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Short Communication

Fixed-cell immunoperoxidase technique for the study of surface antigens induced by the coronavirus of transmissible gastroenteritis (TGEV)

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ABSTRACT

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An immunoperoxidase technique performed on the TGEV-infected cells was developed for detection of virus-induced antigens. The presence of M antigen of TGEV on the surface of infected cells was demonstrated by this technique. This finding is in contrast to the M protein of murine hepatitis coronavirus which migrates to the Golgi apparatus but is not transported to the plasma membrane. The time course of appearance M and S antigens on the surface of TGEV-infected cell can be studied by this technique.

INTRODUCTION

Three major structural proteins have been described for all coronaviruses, two glycoproteins (S and M) and one phosphorylated nucleoprotein (N) (for review, see Sturman, 1981; Garwes, 1982; Holmes et al., 1984; Laude et al., 1990). For mouse hepatitis virus (MHV), a well studied coronavirus, the M protein migrates to the Golgi apparatus, but is not transported to the plasma membrane as readily as is the S protein (Sturman and Holmes, 1983).

For porcine TGEV, the presence of the virus envelope S antigen on the surface of infected cells was demonstrated by immunofluorescence (Laude et al., 1986), while the presence of M antigen on the plasma membrane has only been suspected by unspecified monoclonal antibodies (mAbs) (Welch and Saif, 1988).

Recently, the expression of the TGEV envelope M protein on the surface of infected cells has been demonstrated by isotope labelling (Laviada et al., 1990). There has not been any published report concerning the presence of N antigen on the plasma membrane of infected cells.

We describe in the present study a more convenient immunoperoxidase test (IPT) for the confirmation of the location of TGEV M antigen at the cell surface. Moreover, this test, performed on 0.1% paraformaldehyde-fixed, TGEV-infected cells can be used not only for detecting but also for quantifying the expression of two viral M and S glycoproteins on the plasma membrane.

MATERIALS AND METHODS

Virus, cells and monoclonal antibodies

The swine testis (ST) cell line and Purdue-115 virus strain which have been described elsewhere (Laude et al., 1981) and three mAbs, anti-S (51–13), anti-M (25–22) (Delmas et al., 1986) and anti-N (22–6) (Laude et al., 1986) were used in this study.

Infection of cells

Confluent monolayers of ST cells in 96-well, flat-bottomed plastic plates (Falcon 3072, Becton Dickinson) were inoculated with 0.1 ml of virus suspension containing a multiplicity of infection (m.o.i) of either 2, 10 or 100. The uninfected cells served as controls. After 30 min of incubation at 37°C under 5.5% CO_2, the inoculum in each well was removed from the cell monolayer by two washes with Dulbecco's phosphate buffered saline (DPBS) and was replaced with 0.1 ml of Eagle's minimum essential medium containing 5% heat-inactivated normal calf serum. The infected cells were then incubated at 37°C under 5.5% CO_2 for 18 hours unless otherwise specified.

Fixation of cells with paraformaldehyde for detection of virus induced-antigens on surface

Paraformaldehyde (PFA) powder (Prolabo-France) was dissolved in DPBS by heating at 80°C and used as a fixative for the detection of virus-induced antigens on the surface of TGEV-infected cells. The desired concentration (0.1%) of the fixative was prepared by dilution in DPBS. The fixative solution was freshly made just before use for each experiment. The monolayers were carefully washed twice with DPBS and the cells were fixed with the appropriate fixative at 4°C for 30 min and then saturated with 5% skimmed milk in PBS without calcium and magnesium for 15 min at room temperature.

Fixation of cells with 80% acetone for detection of virus-induced antigens in cytoplasm

For the detection of virus-induced antigens in cytoplasm, the infected cells, after incubation for virus replication, were washed as for surface antigens and

then fixed with 80% acetone at −20°C for 30 min. After fixation the cells were washed and then saturated as mentioned above.

