Monoclonal Antibodies to Sindbis Virus Glycoprotein E1 can Neutralize, 
Enhance Infectivity, and Independently Inhibit Haemagglutination or 
Haemolysis

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SUMMARY

Two monoclonal antibodies raised against Sindbis virus were shown to be specific 
for the envelope glycoprotein E1 by radioimmunoprecipitation (RIP). They had a 
number of contrasting biological properties. One of them was capable of neutralizing 
virus infectivity and inhibiting haemagglutination, while the other had no significant 
nutralizing or haemagglutination-inhibiting capability, but did inhibit virus-mediated 
haemolysis. Both monoclonal antibodies could enhance virus infectivity of Fc 
receptor-bearing macrophage-like cells when present at suitable dilutions.

INTRODUCTION

Sindbis virus is an alphavirus consisting of an RNA-containing nucleocapsid surrounded 
by a lipid-containing membranous envelope which contains two virus-specified glycoproteins, 
E1 and E2 (Schlesinger & Schlesinger, 1972). Biological functions associated with the surface 
of the virus include neutralization of infectivity, haemagglutination (Clarke & Casals, 1958) 
and haemolysis (Karabatsos, 1963, 1965, 1973; Goto et al., 1979; Väänänen & Kääriäinen, 
1980; Yoshinaka et al., 1978).

The precise roles of the two surface glycoproteins in these biological functions are not fully 
understood, but studies of virus–antibody interactions may provide a rational basis for their 
investigation. Monospecific antiserum to purified E2 neutralizes infectivity, while similar 
antiserum raised against purified E1 inhibits haemagglutination (Dalrymple et al., 1976). 
Antiserum against purified envelope proteins inhibits haemolysis (Väänänen & Kääriäinen, 
1980). However, the wide diversity of antibodies in conventional hyperimmune sera makes 
the unambiguous interpretation of the various effects difficult, and the denaturing processes 
associated with purification of antigens for the production of monospecific antisera may result 
in reagents which are poorly neutralizing or of low avidity (Symington et al., 1977). It has 
also been shown that at least one epitope present on intact Sindbis virus is lost upon 
disruption (Roehrig et al., 1980). Monoclonal antibodies can be used to circumvent these 
problems and to define precisely the function of the surface proteins of the virus.

Here we show that monoclonal antibodies specific for the E1 glycoprotein of Sindbis virus 
can neutralize infectivity, enhance infectivity for macrophage-like cells and separately inhibit 
hemagglutination and haemolysis.

METHODS

Virus and hyperimmune serum. Sindbis virus AR339 obtained from Dr C. Leake (London 
School of Hygiene and Tropical Medicine) was inoculated intracerebrally into 1-day-old
mice. After 36 h a suspension of infected brain was prepared in phosphate-buffered saline (PBS). A working stock of Sindbis virus was made by inoculation of this suspension on to Vero cell monolayers at an input multiplicity of 0-01 p.f.u./cell. After incubation for 42 h at 37 °C, when maximal c.p.e. was observed, the supernatant medium was clarified by centrifugation at 2000 g for 10 min. Stock virus was stored at -70 °C. Rabbit hyperimmune serum against infectious Sindbis virus was raised by multiple inoculations of infectious suckling mouse brain suspension.

**Monoclonal antibodies.** Female Balb/c mice for fusion were inoculated intraperitoneally with 0-1 ml of infected suckling mouse brain suspension. Animals were rested for 4 to 5 weeks before intravenous inoculation with a further 0-1 ml of infected mouse brain suspension. Spleens for fusion were removed 3 days later. The procedure for the production of monoclonal antibodies was based on that of Fazekas de St Groth & Scheidegger (1980), except that the non-secretor myeloma cell line P3-X63-Ag8-653 (Kearney et al., 1979) was used. Antibody production was detected by indirect immunofluorescence approx. 4 weeks after fusion. Antibody-positive cultures were cloned three times by isolation of single cells and grown in L15 medium containing 15% foetal calf serum (FCS). To assist growth of the clones peritoneal macrophages were added to these cultures. Mouse ascites was prepared by intraperitoneal inoculation of 10^6 to 10^7 hybrid cells in Balb/c mice pretreated with 0-5 ml pristane (2,6,10,14-tetramethylpentadecane, Aldrich Chemical Company, Milwaukee, Wis., U.S.A.) 3 to 6 days previously.

