methionine in methionine-free medium for 3 to 6 h
post-infection. After an additional incubation for 1 h in complete medium the infected cells were harvested. The
$^{35}$S methionine-labelled poliovirus was then purified by isopycnic banding in caesium chloride as described by

Virus stocks of wild-type poliovirus type 1/Mahoney, type 2/MEF and type 3/Leon and of the Sabin strain of
poliovirus type 1, coxsackievirus B5, echovirus type 30, vesicular stomatitis virus, herpes simplex virus type 1 and
adenovirus type 5 were propagated in HeLa cells and frozen as clarified cell lysates. Virus titres were determined
by a plaque assay as described previously (Koch et al., 1966).

*Production of monoclonal antibodies.* BALB/c mice were primed by intraperitoneal and subcutaneous injection of
3 x 10$^7$ viable HeLa cells from suspension cultures without adjuvant. Three, 6 and 9 weeks later the mice were
inoculated in a similar fashion. Three days after the last immunization the spleens were removed and splenic
mononuclear cells were fused with the NS-1 myeloma line following a procedure described by Goding (1980).
Hybridomas were selected in HAT medium in 384-well plates (product number 704160, Greiner,
F.R.G.).

*Initial screening of hybridomas.* HeLa cells were seeded into flat-bottom microtitre plates at a density of 10$^5$ cells
in 100 $\mu$l DME/M per well. The next day, 100 $\mu$l of hybridoma supernatant per well was added. After 1 h incubation
at 37 °C the cells were infected with 500 p.f.u. of poliovirus in 20 $\mu$l DME/M. Twenty and 48 h after virus addition,
the cells were screened with a phase-contrast microscope. Virus replication was evaluated by the observation of
the virus-induced c.p.e.

*Purification of immunoglobulin and preparation of Fab fragments.* One of the hybridomas (D171) inhibiting the
replication of poliovirus in HeLa cells was cloned twice by limiting dilution. The antibody was used as hybridoma
supernatant or as ascites fluid after growing the hybridoma cells in Pristane-primed BALB/c mice. By an
Monoclonal antibody to poliovirus receptor

Monoclonal antibody to poliovirus receptor

immunodot assay (McDougal et al., 1983) the antibody D171 was found to be of the IgG1 isotype. IgG was purified by passing the ascites fluid over a Protein A–Sepharose column and eluting the adsorbed IgG with 0.1 M-citrate buffer at pH 4.5 (Ey et al., 1978). Protein A–Sepharose-purified IgG from hybridoma D171 was digested with cellulose-bound papain as described by Mage (1980). The Fab fragments were separated from undigested IgG by Protein A–Sepharose chromatography.

The monoclonal antibody L5.1 with specificity against the human transferrin receptor (Lebman et al., 1982) was obtained from the American Type Culture Collection.

Analysis of the protective activity of antibody D171 on HeLa cells. HeLa cells were seeded at 4 x 10^4 in 0.5 ml DMEM per well in 48-well tissue culture plates (product number 3548, Costar). Twenty-four h later, increasing amounts of purified IgG of D171 or its Fab fragment were added. After 2 h incubation with the antibody, different viruses were added at multiplicities of 1 or 10 p.f.u./cell. The incubation was continued and the c.p.e. was monitored using phase-contrast microscopy for several days. All incubations were carried out at 37°C.

Radioactive labelling of antibodies. Purified IgG or Fab fragments of hybridomas D171 or L5.1 were labelled with 125I using the iodogen® method (Fraker & Speck, 1978). Radiolabelled antibody was purified by Bio-Gel P-4 gel filtration. Specific activities of 20 to 30 μCi/μg protein were obtained.

Binding assay for radioactively labelled monoclonal antibody or poliovirus. HeLa or other cells were seeded in 24-well tissue culture plates at 10^3 cells/well. The next day the medium was removed and the plates were put on ice. Radioactive virus or antibody were suspended in 5% FCS containing DMEM in which bicarbonate had been replaced by 25 mM-HEPES pH 7.2 and 250 μl of this suspension per well was added to the cells to be tested. After 1 h of incubation at 0 °C on a rotating shaker the supernatant was aspirated and the cells washed three times with DMEM containing 5% FCS. The adherent cells were solubilized in 250 μl 0.2% SDS and the radioactivity bound was determined.