Detection of viral antigens by an immunoperoxidase test (IPT)

The surface or cytoplasmic antigens induced by the virus were detected by an IPT as follows: the monolayers were overlaid with 100 µl of mAb at working dilution for 90 min at 4°C. The reagents were removed from the plates with two rinses with tap-water and two washes with PBS containing 0.05% Tween 20 (Serva) and were then replaced with 0.1 ml per well of an optimal dilution of peroxidase-labelled goat anti-mouse Fc serum (ICN Immunobiologicals, Israel). After a further 90 min of incubation at 4°C, the plates were washed as before and the enzymic reaction was developed by incubation at 37°C for 1 hour with 2,2′-azino-bis(3-ethyl benzthiazoline-6 sulfonic acid) [ABTS, (Boehringer Mannheim)]/H_2O_2 substrate solution. The supernatant was transferred to another plate containing 0.02 ml of sodium dodecyl sulfate (SDS) to stop the enzymic reaction and to permit the reading of the plate. The peroxidase was quantified by measuring the OD at 415 nm with Titertek Multiscan (Flow Laboratories, Irvine, Scotland, UK). Each antigen quantity, tested in quadruplicate, was expressed as the difference between the OD at 415 nm of virus- and mock-infected cells using the formula: OD at a given timepoint = [(OD of virus-infected cells − OD background of virus-infected cells) − (OD of mock-infected cells − OD background of mock-infected cells)].

RESULTS

Expression of representative cytoplasmic N and surface M and S antigens was measured by an IPT performed on fixed cells which were infected with 2, 10 or 100 m.o.i. The results are shown in Fig. 1. These antigens were detected

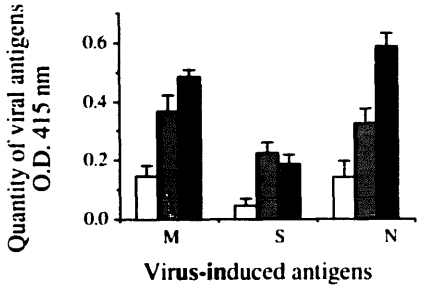

Fig. 1. Expression of representative cytoplasmic N and surface M and S antigens on swine testis (ST) cells. Cells were infected with 2 (open symbol), 10 (shaded symbol) or 100 (solid symbol) m.o.i. The detection of virus-induced antigens was carried out at 10 h post-infection by immunoperoxidase test (IPT) performed on infected cells fixed with 80% acetone for cytoplasmic antigen (N) or with 0.1% paraformaldehyde for surface antigens (M and S).

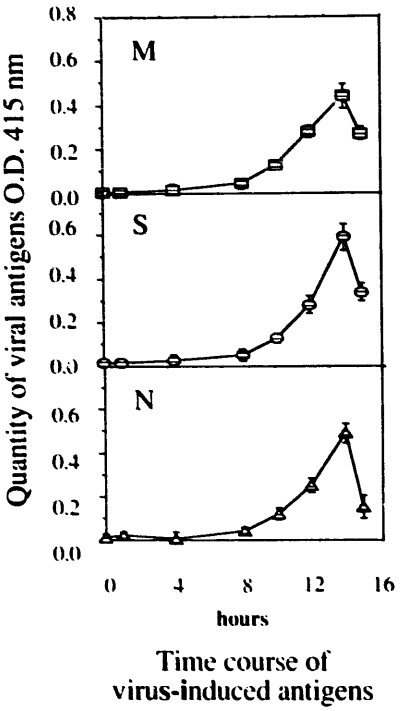

Fig. 2. Kinetic expression of virus-induced antigens. ST cells were infected with 2 m.o.i. and incubated for 18 hours. At indicated time, cells were fixed with either 80% acetone or 0.1% paraformaldehyde and detection of N (△), M (□) and S (○) antigens was carried out by IPT.

randomly at 10 h post-infection. It is clear that the quantity of antigens detected varied non-linearly with the m.o.i used.