**Ouchterlony gel diffusion tests.** These were performed on tissue culture fluids from antibody-positive hybridoma cultures. Fluids were concentrated five- to tenfold with Lyphogel (Gelman Instruments) and tested against immunoglobulin class-specific antisera obtained from Nordic Immunological Laboratories (Maidenhead, Berks, U.K.).

**Plaque reduction neutralization and enhancement of infectivity assays.** A range of antisera dilutions was tested against a range of virus suspension dilutions in two-dimensional checkerboard pattern neutralization tests. These were performed in either Vero or P388D1 cells using serial fivefold virus dilutions and serial twofold antisera dilutions. The mouse macrophage-like P388D1 cells were obtained from Dr J. S. Porterfield (Sir William Dunn School of Pathology, University of Oxford). The method was essentially as described by de Madrid & Porterfield (1969) and Peiris & Porterfield (1979) adapted to micro-tissue culture plates (Falcon Microtest II) or multi-test plates (Linbro TC multidish). In brief, 1 vol. virus suspension in L15 medium was incubated with an equal volume of antiserum at 37 °C for 1 h. One vol. cell suspension in L15 medium (5% FCS + 10% tryptose phosphate broth and antibiotics) was then added, followed by further incubation for 3 h. Overlay medium (2 vol.) consisting of L15 medium containing carboxymethylcellulose (1-5%) was then added and the plates were incubated for 2 days before staining with naphthalene black solution. In experiments where a second step reagent was used to mediate neutralization, it was added after the virus–antibody mixtures had been incubated for 1 h at 37 °C. A further incubation for 30 min then followed.

**Immunofluorescence (IF) tests.** The indirect test was used on unfixed and acetone-fixed infected Vero cells grown on glass coverslips. Test fluids were added to infected cells for 40 min at 37 °C, followed by the appropriate fluorescein-conjugated antiglobulin obtained from Nordic Immunological Laboratories.

**Haemagglutination inhibition (HI) tests.** This followed the procedure of Clarke & Casals (1958) using gander erythrocytes. Haemagglutinin was concentrated from infected Vero cell supernatant fluid by polyethylene glycol precipitation (6%) in the presence of sodium chloride (0-4 m).

**Haemolysis inhibition (HL1) tests.** These were initially performed as above, but after the erythrocytes had settled they were resuspended by vigorous agitation every 15 min for 2 h.
The erythrocytes were then sedimented by centrifugation at 1000 g for 3 min and lysis was detected visually or spectrophotometrically at 410 nm.

**Complement.** Fresh guinea-pig serum at a dilution of 1/20 served as complement source.

**Antiglobulins.** These were obtained from Miles Laboratories and used at 1/20 dilution.

**Protein A.** Purified protein A obtained from Pharmacia was dissolved in serum-free L15 medium at a concentration of 1 mg/ml and used at a final concentration of 300 μg/ml during virus–antibody reactions.

**Mouse inoculations.** Equal volumes of serial dilutions of virus and antisera were mixed at 37 °C for 1 h. Amounts of 0.02 ml were inoculated intracerebrally into groups of 10 randomized newborn mice (strain TO).

**Radioimmunoprecipitations (RIP).** Confluent monolayers of BHK cells were infected at an input multiplicity of 10 to 20 p.f.u./cell and incubated at 37 °C in Plaisner’s modified Eagle’s medium with 2% foetal calf serum. To label intracellular virus-specific proteins, the medium was replaced 6 h post-infection with HEPES-buffered Earle’s salts solution containing 2% dialysed foetal calf serum (HED). At 7 h post-infection this was in turn replaced with HED containing 10 μCi/ml [35S]methionine. After 10 min at 37 °C the cells were washed twice with ice-cold PBS and lysed in 200 mM-glycine, 50 mM-tris, 100 mM-NaCl, 1 mM-EDTA (GTNE) containing 1% Triton X-100. The cell extract was centrifuged at 20000 g for 2 min and stored frozen at −20 °C. Labelled virus was obtained from cells given [35S]methionine (10 μCi/ml) 3 to 6 h post-infection and then further incubated in maintenance medium, which was harvested 20 h post-infection. The virus was concentrated from clarified medium by centrifugation at 80000 g for 60 min, resuspended in a small volume of GTNE and stored at −20 °C.

