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Expression of swine transmissible gastroenteritis virus envelope antigens on the surface of infected cells: epitopes externally exposed

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(Accepted 26 March 1990)

Summary

The peplomer protein (S) and the transmembrane protein (M) of transmissible gastroenteritis virus (TGEV) of swine were identified by iodination and serologically on the surface of infected cells. Of a total of 4 monoclonal antibodies (mAb) directed against four antigenic sites of S protein (Correa et al., 1988), 3 specific for sites A, B and D attached to the plasma membrane of infected cells, as disclosed by indirect immunofluorescence and by complement-mediated cytolysis. Four of the mAbs assayed were specific for the viral protein M and two of them gave plasma membrane immunofluorescence and mediated cytolysis in the presence of complement. The viral nucleoprotein N could not be demonstrated on the surface of infected cells either by iodination or employing 3 mAbs against this protein. Finally, a time course infection experiment demonstrated that S and M proteins were expressed on the surface of infected cells at 4 h after infection, before infective virus was released from infected cells.
Porcine transmissible gastroenteritis virus, a coronavirus, is highly infectious and causes mild or inapparent infections in adults but severe diarrhoeal disease in newborn or infant animals (Bohl, 1975; Siddell et al., 1983).

Three major structural proteins have been described for transmissible gastroenteritis virus (TGEV), two glycoproteins (S and M) and one phosphorylated nucleoprotein (N) (Garwes et al., 1975, 1979). The large complex protein S forms the characteristic surface projections responsible for attachment to cells, induction of neutralizing antibodies and cell fusion (Garwes et al., 1979; Holmes et al., 1981). M glycoprotein may also be important in neutralization, especially if complement is part of the virus-antibody reaction (Woods et al., 1987). Previous reports (Laude et al., 1986; Welch and Saif, 1988) have demonstrated the presence of S protein on the surface of infected cells by immunofluorescence, while the fluorescence pattern with anti-M mAbs was not clearly identified.

The antigenic structure of S glycoprotein has been characterized. Four antigenic sites (A, B, C and D) were defined by competitive radioimmunoassay using monoclonal antibodies (mAb) (Correa et al., 1988). Most of the neutralizing mAbs were specific for site A, which was one of the antigenically dominant determinants (Correa et al., 1988).

The purpose of the present study was to determine which of the TGEV-induced proteins becomes expressed on plasma membranes of infected cells, and which of the antigenic sites of these proteins are exposed. Each of these antigens can be postulated to play a role in the induction of alpha interferon (Charley and Laude, 1988), or to mediate lysis of infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC), natural killer activity (NK) (Cepica and Derbyshire, 1983), or complement-mediated cytotoxicity.

Three procedures were employed for analyzing the proteins exposed on the plasma membranes of swine testicle (ST) cells infected with TGEV. In the first, ST cells were infected with the Purdue strain (PUR 54-C1P1) of TGEV (Jimenez et al., 1986). The infected cells were labeled at various times after infection with Na$^{125}$I as previously described (Alcaraz et al., 1989). After 15 min at 4°C, the reaction was terminated by the addition of 4 mg of 2-mercaptoethanol/mg of chloramine T. Cells were washed with phosphate-buffered saline and lysed by adding lysis buffer (50 mM Tris-HCl pH 7.5, 5 mM disodium EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate) in the presence of 1 mM phenylmethylsulfonyl fluoride. Immunoprecipitates of labeled cell extracts, with serum from a sow immunized with the virulent TGEV strain MAD 88 (Laviada et al., 1988; Sanchez et al., 1989), were analyzed by SDS-PAGE electrophoresis (Escribano and Tabarts, 1987). The kinetics of TGEV protein synthesis in infected cells were determined in parallel using $^{35}$S-methionine labeling (100 μCi/ml) (Laviada et al., 1988).