To understand better the expression of virus-induced antigens in TGEV-infected cells over a period of time, a comparative study of kinetic expression was carried out in ST cells infected with 2 m.o.i. Cells were incubated at 37°C under 5.5% CO_2 for 18 h. The representative results are shown in Fig. 2. The expression of M, S and N antigens appeared in multimodal patterns with peaks at 14, 16 and 18 h post-infection. When cells were infected with 10 m.o.i., the curve became hyperbolic until 12 h post-infection for surface antigens and 14 h post-infection for cytoplasmic antigens (data not shown).

DISCUSSION

Most authors use immunofluorescent technique on living cells (Ishimoto, 1969; Hahon, 1970; Ito et al., 1972; Hahon et al., 1972) or on fixed cells (Collins et al., 1982; Laude et al., 1986; Welch and Saif, 1988) for the identification and study of virus-specific surface antigens in infected cells. Similarly, other techniques such as the immune adherence test (Tachibana and Klein, 1970 and O'Neill, 1968), radioimmunoassay (Yewdell et al., 1981; Laviada et al., 1990), scanning electron microscopy (Pringle and Pareny,

1982) and antibody-dependent-cell-mediated cytotoxicity (Charley et al., 1983) have been mentioned. These techniques are generally laborious, time-consuming and require specialized equipment.

In recent years the fixed-cell ELISA system has been applied widely to virological and immunological investigations. This method is frequently used to demonstrate surface antigens unless endogenous peroxidases of the cells under study prevents its application (Epstein and Lunney, 1985; Nibbering et al., 1990). Therefore, we developed a more convenient immunoperoxidase technique to study the expression of virus-induced antigens on the plasma membrane of TGEV-infected cells. It is clear that the presence of M and S antigens (Fig. 1) can be demonstrated on the plasma membrane of TGEV-infected cells, fixed with 0.1% PFA; while the presence of N antigen can not (data not shown). In contrast, the N (Fig. 1), M and S (data not shown) antigens could be easily detected by IPT in the cytoplasm of TGEV-infected cells which were fixed with 80% acetone. However, the results depend on the number of cells being infected. In other words, for the comparative study, the synchronization of cells plays an important role in estimation of the results. It is clear that the antigen quantity detected depends upon number of the cells infected rather than upon the antigen quantity expressed by a single cell. The curve over a period of time appeared in hyperbolic form with the peak at 14 h post-infection (Fig. 2). Under standardized conditions of cell number, m.o.i., and incubation time, the results obtained are easily reproducible.

The described IPT performed on 0.1% PFA-fixed cells appeared to be a reliable and useful technique for studying the expression of virus-induced antigens on the membranes of infected cells. Furthermore, in comparison with the immunofluorescent technique (data not shown), IPT is less tiring and easily automated and gives similar results for the comparative study of surface antigens expressed in cells infected with different TGEV strains (data not shown). Also, this technique performed on unfixed cells can be alternatively used in tests like ADCC, lymphocyte cytotoxicity (LCT), etc., to study surface antigens and can be applied to explore the presentation of the surface TGE viral antigens to the immunocompetent cells.

For S antigen, its presence on the surface of TGEV-infected cells has been demonstrated by immunofluorescence in the previously published reports (Laude et al., 1986; Welch and Saif, 1988) while the presence of the M antigen on the plasma membrane has only been suspected by four unspecified mAbs (Welch and Saif, 1988). What is interesting in our study is that not only S but also M antigens could be found, by an IPT, present on the surface of TGEV-infected cells. This result confirmed the recent findings of Laviada et al. (1990) on the presence of S and M proteins of TGEV on the surface of infected cells by isotope labelling. The MAb directed against TGEV M antigen (25–22) used in our study was postulated to play a key role in alpha interferon induction in a previous report (Charley and Laude, 1988). These