Immediately before immunoprecipitation, cell extracts of virus suspensions were brought to 1% Triton X-100, 0.1% SDS and centrifuged at 12000 g for 2 min. Then, 25 to 150 μl samples of the supernatant were incubated with 2-5 μl amounts of monoclonal ascitic fluids (or suitable dilutions thereof) overnight at room temperature. To each incubation mixture 20 μl of a 50% (v/v) suspension of protein A coupled to Sepharose CL4B (Pharmacia) was added. After 60 min further incubation with occasional agitation, the beads were recovered by centrifugation (12000 g for 2 min), washed three times in GTNE containing 1% Triton X-100 and the precipitated proteins dissolved in 50 μl 2% SDS, 1% 2-mercaptoethanol, 15% glycerol, 0.05% bromophenol blue by heating at 100 °C for 2 min. Proteins were analysed on 8% polyacrylamide gels in the buffer system of Laemmli (1970) which were dried and autoradiographed or impregnated with sodium salicylate (Chamberlain, 1979) and fluorographed at −70 °C.

**RESULTS**

**Monoclonal antibodies**

The general characteristics of the immune fluids used are summarized in Table 1. Ouchterlony gel diffusions against specific antiglobulins produced precipitation only with anti-IgG2a. Both supernatant media from hybridoma cultures and ascitic fluids from Balb/c mice injected with clones 30.12 and 30.11 produced specific cytoplasmic immunofluorescence in acetone-fixed Sindbis virus-infected Vero cells. Early after infection (6 to 10 h) the majority of the infected cells were stained around the perinuclear region (Fig. 1). Later, as cytopathic changes became extensive, both monoclonal antibodies stained a larger zone around the nucleus extending to the cell plasma membrane. The test fluids also produced fluorescence on the surface of unfixed infected Vero cells.

**Radioimmunoprecipitation**

The specificity of the monoclonal antibodies was determined by immune precipitation of [35S]methionine-labelled cell extracts or partially purified virus preparations. As shown in Fig.
Fig. 1. Immunofluorescence of Sindbis virus-infected Vero cells 6 h post-infection, using anti-E1 monoclonal antibodies.

Table 1. Properties of immune ascitic fluids and hyperimmune serum

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Protein specificity by RIP</th>
<th>Ig class</th>
<th>IF titre (fixed cells)</th>
<th>IF titre (non-fixed cells)</th>
<th>HI titre</th>
<th>HLI titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.11</td>
<td>E1</td>
<td>IgG2a</td>
<td>40,000</td>
<td>25,000</td>
<td>10,240</td>
<td>NA†</td>
</tr>
<tr>
<td>30.12</td>
<td>E1</td>
<td>IgG2a</td>
<td>20,000</td>
<td>5,000</td>
<td>&lt; 20</td>
<td>640</td>
</tr>
<tr>
<td>Hyperimmune serum</td>
<td></td>
<td>ND</td>
<td>5000</td>
<td>5000</td>
<td>160</td>
<td>NA</td>
</tr>
</tbody>
</table>

* ND, Not done. † NA, Not applicable.

2 (a to d), a short pulse of radioactive precursor 7 h post-infection predominantly labelled the virus-specific proteins p120 (containing envelope protein sequences), PE2, E1 and C (the capsid protein). Of these, only E1 was precipitated by the monoclonal antibodies 30.11 and 30.12. The specificity of the reaction was demonstrated by the fact that another monoclonal antibody, 30.22 (A. C. Chanas et al., unpublished results), precipitated only capsid protein from infected cell extract (Fig. 2.d). These results were confirmed by immunoprecipitation of a disrupted virus preparation. Although the envelope proteins were not well separated, it is apparent that even mature E2 was not precipitated significantly by 30.11 or 30.12 (Fig. 2 f, g).