In the second procedure, a panel of mAbs (Table 1) were used in an indirect immunofluorescence assay, using TGEV-infected cells (m.o.i. 0.3) at 14 h, after infection. For localizing viral antigens on the surface of infected cells, the cells were not fixed prior to adding the immune reagent as the first step in the procedure of indirect immunofluorescence. The mAb immunocomplexes were developed by ad-
TABLE 1

Properties of monoclonal antibodies used in recognition of viral epitopes exposed on the surface of infected cells

| mAbs     | Specificity | Ig isotype | Titer in RIA $^c$ | Titer in neutralization $^d$ | Antigen recognition on cell surface $^e$ |
|----------|-------------|------------|-------------------|-----------------------------|----------------------------------------|
| 6AC3     | S (A) $^a$  | IgG1       | $10^4$            | 4.5                         | +                                      |
| 1DB12    | S (B)       | IgG1       | $10^5$            | 0.8                         | +                                      |
| 6AA6     | S (C)       | IgG1       | $10^3$            | 0.3                         | -                                      |
| 1DG3     | S (D)       | IgG1       | $10^4$            | 0.6                         | +                                      |
| 3BD3     | M           | IgG1       | $10^4$            | 0.3                         | -                                      |
| 3BB3     | M           | IgG1       | $10^5$            | 0.3                         | +                                      |
| 9DB4     | M           | ND         | ND                | ND                          | +                                      |
| 3DE3     | M           | IgG2b      | $10^5$            | 0.3                         | -                                      |
| 3DH10    | N           | IgG1       | $10^4$            | 0.3                         | -                                      |
| 3DC10    | N           | IgG1       | $10^4$            | 0.3                         | -                                      |
| 3BB5     | N           | IgG1       | $10^4$            | 0.3                         | -                                      |

$^a$ A, B, C and D correspond to different antigenic sites of S glycoprotein, determined previously by competitive RIA by Correa et al., 1988.

$^b$ ND, not determined.

$^c$ The mAb titers were determined by RIA on hybridoma culture supernatants.

$^d$ The neutralization index was determined by dividing the number of PFU of virus per milliliter mixed with normal medium by the number of PFU of virus per milliliter in the presence of a mAb and complement, and is expressed as the log$_{10}$ of this ratio.

$^e$ Disclosed by the indirect immunofluorescence procedure.

TABLE 2

Lysis of TGEV-infected ST cells by complement-mediated cytotoxicity with monoclonal antibodies, sera and colostra specific for TGE virus $^a$

| Specific $^{51}$Cr release (%) $^b$ | Serum $^c$ of sow number | Colostrum $^d$ of sow number |
|-------------------------------------|---------------------------|-----------------------------|
| Monoclonal antibody specificity     | M protein                |                            |
| S protein                           | 3BB3 9DB4                | 31 33                       |
| 6AC3 1DB12 6AA6 1DG3 3BD3 3BB3 9DB4 | 17.8 23.4 0.7 17.1 16.6 13.1 | 23.9 22.9 30.8 28.5 |

$^a$ TGEV-infected cells were incubated with the antibody. After this incubation, heat inactivated rabbit anti-mouse serum was added at 1:30 final dilution, and then, rabbit serum diluted 1:32 was added as source of complement.

$^b$ The specific $^{51}$Cr release was defined in Materials and Methods. The results shown are the medium values of three experiments. The standard deviation was less than 20% in all cases.

$^c$ Pig serum from sows numbered 31 and 33, infected with the MAD 88 strain of TGEV at days 83 and 104 of pregnancy, collected one day after farrowing.

$^d$ Colostrum from the same sows collected the day of farrowing.
could play a role in the lysis of infected cells. Briefly, virus-infected and $^{51}$Cr-labeled target cells were incubated with the mAbs, and heat-inactivated rabbit anti-mouse serum was added at 1:30 final dilution. Rabbit serum diluted 1:32 was added as a source of complement, and incubated for 30 min at 37°C, after which the quantity of released radioactive label was measured with a γ-ray scintillation counter. The specific release was calculated with the formula

$$\frac{100 \times (a - b)}{(c - b)} - \frac{100 \times (d - b)}{(c - b)}$$

where a equals release from infected cells in the presence of immune reagents; b, spontaneous release; c, maximum release determined after treatment of the cells with detergent; and d, release from uninfected cells in the presence of immune reagents (Zeller et al., 1988).

![Fig. 1. Identification of TGEV-induced proteins on plasma membranes of infected cells by labeling with $^{125}$I-chloramine T. (A) Kinetics of synthesis of TGEV-induced proteins in infected ST cells at different hours post infection (0–8 hpi) and labeled with $^{35}$S-methionine in parallel to iodination; (B) Pattern of proteins labeled with $^{125}$I-chloramine T, as described in Materials and Methods, at different hours post infection (0–8 hpi); (C) Immunoprecipitates of $^{125}$I-labeled infected ST cell extracts at different hours post infection (0–8 hpi) with an immune serum; (D) Immunoprecipitate of $^{35}$S-methionine infected ST cell extract at 8 h post infection with the same immune serum.](image)
Protein labeling with Na$^{125}$I showed that proteins S and M were exposed on the surface of infected cells (Fig. 1B). Both proteins were specifically immunoprecipitated by a hyperimmune serum after $^{125}$I-labeling (Fig. 1C), and both were detected since 4 h after infection at the same time that these proteins were detected by $^{35}$S-methionine labeling (Fig. 1A). The kinetics of protein expression on the plasma membranes was corroborated by a time course experiment using an indirect immunofluorescence assay employing the mAbs 6AC3 and 3BB3 directed against S and M proteins, respectively (Fig. 2). After parallel titration of infected cell supernatants at the same hours after infection virus release was not detected before 7 h after infection (data not shown).