authors suggested that interferon induction by TGEV resulted from interaction between the peripheral blood mononuclear cell membrane and an outer membrane domain of the M protein by the fact that anti-M mAbs 25–22 and 49–22 could block alpha interferon induction by infectious or inactivated virions. However, the presence of TGEV M antigen on the surface of infected cells was in contrast to the location of the M protein of MHV, a well studied coronavirus. The MHV M protein migrates to the Golgi apparatus, but is not transported to the plasma membrane as readily as is the S protein (Sturman and Holmes, 1983). Transmission electron micrographs presented in a previous report (Holmes et al., 1981) on MHV maturation showed virions in the lumen of the rough endoplasmic reticulum, in smooth walled vesicles and adsorbed in large number to the plasma membrane by the tips of the peplomers in the 17 Cl 1 cells 24 h after infection. The question still remains whether the TGEV M protein is virion-associated M or this antigen itself is inserted into the plasma membrane. Our results (Fig. 2) suggested that the TGEV M protein would insert into the plasma membrane as we could detected this antigen at 4 h after infection, long before infectious virus was released from infected cells by plaque assay (data not shown). Moreover, the possible insertion of M protein into the plasma membrane could be explained by its predicted amino acid sequence. According to this, although the TGEV M glycoprotein is mainly buried in the viral lipid membrane (Laude et al., 1987) but is might protrude through the lipid membrane with a short (around 30 residues) amino-terminal domain (Charley and Laude, 1988). For TGEV, the insertion of M protein into the plasma membrane is more likely as its amino terminus 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 16 amino-terminal amino acids are hydrophobic and the positions of charged amino acids around this sequence suggest that the first sixteen amino acids comprise a potentially cleavable signal peptide for membrane insertion. A similar sequence is not found in the M protein of BCV, MHV, or IBV. This finding suggested that TGEV M protein may behave differently from its BCV, MHV or IBV counterparts with regard to intracellular trafficking (Kapke et al., 1988). Studies on morphogenesis and M protein migration of TGEV should be done to clarify this in vitro phenomenon. Beside this, researches on in vivo expression of TGEV-induced antigens in intestinal cells should be carried out to understand their role in immune response of swine against TGEV infection.

REFERENCES

Charley, B. and Laude, H., 1988. Induction of alpha interferon by transmissible gastroenteritis coronavirus: role of transmembrane glycoprotein E1. J. Virol., 62: 8–11.

Charley, B., Petit, E., Laude, H. and La Bonnardière, C., 1983. Myxovirus and coronavirus induces in vitro stimulation of spontaneous cell-mediated cytotoxicity by porcine blood leukocytes. Ann. Inst. Pasteur/Virol., 134E: 119–126.

Collins, A.R., Knobler, R.L., Powell, H. and Buchmeier, M.J., 1982. Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell–cell fusion. Virology, 119: 358–371.

Delmas, B., Gelfi, J. and Laude, H., 1986. Antigenic structure of transmissible gastroenteritis virus: II. Domains in the peplomer glycoprotein. J. Gen. Virol., 67: 1405–1418.

Epstein, S.L. and Lunney, J.K., 1985. A cell surface ELISA in the mouse using only Poly-L-Lysine as cell fixative. J. Immunol. Methods, 76: 63–72.

Garwes, D.J., 1982. Coronaviruses in animals. In: D. Tyrell and A. Kapikian (Editors), Virus infection of the gastrointestinal tract. Marcel Dekker, New York. Basel., 315–360.

Hahon, N., 1970. Cell surface antigen induced by Venezuelan equine encephalomyelitis virus. Infect. Immun., 2: 713–715.

Hahon, N. and Eckert, H.L., 1972. Cell surface antigen induced by influenza virus. Infect. Immun., 6: 730–737.

Holmes, K.V., Doller, E.W. and Behnke, J.N., 1981. Analysis of the function of coronavirus glycoproteins by differential inhibition of synthesis with tunicamycin. In: V. ter Meulen, S. Siddell and H. Wege (Editors), Biochemistry and Biology of Coronavirus. Plenum, pp. 133–142.