**HI and HLI tests**

HI activity was detected in both the monoclonal ascitic fluid 30.11 and the rabbit hyperimmune serum. None was detected with the monoclonal ascitic fluid 30.12, although inhibition of red cell lysis by this antibody was detectable up to a dilution of 1/640 (Table 1). No HLI activity was detected with monoclonal antibody 30.11 or rabbit hyperimmune serum since their inhibition of haemagglutination obscured any possible effect on haemolysis. Apart from standard HI tests in which 8 haemagglutination (HA) units were used, the activity of the monoclonal antibodies was also estimated using constant antibody-variable antigen dilutions. At a dilution of 1/1000 the monoclonal ascitic fluid 30.11 inhibited haemagglutination by more than 1064 HA units. Interference with the HI activity of this
Monoclonal antibodies to Sindbis

Fig. 2. Radioimmunoprecipitation of Sindbis virus-specific polypeptides by monoclonal antibodies. Labelling of cells or virus, immunoprecipitation and analysis by polyacrylamide gel electrophoresis were carried out as described in Methods. Tracks (a to d) are derived from infected cell extracts, and (e to g) from partially purified virus preparations (in a separate experiment). (a) Total infected cell extract; (b to d) polypeptides precipitated from it by monoclonal antibodies (b) 30.11, (c) 30.12 and (d) 30.22; (e) partially purified virus preparation; (f, g) polypeptides precipitated from it by (f) 30.11 and (g) 30.12.

antibody was not demonstrated by monoclonal antibody 30.12 when this was added at a similar dilution 1 h previously. In separate experiments using monoclonal ascitic fluid 30.12 only, the subsequent addition of anti-mouse globulin led to an inhibition of 32 HA units.

Plaque reduction neutralization on Vero cells

The two monoclonal antibodies and the hyperimmune rabbit antiserum against Sindbis virus were each tested for their ability to neutralize Sindbis virus infectivity in Vero cells (Fig. 3). The order of potency of the two reagents showing neutralization changed according to the dilution; the monoclonal antibody (30.11) was more effective than the hyperimmune antiserum when used at higher concentrations, but its potency declined more rapidly than the hyperimmune serum when diluted. Conversely, the hyperimmune serum initially had a lower activity than the monoclonal antibody but retained its effectiveness over a wider range of dilutions. The monoclonal antibody 30.12 did not neutralize at any dilution tested. The neutralizing activity of the monoclonal antibody 30.11 was also examined when mixed with an equal amount of monoclonal antibody 30.12. The characteristics of the neutralization reaction under these conditions resembled more closely those of the hyperimmune serum. An eightfold decrease in relative neutralization occurred at a dilution of 1/10, whereas a significant increase in neutralization occurred at a dilution of 1/100. In separate experiments, mouse or rabbit antiglobulin (as appropriate) was added to each of the antisera before inoculation of cells. This increased the neutralizing potential of monoclonal 30.11 and the hyperimmune serum. The addition of complement mediated neutralization of both monoclonal antibodies and hyperimmune serum (Table 2).
Dose-response neutralization curves obtained using the macrophage-like P388D1 cell line are shown in Fig. 4. These cells, unlike Vero cells, possess Fc receptors which can mediate enhancement of infectivity by antibody (Peiris & Porterfield, 1979; Peiris et al., 1981). Thus, there are two distinct components for each curve, neutralization of infectivity at the lower antiserum dilutions and enhancement of plaque formation at the higher dilutions. Comparison of data obtained with the two cell lines indicated that maximum virus titres and plaque reduction neutralization were higher in the P388D1 cells. All antibodies performed better in this cell line than in Vero cells and there was a synergistic effect between the mixture of the two monoclonal antibodies throughout the neutralizing range. A slight neutralizing activity was also shown by monoclonal antibody 30.12 alone. There was enhancement of plaque formation by each antibody at dilutions between 1/640 and 1/40,000. The broadest range of dilutions showing this effect was produced by the hyperimmune serum. Confirmation that this enhancement was Fc receptor-mediated, as shown by Peiris et al. (1981), was made in separate experiments by the addition of pure protein A at a final concentration of 300 μg/ml to the virus–antibody mixtures. This resulted in a substantial suppression of the enhancing effect (Table 3).
Monoelonal antibodies to Sindbis