Preparation of human–mouse hybrid cell lines. A 431 human carcinoma and NIH 3T3 TK− mouse fibroblast cells were seeded at a density of 3 x 10^5 each per 9 cm Petri dish and fused using polyethylene glycol as described by Noda et al. (1983). After selection in HAT medium containing 5 x 10^-7 M-ouabain, individual colonies were picked and propagated for 5 months in HAT medium to allow chromosome segregation. The individual cultures were cloned twice and replica cultures were exposed to poliovirus (100 p.f.u./cell). Clones repeatedly showing the same feature, i.e. uniform susceptibility or resistance to virus, were selected and tested for the binding of monoclonal antibodies D171 and L5.1.

RESULTS

Establishment of the antibody-producing hybridoma line D171

Spleen cells from two hyperimmunized mice were fused with NS-1 myeloma cells. After selection in HAT medium, close to 1400 clones were obtained. The hybridoma supernatants were tested for their ability to inhibit poliovirus-induced c.p.e. on cells. The spent tissue culture medium of 12 wells inhibited c.p.e. for at least 24 h. One of these hybridomas was cloned twice by limiting dilution and established as an antibody-producing stable hybridoma cell line (D171). The data presented in this paper indicate that screening hybridoma supernatants for their ability to prevent the c.p.e. of poliovirus permits the detection of antibodies interfering with virus attachment to the cellular receptor. While our study was in progress the establishment of monoclonal antibodies that inhibit attachment of group B coxsackieviruses using the same rationale was reported (Campbell & Cords, 1983).

Protection of HeLa cells by antibody D171 against virus-induced cytopathic effect

HeLa cells were preincubated with various amounts of purified IgG of D171 or its Fab fragment. After 2 h the cells were challenged with different viruses and the appearance of c.p.e. was observed. The effect of antibody D171 on the extent of c.p.e. caused by different viruses is shown in Table 1. Even at the highest concentration of antibody we could not observe any effect on the severe c.p.e. of cells infected with coxsackievirus B5, adenovirus type 5, herpes simplex virus type 1, echovirus type 30 or vesicular stomatitis virus. However, the destruction of HeLa cells by poliovirus types 1, 2 and 3 and by the Sabin attenuated vaccine strain of poliovirus type 1 was clearly inhibited by purified IgG of D171 or by its Fab fragments. The weaker protection against poliovirus type 2, which we observed twice, as yet remains to be explained.

Specific binding of monoclonal antibody D171 to cell lines of various origins

The monoclonal antibody D171 was analysed for the specificity of its binding to different cell lines. Table 2 shows the reactivity of the radiolabelled antibody with cell lines of primate and
Table 1. Effect of antibody D171 on the virus-induced cytopathic effect in HeLa cells*

<table>
<thead>
<tr>
<th>Virus type and strain†</th>
<th>Multiplicity (p.f.u./cell)</th>
<th>Cytopathic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50‡</td>
<td>10</td>
</tr>
<tr>
<td>Polio 1</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Polio 2</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Polio 3</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Sabin 1</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>HSV-1</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>VSV</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>Ad5</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>Echo 30</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>CB5</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>Polio 1§</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
</tr>
</tbody>
</table>

* The cells were observed 48 h after infection. The c.p.e. is expressed as (+ + +) all cells destroyed, (+ +) 50 to 100%, (+) 10 to 50% and (−) no cells destroyed.
† HSV-1, herpes simplex virus type 1; VSV, vesicular stomatitis virus; Ad5, adenovirus type 5; Echo 30, echovirus type 30; CB5, coxsackievirus type 5.
‡ Amount of antibody (µg) added to cells.
§ D171 Fab fragments were tested instead of D171 immunoglobulin.