Holmes, K.V., Frana, M.F., Robbins, S.G. and Sturman, L.S., 1984. Coronavirus maturation. In: P.J.M. Rottier, B.A.M. Van der Zeijst, S.J. Spaan and M. Horzinek (Editors), Molecular Biology and Pathogenesis in Coronaviruses. Plenum. New York, pp. 37–52.

Ishimoto, A. and Ito, Y., 1969. Specific surface antigen in Shope papilloma cells. Virology, 32: 595–597.

Ito, M. and Barron, A.L., 1972. Surface antigen produced by herpes simplex virus (HSV). J. Immunol., 108: 711–718.

Kapke, P.A., Tung, F.Y.T., Hogue, B.G., Brian, D.A., Woods, R.D. and Wesley, R., 1988. The amino-terminal signal peptide on the porcine transmissible gastroenteritis coronavirus matrix protein is not an absolute requirement for membrane translocation and glycosylation. Virology, 165: 367–376.

Laude, H., Gelfi, J. and Aynaud, J.M., 1981. In vitro properties of low and high passaged strains of transmissible gastroenteritis coronavirus of swine. Am. J. Vet. Res., 42: 447–449.

Laude, H., Chapsal, J.M., Gelfi, J., Labiau, S. and Grosclaude, J., 1986. Antigenic structure of transmissible gastroenteritis virus: I Properties of MAb directed against virion proteins. J. Gen. Virol., 67: 119–130.

Laude, H., Rasschaert, D. and Huet, J.C., 1987. Sequence and N-terminal processing of the transmembrane protein E1 of the coronavirus transmissible gastroenteritis virus. J. Gen. Virol., 68: 1687–1693.

Laude, H., Rasschaert, D., Delmas, B., Godet, M., Gelfi, J. and Charley, B., 1990. Molecular biology of transmissible gastroenteritis virus. Vet. Microbiol., 23: 147–154.

Laviada, M.D., Videgain, S.P., Moreno, L., Alonso, F., Enjuanes, L. and Escribano, J.M., 1990. Expression of swine transmissible gastroenteritis virus envelope antigens on the surface of infected cells: epitopes externally exposed. Virus Res., 16: 247–254.

Nibbering, P.H., Van de Gavel, J.S. and Van Furth, R., 1990. A cell-ELISA for the quantification of adherent murine macrophages and the surface expression of antigens. J. Immunol. Methods, 131: 25–32.

O'Neill, C.H., 1968. An association between viral transformation and Forssman antigen detected by immune adherence in culture BHK 21 cell. J. Cell. Sci., 3: 405.

Pringle, C.R. and Pareny, J.E., 1982. Measurement of surface antigen by specific bacterial ad-

herence and scanning electron microscopy (SABA/SEM) in cells infected by vesiculovirus ts mutants. J. Gen. Virol., 59: 207–211.

Sturman, L.S., 1981. The Structure and Behavior of Coronavirus A59 Glycoproteins. In: V. ter Meulen, S. Siddell and H. Wege (Editors), Biochemistry and Biology of Coronavirus. Plenum pp. 1–17.

Sturman, L.S. and Holmes, K.V., 1983. The molecular biology of coronaviruses. J. Gen. Virol., 101: 221–235.

Tachibana, T. and Klein, E., 1970. Detection of cell surface antigen on monolayer cells. Immunology, 19: 771–782.

Welch, S.K.W. and Saif, L.J., 1988. Monoclonal antibodies to a virulent strain of transmissible gastroenteritis virus: comparison of reactivity with virulent and attenuated virus. Arch. Virol., 101: 221–235.

Yewdell, J.W., Frank, E. and Gerhard, W., 1981. Expression of influenza A virus internal antigens on the surface of infected P815 cells. J. Immunol., 126: 1814–1819.