Table 2. Neutralization of Sindbis virus and the effect of addition of antiglobulin or complement

<table>
<thead>
<tr>
<th>Reagent added to virus</th>
<th>Infectivity in Vero cells (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibody 30.11*</td>
<td>7 x 10^5</td>
</tr>
<tr>
<td>Monoclonal antibody 30.11 + anti-mouse serum</td>
<td>5 x 10^3</td>
</tr>
<tr>
<td>Monoclonal antibody 30.11 + complement</td>
<td>1 x 10^4</td>
</tr>
<tr>
<td>Monoclonal antibody 30.12*</td>
<td>6 x 10^7</td>
</tr>
<tr>
<td>Monoclonal antibody 30.12 + anti-mouse serum</td>
<td>4 x 10^7</td>
</tr>
<tr>
<td>Monoclonal antibody 30.12 + complement</td>
<td>5 x 10^4</td>
</tr>
<tr>
<td>Hyperimmune rabbit serum</td>
<td>4 x 10^4</td>
</tr>
<tr>
<td>Hyperimmune rabbit serum + anti-rabbit serum</td>
<td>2 x 10^2</td>
</tr>
<tr>
<td>Hyperimmune rabbit serum + complement</td>
<td>9 x 10^3</td>
</tr>
<tr>
<td>Control ascitic fluid*</td>
<td>7 x 10^7</td>
</tr>
<tr>
<td>Control ascitic fluid + anti-mouse serum</td>
<td>7 x 10^7</td>
</tr>
<tr>
<td>Control ascitic fluid + complement</td>
<td>4 x 10^7</td>
</tr>
<tr>
<td>Control ascitic fluid + anti-rabbit serum</td>
<td>5 x 10^7</td>
</tr>
</tbody>
</table>

* Ascitic fluid used at 1/100 dilution.

Table 3. Effect of protein A on enhancing antibody concentration

<table>
<thead>
<tr>
<th>Reagent added to virus</th>
<th>Infectivity in P388D1 cells (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibody 30.11*</td>
<td>9 x 10^6</td>
</tr>
<tr>
<td>Monoclonal antibody 30.11 + protein A</td>
<td>8 x 10^5</td>
</tr>
<tr>
<td>Monoclonal antibody 30.12*</td>
<td>2 x 10^6</td>
</tr>
<tr>
<td>Monoclonal antibody 30.12 + protein A</td>
<td>4 x 10^5</td>
</tr>
<tr>
<td>Hyperimmune rabbit serum*</td>
<td>1 x 10^7</td>
</tr>
<tr>
<td>Hyperimmune rabbit serum + protein A</td>
<td>8 x 10^5</td>
</tr>
<tr>
<td>Control ascitic fluid</td>
<td>2 x 10^5</td>
</tr>
<tr>
<td>Control ascitic fluid + protein A</td>
<td>2 x 10^5</td>
</tr>
</tbody>
</table>

* Used at 10^-3 dilution.

Plaque size reduction using Vero cells

In order to examine the effect of the antibodies on the terminal stages of infection, they were incorporated at different concentrations into the overlay medium of Vero cell monolayers infected with Sindbis virus 1 h previously. Plaque development was completely inhibited in the presence of either the hyperimmune serum or monoclonal antibody 30.11 at concentrations as low as 1/200 and 1/400 respectively. A small decrease in plaque development was also noted in the presence of monoclonal antibody 30.12 when this was used at final dilution of 1/50. No decrease was observed when either normal ascitic fluid or monoclonal ascitic fluid against the capsid protein of Sindbis virus was used.

