Neutralizing Secretory IgA and IgG Do Not Inhibit Attachment of Transmissible Gastroenteritis Virus

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(Accepted 23 January 1986)

SUMMARY

Secretory IgA (sIgA) and IgG from porcine milk and serum, respectively, [3H]uridine-labelled virus, swine testis and pig kidney cell lines were used to examine the neutralized virus-cell interaction. Transmissible gastroenteritis virus (TGEV), 99.99% neutralized by immunoglobulin, was able to attach to the cells. Moreover, sIgA enhanced virus attachment. However, the neutralized virus was unable to enter cells, as demonstrated by the action of proteinase K which removed it from the cell surface. It was also found that pre-attached virus was still neutralizable and that IgG and sIgA had similar TGEV-neutralizing capacities.

Antibody binding may render a virus non-infectious and represents one of the major defence mechanisms against viral infections. The mechanism of virus neutralization is complex and general rules have not yet been established. The reaction depends greatly on the nature of each of the three participating elements, namely the virus, the antibody and the host cell in which the residual infectivity is tested. In many instances, it has been demonstrated that neutralizing antibodies alone act by impairing attachment, entry, uncoating or transcription of the virus (for reviews, see Dimmock, 1984; Lachmann, 1985).

Transmissible gastroenteritis virus (TGEV) of swine (a member of the family Coronaviridae) causing acute and severe diarrhoea in newborn piglets (Caletti et al., 1970) can be neutralized by anti-TGEV antibodies (Cartwright et al., 1965). It has been shown that passive immunity conferred by immune sow milk is the most important mechanism protecting suckling piglets against TGEV infection (Bohl et al., 1972). IgA was reported to be more effective than IgG (Saif & Bohl, 1981). Our intention was to examine the interaction between TGEV neutralized by immunoglobulins (A and G) and swine testis (ST) and pig kidney (RPD) cells with the aim of understanding better the protective mechanisms against TGEV infection.

For IgG and IgA purification, serum and milk from animals used in previous studies (Aynaud et al., 1985) were used. The purification of IgG by (NH₄)₂SO₄ precipitation and ion-exchange chromatography was carried out according to Corthier et al. (1984). IgA was purified as follows. Sow milk was skimmed by low-speed centrifugation (2500 g) for 10 min at 4 °C and casein was removed by pelleting at high speed (85000 g) for 1 h. After precipitation with a half-saturated ammonium sulphate solution, the IgA was separated by using a DEAE-Triacryl M column (LKB). The fractions eluted with 0·14 M-NaCl were collected, pooled and used as the IgA solution. Ig class characterization using ELISA was performed as described previously (Bernard & Lantier, 1985). The results of the analysis are shown in Table 1. To determine the mol. wt. of the Ig present in the solution, a 1·6 × 20 cm column was constructed with AcA 2.2 gel (LKB) in 0·035 M-Tris–HCl and 0·15 M-NaCl pH 8·3; it was calibrated with blue dextran 2000 (mol. wt. 2000000) thyroglobulin (mol. wt. 690000) and aldolase (mol. wt. 158000) (calibration kit from Pharmacia) at a flow rate of 0·2 ml/min. The IgA solution (0·2 ml) was loaded on the column, the eluted fractions were collected and the protein content (monitored at A₂₈₀) and virus-
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**Fig. 1.** Characterization of IgA by AcA 2.2 gel chromatography. The protein content (●) and neutralizing capacity (histogram) of the eluted fractions were determined by A280 and seroneutralization tests, respectively. Note that the fraction of maximal neutralizing capacity had a mol. wt. of about 400 000 which suggests that the IgA was dimeric.