Table 2. Binding of 125I-labelled antibody D171 to different cell lines

<table>
<thead>
<tr>
<th>Cell</th>
<th>Bound antibody D171 (c.p.m./10^5 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human: HeLa</td>
<td>2360</td>
</tr>
<tr>
<td>SV 80</td>
<td>1410</td>
</tr>
<tr>
<td>HEp-2</td>
<td>2970</td>
</tr>
<tr>
<td>RD</td>
<td>2130</td>
</tr>
<tr>
<td>A 431</td>
<td>1110</td>
</tr>
<tr>
<td>HL-60</td>
<td>1530</td>
</tr>
<tr>
<td>U-937</td>
<td>1990</td>
</tr>
<tr>
<td>Monkey: Vero</td>
<td>1820</td>
</tr>
<tr>
<td>GMK</td>
<td>2440</td>
</tr>
<tr>
<td>TC-7</td>
<td>1980</td>
</tr>
<tr>
<td>Non-primate*</td>
<td>&lt;120</td>
</tr>
<tr>
<td>Blank†</td>
<td>&lt;180</td>
</tr>
</tbody>
</table>

* Cells of non-primate origin were from pig (PK 15), dog (MDCK), rabbit (SIRC), rat (NRK and XC), hamster (BHK, WOBR-5 and CHO) or mouse (NIH 3T3 TK− and SC-1).
† Empty tissue culture well.

non-primate origin. All of the 10 tested cell lines of non-primate origin showed very low binding of the radioactively labelled antibody, comparable to background binding in wells without cells. In contrast, all cell lines of human or other primate origin bound significant amounts of the radioactively labelled monoclonal antibody D171. Utilizing indirect immunofluorescence it could be seen (data not shown) that D171 binds to the cell surface of native or glutaraldehyde-treated HeLa cells.

Competition between poliovirus and monoclonal antibodies for binding sites on HeLa cells

Since D171 bound to all cells susceptible to poliovirus, and inhibited virus replication in HeLa cells we investigated whether this antibody competed with poliovirus for the same cellular receptor sites. To prevent endocytosis the cells were kept on ice. HeLa cells (10^5) were
Monoclonal antibody to poliovirus receptor

Fig. 1. HeLa cells were preincubated with increasing amounts of either poliovirus (■), antibody D171 (□), D171 Fab fragments (○) or antibody L5.1 (▲). The effect of this preincubation on the binding of (a) 125I-labelled D171 Fab fragments, (b) 125I-labelled D171 IgG and (c) 35S-labelled poliovirus was determined. The degree of binding is expressed as the percentage of maximal ligand binding without the addition of competing protein. The maximal binding was (a) 16000, (b) 24000 and (c) 2400 c.p.m. The amount of competing protein (in ng) added to the cells is indicated on the abscissa.

Table 3. Correlation of poliovirus susceptibility with the binding of antibodies D171 and L5.1 on human–mouse hybrid cell lines

<table>
<thead>
<tr>
<th>Group</th>
<th>Susceptibility to poliovirus</th>
<th>Binding of antibody</th>
<th>Number of hybrid cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>−</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
<td>−</td>
<td>−</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>−</td>
<td>−</td>
<td>14</td>
</tr>
</tbody>
</table>

* As the D171 binding and poliovirus susceptibility correlated in all 40 hybrid cell lines we sorted the lines into four groups (A to D) according to the reactivity of the two antibodies.

preincubated for 20 min with various amounts of either unlabelled virus, immunoglobulins or the Fab fragments of D171 in 100 μl DMEM. As a control HeLa cells were preincubated with the monoclonal antibody L5.1. The incubation was continued for 60 min after the addition of 250 μl DMEM containing either 125I-labelled antibody, 125I-labelled Fab fragments or 35S-labelled poliovirus. The amount of radioactivity bound was determined. The results are shown in Fig. 1. Even at high concentrations L5.1 did not compete either with poliovirus, antibody D171 or the D171 Fab fragments for binding sites on HeLa cells. However, the binding of 35S-labelled poliovirus to HeLa was inhibited after preincubation of the cells with antibody D171, and D171 Fab fragments or with unlabelled poliovirus (Fig. 1c). Conversely, the binding of labelled antibody D171 or its Fab fragment to HeLa cells could be competed with increasing amounts of unlabelled poliovirus, antibody D171 or the D171 Fab fragments (Fig. 1a, b).