Sindbis virus neutralization in newborn mice

Table 4 shows the average survival times of newborn mice inoculated intracerebrally with different combinations of virus and antibody. The antibodies were diluted 1/50 and incubated at 37 °C for 30 min with an equal volume of virus. These mixtures were inoculated into groups of 10 newborn mice. The monoclonal antibody 30.11 gave almost complete protection against challenge with Sindbis virus; the hyperimmune serum was also effective,
Table 4. Newborn mouse average survival time after intracerebral inoculation with virus–antibody mixtures

<table>
<thead>
<tr>
<th>Virus dose LD₅₀/0.02 ml</th>
<th>Control ascitic fluid</th>
<th>Monoclonal antibody ascitic fluid</th>
<th>Hyperimmune serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>10000</td>
<td>1.0†</td>
<td>2.7</td>
<td>14.8</td>
</tr>
<tr>
<td>1000</td>
<td>1.5</td>
<td>3.6</td>
<td>17.6</td>
</tr>
<tr>
<td>100</td>
<td>1.8</td>
<td>2.0</td>
<td>17.2</td>
</tr>
</tbody>
</table>

* Ascitic fluids and hyperimmune serum were used at a dilution of 1/50.
† Average survival time in days.

but to a lesser degree. A slight but consistent prolongation in average mouse survival time was also conferred by monoclonal antibody 30.12.

**DISCUSSION**

Although the two monoclonal antibodies described here are both directed against the E1 glycoprotein of the virus envelope, marked differences in their biological properties are apparent. These can be used to extend results obtained with conventional immunological reagents. The neutralizing activity of the monoclonal antibody 30.11 demonstrates that antibodies against Sindbis virus E1 protein can be neutralizing in contrast to the findings of Dalrymple et al. (1976). The absence of neutralizing activity in antibody 30.12, however, shows that only antibodies to a subset of epitopes on the protein are capable of neutralization. Haemagglutination inhibition by antibody 30.11 is consistent with the HA activity of isolated E1 (Dalrymple et al., 1976), whereas the inhibition of haemolysis but not haemagglutination by antibody 30.12 discriminates between these two phenomena in a way impossible with conventional antisera. The haemolytic activity of the virus was clearly located on the E1 glycoprotein and was physically distinct from, although functionally dependent on, the haemagglutinating activity of the protein. This has been reported for the alphavirus of Western equine encephalitis in reconstitution experiments (Yamamoto et al., 1981). The detection of the E1 polypeptide, by indirect immunofluorescence, in the perinuclear region of the cytoplasm using the monoclonal antibodies is in accord with previous reports of the early intracellular location of alphavirus glycoproteins (Kääriäinen & Renkonen 1977; Pruslin & Scherer 1980).

Despite considerable advances in our understanding of antibody–virus–cell interactions many basic questions remain unanswered. It is now possible, however, to suggest that Fc receptor-mediated enhancement of virus infectivity in macrophage-like cells can be demonstrated by a variety of antibody specificities. These include neutralizing antibodies at subneutralizing dilutions, as well as non-neutralizing antibodies such as 30.12 and others (A. C. Chanas et al., unpublished observations) at certain concentrations. The broader enhancing response obtained with the hyperimmune serum indicates that different antibodies in the hyperimmune serum reach optimum proportions at different dilutions. That this was the result of an improvement in virus thermostability in the presence of some antibodies was considered unlikely. Virus infectivity in equivalent experiments using Vero cells (Fig. 3) never exceeded control levels and the enhancement effect could be substantially negated by pure protein A.