**Table 1.** Characteristics of slgA and IgG purified from immunized sow milk and serum, respectively

<table>
<thead>
<tr>
<th>Immunoglobulin solution</th>
<th>Total immunoglobulin*</th>
<th>Anti-TGEV immunoglobulin*</th>
<th>Neutralizing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. (mg/ml)</td>
<td>Impurities (µg/ml)</td>
<td>Conc. (µg/ml)</td>
</tr>
<tr>
<td>slgA</td>
<td>5.6</td>
<td>IgG : 1.25</td>
<td>11</td>
</tr>
<tr>
<td>IgG</td>
<td>5.7</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

* Determined by ELISA.
† Inverse dilution neutralizing 100 TCID₅₀ of TGEV in ST cells.
‡ Virus-neutralizing titre of 1 µg of immunoglobulin.

neutralizing capacity were determined. Fig. 1 shows that the maximal neutralizing capacity coincided with the fraction which contained an Ig with a mol. wt. of about 400 000. An immunodiffusion (Ouchterlony) test was conducted to determine the presence of secretory component (SC) in the IgA solution; using anti-SC antibodies (kindly provided by Dr J. Bourne, Bristol, U.K.) a single precipitation line was observed (data not shown). All these results indicated that the neutralizing antibody in the solution was slgA.

The TGEV strain Purdue-115 and the ST and RPD cell lines have been described before (Aynaud et al., 1985), as has the production of the virus. For radiolabelled virus preparation, [5-³H]Juridine (Amersham, sp. act. 26 Ci/mmol) was added to the minimum essential medium (MEM) used for production of virus, at a final concentration of 0.2 µCi/ml. The clarified virus suspension was centrifuged at 85 000 g for 4 h at 4 °C in an R30 rotor (Beckman). The pellet was resuspended in MEM by vortexing and diluted to obtain a titre of about 1 × 10⁸ p.f.u./ml, corresponding to 10⁶ c.p.m./ml. The radioactivity of the labelled virus was measured using ACS scintillation fluid (Amersham) in an LKB liquid scintillation counter.

Equal volumes of Ig (A or G) solutions and virus suspension were mixed. Following incubation for 1 h at 37 °C, the residual infectivity of these mixtures was determined by plaque assay (Aynaud et al., 1985). Mock virus neutralizations using non-immune Ig (from serum and
Table 2. Neutralizing efficacy of slgA and IgG solutions*

<table>
<thead>
<tr>
<th>Immunoglobulin solution</th>
<th>Residual titre of virus (p.f.u./ml)</th>
<th>Neutralizing efficacy $[(a-b)/a \times 100]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mock-neutralized (a)</td>
<td>Neutralized (b)</td>
</tr>
<tr>
<td>slgA</td>
<td>$1 \times 10^8$</td>
<td>$1.6 \times 10^2$</td>
</tr>
<tr>
<td>IgG</td>
<td>$1 \times 10^8$</td>
<td>$0.8 \times 10^2$</td>
</tr>
</tbody>
</table>

* Equal volumes of slgA or IgG solution and virus suspension were mixed. Following incubation for 1 h at 37 °C, the residual virus infectivity in the mixture was titrated.

To determine whether virus could be neutralized after it had attached to the cell surface, monolayers of cells in six-well plates (Costar) were incubated with TGEV (about 100 p.f.u./well) for 1 h at 4 °C, washed with MEM and incubated for another hour with the Ig (A or G) solutions (about 200 units/well, one unit being able to neutralize 100 TCID$_{50}$ of TGEV). After a second rinse with MEM, the cells were covered with agar overlay medium and incubated at 37 °C for plaque formation (Aynaud et al., 1985). This experiment showed that when immune slgA or IgG was added no plaques were observed; there was plaque formation (mean values 54 and 40 plaques/well in ST and RPD cells, respectively, for a similar inoculum) when non-immune IgA or IgG was used. These results suggested that the pre-attached virus was still neutralizable.

The interaction between neutralized virus and cells was studied further. Equal volumes of virus suspension and Ig (A or G) solution were mixed and incubated for 1 h at 37 °C resulting in more than 99.99% of the virus being neutralized. A volume of 0.2 ml of this Ig–TGEV complex was inoculated onto monolayers (ST or RPD cells) grown in 24-well plates (Costar). Mock-neutralized virus was used as a control. The reaction was allowed to proceed for 1 h at 37 °C. The cells were then washed three times with MEM. Following addition of MEM (0.5 ml/well) the cells were again incubated for 1 h at 37 °C to permit immune complex penetration. In order to remove the unpenetrated virus, proteinase K (Boehringer) at a concentration of 2 µg/ml in MEM was used (200 µl/well). After incubation with the enzyme for 45 min at 4 °C and two rinses with MEM, the cells were solubilized with 0.1 N-NaOH and neutralized with 1 N-HCl. The entire contents of each well were transferred to a scintillation vial and the radioactivity was measured (Fig. 2a). The IgG-neutralized, mock-neutralized virus and virus alone were found to attach equally well to the cells as indicated by similar amounts of cell-associated radioactivity, suggesting that neutralizing IgG had no effect on virus attachment. However, when slgA was used, the cell-associated radioactivity was increased (910 compared to 310 c.p.m./well containing mock-neutralized virus). After proteinase K treatment residual intracellular radioactivity was unchanged when mock-neutralized virus was used whereas it was reduced by 90% in the preparations containing neutralizing immunoglobulins. This observation suggests that slgA as well as IgG impaired penetration of TGEV into the cells, thereby rendering the virus susceptible to proteinase K action.