As disclosed by the indirect immunofluorescence procedure using four mAbs, representative of the antigenic sites A, B, C and D of the S protein (Correa et al., 1988), only sites A, B and D were accessible on the surface of the infected cells (Fig. 3, panel A). These results are in agreement with the presence of the antigenic sites A, B and D on the surface of native virus, while site C is only exposed when the virus is slightly denatured by binding to plastic surfaces (I. Correa and L. Enjuanes, to be published). When four mAbs specific for different epitopes of protein M were used in an indirect immunofluorescence assay, only the mAbs 3BB3 and 9DB4 gave a positive reaction (Fig. 3, panel B). None of the three N protein specific mAbs gave a positive immunofluorescence pattern when assayed on the unfixed infected cells (Fig. 3, panel C).

All the mAbs that reacted with the surface of infected cells were able to mediate complement-mediated cytotoxicity (Table 2). In contrast, mAbs such as 6AA6, specific for a non-exposed epitope of the S protein, did not induce this reaction.

The above results demonstrated the localization of S and M proteins on the plasma membrane of TGEV-infected cells, as well as the antigenic sites of the S protein.
protein and some epitopes of the M protein that are accessible on the surface of infected cells. Charley and Laude (1988) were able to induce high alpha interferon production using epithelial cells infected with the TGEV and fixed by glutaraldehyde. Two mAbs directed against the M protein inhibited the interferon-inducing capacity of both TGEV-infected cells and viral particles. The authors suggested that interferon induction by TGEV resulted from interactions between an outer membrane domain of the M protein and the peripheral blood mononuclear cell membranes. Some of the identified protein epitopes, as exposed on the surface of infected cells, could play a key role in the induction of alpha interferon.

According to its predicted amino acid sequence, the TGEV M glycoprotein is mainly buried in the viral lipid membrane (Laude et al., 1987). However, the amino terminus of the TGEV M protein extends 54 amino acids from the virion envelope which compares with only 28 for bovine coronavirus (BCV), 26 for MHV, and 21 for avian infectious bronchitis coronavirus (IBV) (Kapke et al., 1988). Eleven of the sixteen amino-terminal amino acids are hydrophobic and the positions of charged amino acids around this sequence suggest that the first 16 amino acids comprise a potentially cleavable signal peptide for membrane insertion. A similar sequence is not found in the M proteins of BCV, MHV, or IBV. Because of this modification in the amino-terminal hydrophobic sequence of the TGEV M protein it has been suggested that this protein may behave differently from its MHV, BCV or IBV counterparts with regard to intracellular trafficking (Kapke et al., 1988). These findings could explain the insertion of TGEV M protein into the plasma membrane of infected cells, in contrast to the M protein of MHV that migrates to the Golgi apparatus, but is not transported to the plasma membrane (Sturman and Holmes, 1983). Further experiments in morphogenesis and M protein migration of TGEV must be done to clarify this phenomenon.

Acknowledgement

This work was supported by a grant from the Instituto Nacional de Investigaciones Agrarias, Madrid, Spain. We thank M. Sevilla and J. Tomillo for expert technical assistance.

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Fig. 3. Plasma membrane immunofluorescence patterns of mAbs directed against S, M and N proteins. Unfixed TGEV-infected ST cell monolayers were used in an indirect immunofluorescence assay at 14 h post infection. Panel A: Patterns of mAbs 6AC3, 1DB12, 6AA6 and 1DG3, representative of the antigenic sites A, B, C and D of the S protein respectively. Panel B: Patterns of mAbs 3BD3, 3BB3, 9DB4 and 3DE3 directed against M protein. Panel C: Patterns of mAbs 3DH10, 3DC10 and 3BB5 directed against N protein.
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(Received 27 November 1989; revision received 26 March 1990)