Human–mouse cell hybrids: correlation of susceptibility to poliovirus and binding of monoclonal antibodies D171 and L5.1

Somatic human–mouse cell hybrids were derived by fusion of A 431 epidermoid carcinoma and NIH 3T3 TK− cell lines. The hybrids were cloned twice by limiting dilution. Replica cultures of 40 clones were tested for susceptibility to infectious poliovirus and for the binding of monoclonal antibodies D171 and L5.1. The results are shown in Table 3. All 17 clones susceptible to poliovirus bound the 125I-labelled antibody D171 as did the parental human cell line, whereas clones resistant to poliovirus bound only background levels of radioactivity comparable to the
parental poliovirus-resistant mouse cell line NIH 3T3 TK−. It can be seen in Table 3 that the binding of antibody L5.1 with specificity against the human transferrin receptor did not correlate with the binding of D171.

**DISCUSSION**

Our goal was to obtain monoclonal antibodies that interfere with the specific binding of poliovirus to susceptible cells. Several lines of evidence indicate that the monoclonal antibody D171 described in this paper recognizes specifically the poliovirus binding site on primate cells.

The evidence may be summarized as follows. D171 immunoglobulin and Fab fragments protect cells against c.p.e. caused by polioviruses of types 1, 2 and 3, but do not protect against five other viruses. This result is in good agreement with the observation that all three serotypes of poliovirus compete for the same cellular receptor which is not recognized by other picornaviruses (Crowell & Siak, 1978), and corresponds to the properties of the antibodies described by Minor et al. (1984). It also indicates that the antibody D171 does not interfere with receptor-mediated endocytosis in general. The susceptibility of primates to poliovirus infections is paralleled by the binding of D171 to cells of primate origin but not to cells of non-primate origin. The competition data in Fig. 1 demonstrate that polioviruses as well as antibody can block one another’s binding to HeLa cells. More than 95% of the binding sites of D171 can be blocked by poliovirus, indicating that most of the epitopes recognized by antibody D171 also function as (or are at least part of) specific binding sites for poliovirus. This argues against the possibility that a substantial percentage of epitopes recognized by D171 are also found on other membrane structures not functioning as viral binding sites, as is the case for antibodies against the receptor of the epidermal growth factor (EGF). Several monoclonal antibodies against the EGF receptor of the epidermoid carcinoma cell line A431 have been shown to recognize blood group-related carbohydrate structures (Childs et al., 1984). The binding of antibody D171 correlates clearly with the segregating susceptibility to poliovirus of 40 human–mouse hybrid cell lines. If the binding site for antibody D171 and the receptor for poliovirus were to be coded for by independent loci one should expect segregation of the binding on individual hybrid cell lines. As a control this expected segregation was demonstrated for an unrelated membrane receptor. The binding of antibody L5.1 specific for the human transferrin receptor is segregated randomly in the poliovirus-susceptible or -resistant clones. On the basis of these results we conclude that essentially all epitopes recognized by antibody D171 function as specific poliovirus binding sites on HeLa cells.

In many cases the preparation of large quantities of purified virions for receptor studies is laborious. Monoclonal antibodies against plasma membrane components thus are useful tools for biochemical and functional characterization of virus receptors. Using this technique it was shown that the C3d complement receptor is also the cellular receptor for Epstein–Barr virus (Fingeroth et al., 1984). In addition, monoclonal antibodies should be helpful for the analysis of synthesis, endocytosis, turnover and tissue distribution of viral receptors. The sites on poliovirus complementary to the cellular receptor (the receptor recognition site) have not yet been identified. Idiotypic antibodies to D171 might be useful in the elucidation of the receptor recognition site of poliovirus, a possibility which we are currently investigating.

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**REFERENCES**


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