In order to clarify the reasons for the increased cell-associated radioactivity in the presence of slgA, Ig solutions (0.2 ml at different concentrations) were incubated for 1 h at 37 °C with monolayers in 24-well plates (Costar). After three washings with MEM, the cells were incubated with virus. Titrations of the supernatants showed the same titres whether the virus was incubated with untreated or with Ig-treated cells. This observation was supported by the determination of neutralizing activity of Ig solutions before and after incubation with the cells (Fig. 3), which suggests that the TGEV-neutralizing Ig did not attach to the cells tested.

Dimeric IgA has been reported to impair the attachment of viruses (Mandel, 1967a, b; Dimmock et al., 1984; Taylor & Dimmock, 1985) as well as that of other infectious agents such as pathogenic Escherichia coli (Porter et al., 1978). A similar action of IgG has been described by Lee et al. (1981) in studies of the function of the reovirus σ1 protein. Apart from these
observations, neutralization of viral activity by IgG has been found to be due to inhibitory actions on every step in the replication cycle with the exception of the first one, i.e. virus attachment (for review, see Dimmock, 1984). In the present study, IgG did not inhibit virus attachment to the cells and slgA even stimulated it. Since we found no evidence of slgA and IgG binding to the cell, virus attachment in the presence of the immunoglobulins cannot be explained by attachment of an Ig–virus complex to Fc receptor-bearing cells, as has been demonstrated in the case of flavivirus (Kliks & Halstead, 1980; Brandt et al., 1982). The enhanced attachment was decreased as a function of slgA concentration and disappeared when slgA, having a virus neutralization titre of $6 \times 10^4$ per ml, was diluted 64-fold or more, i.e. at low concentrations (Fig. 2). The likely explanation is that virus particles and slgA molecules, in equivalent proportions, are linked to each other to form a complex, such that the attachment of one virus particle will carry along with it many more virions. Also, neutralized virus probably attaches by direct virus–cell interaction. Other possibilities exist, such as Ig having binding activity only after having combined with the virus; alternatively the Ig–virus complex may attach to a cellular site other than that specific for virus alone as has been described for vesicular stomatitis virus (Schlegel & Wade, 1983). However, with fixed amounts of virus and cells, virus attachment would either be Ig concentration-independent or enhanced by Ig at low concentrations in an arithmetically proportional manner due to additional attachment of Ig–virus complex to another cellular site.

We have found that (i) pre-attached virus was still neutralizable and (ii) TGEV-neutralizing antibodies did not inhibit virus attachment. These results suggest that the virus neutralization is independent of virus attachment. What is interesting is that the neutralized virus was unable to enter the cells. Only a few reports are available to explain the inhibition by immunoglobulins of virus penetration into cytoplasm. The cell system used to test the residual infectivity may play a very important role (Appel et al., 1984; Boere et al., 1985). The mechanism by which the virus enters the cytoplasm might also indicate whether or not the virus is neutralized by antibodies (Volkman & Goldsmith, 1985).

Although slgA differed from IgG in forming complexes with TGEV, both were found to have a similar capacity to neutralize viral infectivity, which confirms the observations of Bohl et al. (1972) and Saif & Bohl (1981). According to these authors, the differences in protection against TGEV between the two Ig classes rely on their presence in the mucosal membrane (Bourne, 1977; Newby & Stock, 1984) rather than on their activity per se.
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REFERENCES


(Received 20 August 1985)