The lower neutralizing effectiveness of the hyperimmune serum at low dilution relative to monoclonal antibody 30.11 may be due to either steric hindrance whereby binding of neutralizing antibody is impeded by attachment of non-neutralizing antibodies at adjacent epitopes or lower affinity of the antibody population producing a neutralization curve with a
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slope of lower value. Alternatively, non-neutralizing antibodies may aggregate virus particles, making them inaccessible to neutralizing antibodies. The last alternative seems more attractive, as it provides an explanation for the better relative effectiveness of the hyperimmune serum at higher dilutions when aggregation would not be expected to occur. Under these conditions such attached antibodies may contribute, along with neutralizing antibodies, to the establishment of critical areas in the loss of virus infectivity (Della-Porta & Westaway, 1978). Inability to complete areas of sufficient size by the recruitment of non-neutralizing antibodies may be the reason for the sharp decline in the potency of the monoclonal antibody (30.11) in the dose-response experiments. The better overall effectiveness of the hyperimmune serum becomes apparent when the immunofluorescence and HI data are considered. These support the hypothesis that, despite substantially lower overall antibody levels, synergism between different antibodies improves neutralization at moderate and high dilutions. The slight hindrance exhibited at lower dilutions may be irrelevant in immunologically mature animals when complement or other effector systems are present.

The absence of mediated neutralization by antiglobulin in the case of the monoclonal antibody 30.12 may be attributed to the topological disposition and frequency of recurrence of the corresponding epitope. This may also be the reason for the consistently lower levels of enhancement of infectivity obtained with this antibody. That it may be due to an intrinsic property of the immunoglobulin class involved is unlikely as both monoclonal antibodies were of the same class. Although these results suggest that alphavirus neutralization and haemagglutination inhibition are analogous phenomena, they cannot be considered as identical. This was demonstrated by the involvement of antiglobulin in the HI but not neutralizing activity of this monoclonal antibody (30.12) and the ability of Semliki Forest virus monoclonal antibody to inhibit haemagglutination but not the infectivity of the same virus (A. C. Chanás et al., unpublished data). Moreover, cross-reacting flavivirus antisera do not correlate precisely in HI and neutralization tests (Halstead et al., 1980), and Dalrymple et al. (1976) demonstrated neutralization of Sindbis virus with anti-E2 but not anti-E1 monospecific sera.

The role of Sindbis virus haemolytic activity in virus pathogenesis is not understood. However, as anti-HL antibody does not neutralize infectivity, antibody against ‘haemolysin’ probably does not protect against the initiation of infection. This is not unexpected since it is known that non-haemolytic strains of Semliki Forest virus, which cause cell fusion, can exist (Väänänen & Kääriäinen, 1980). Thus, the haemolytic activity of Sindbis virus may be independent from fusion activity which is essential for the entry of the virus into the cytoplasm of the cell (Helenius et al., 1980). Nevertheless, reduction of plaque size and prolongation of mouse survival time by monoclonal antibody 30.12 may indicate involvement in virus pathogenesis either by aggregation of virus particles, which would effectively reduce the spread of infection, or by blocking or delaying virus release from the cell. The actions of different antibodies in the overlay of virus-infected cells is currently being investigated. Furthermore, the demonstration of enhancement of virus infectivity in P388D1 cells by monoclonal antibody 30.12 suggests that it might be active in vivo in assisting uptake of virus by macrophages. Despite the association of E1 with the lipoprotein envelope, the antigenicity of the ‘haemolysin’ part of E1 protein was not acetone-sensitive since identification of the antibody was originally made using acetone-fixed infected cells. However, we have preliminary evidence that delipidation of Sindbis virus with either the detergent Nonidet P40 or ether destroys the haemolytic activity without affecting the haemagglutinin (A. C. Chanás et al., unpublished results). Thus, the presence of lipid may be essential for Sindbis virus haemolytic activity.

The properties of the two monoclonal antibodies described here may represent extremes in
the spectrum of reactivities that can be demonstrated with antibodies to epitopes of the E1 glycoprotein. It is possible that other antibodies will demonstrate different patterns of independent and combined effects and that antibodies with intermediate reactivities may exist. We are currently producing more monoclonal antibodies to investigate this further.

We are grateful to Miss D. Newman for invaluable assistance throughout the course of this work and to Dr J. S. Porterfield for useful discussions and a culture of the P388D1 cells. The research at the Arbovirus Unit is supported by a grant by the Wellcome Trust.

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