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Effect of fixation on the detection of transmissible gastroenteritis coronavirus antigens by the fixed-cell immunoperoxidase technique

Long-Thành Tô^a and Serge Bernard^b

^a National Institute of Veterinary Research, Department of Virology, Hanoi, Viet Nam, and ^b INRA, Laboratoire de Pathologie Infectieuse et Immunologie, 37380 Nouzilly, France

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The effect of various fixatives and detergents on the *in vitro* detection of the viral determinants which are expressed in swine testis cells infected with the transmissible gastroenteritis coronavirus (TGEV) was studied using a microwell immunoperoxidase technique. When compared with glutaraldehyde and formaldehyde, 0.1% paraformaldehyde was found to be the fixative of choice for the detection of these determinants on the membranes of infected cells. Among dehydrating fixatives, 80% acetone or a mixture of acetone and ethanol or of acetone, methanol and ethanol were found to be the best fixatives for the detection of these viral determinants which are expressed in infected cells. In the case of acetone, the temperature of fixation and its concentration in the fixative preparation were found to be important. The treatment of 0.05% glutaraldehyde-fixed, infected cells with 0.1% saponin or 0.1% paraformaldehyde-fixed, infected cells with 1% NP-40 led to satisfactory detection of viral determinants. Using Triton X-100 to render cells permeable, the quantities of N and M antigen detected in TGEV-infected cells prefixed with either 0.05% glutaraldehyde or 0.1% paraformaldehyde were equal to those of 80% acetone-fixed, TGEV-infected cells while the quantity of S antigen detected was diminished. The effect of other detergents such as zwittergen, emdigen BB, Chaps and *N*-lauroylsarcosine on the detection of viral determinants was also studied.

Key words: Viral determinant detection; Transmissible gastroenteritis coronavirus; Fixative; Immunoperoxidase

Introduction

Transmissible gastroenteritis (TGE) is a highly contagious enteric infection of swine caused by transmissible gastroenteritis coronavirus (TGEV) (Woode, 1969). The TGE virion consists of three

major structural proteins: the nucleocapsid (N), which is associated with the RNA genome; the peplomer glycoprotein (S) and the transmembrane glycoprotein (M) (Laude et al., 1990).

In recent years there has been increasing interest in the development and application of the fixed-cell ELISA system for virological and immunological investigations. For TGE infections, this technique using infected cells as a source of antigen has been used to detect total anti-TGEV

Correspondence to: S. Bernard, INRA, Laboratoire de Pathologie Infectieuse et Immunologie, 37380 Nouzilly, France.

antibodies (Kodama et al., 1980a) or IgA antibodies (Kodama et al., 1980b) in the serum of infected swine. For other viruses, it has been used for screening hybridomas (Russell et al., 1983), for diagnosis (Pauli et al., 1984; Grom and Bernard, 1985; Van Tiel et al., 1988) and for identification of epitopes on enveloped or naked viruses (Anderson et al., 1986; Boere et al., 1986; Oosterlaken et al., 1989; Simkins et al., 1989). Recently, we described a quantitative fixed-cell immunoperoxidase technique for the study of surface viral antigens induced by TGEV (Tô et al., 1991).

As the name of the technique implies, many fixatives have been used by different authors to fix infected cells to the plastic plates. However, there have not been any published reports concerning the effect of fixatives on the detection of viral antigens in infected cells by this technique.

In the present study, we have established the effect of various fixatives and detergents on the detection of TGEV determinants in infected cells, by means of MAbs or PABs in a quantitative fixed-cell immunoperoxidase technique (IPT).

Materials and methods

Virus, cells and infection of cells

The high-passaged Purdue-115 strain of TGEV (Bohl et al., 1972) and the swine testis (ST) cell line (McClurkin and Norman, 1966), supplied by Dr. E.H. Bohl (Wooster, OH, USA) were used in this study. Eagle's minimal essential medium (EMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 IU/ml) and streptomycin (100 µg/ml) was used for cell growth.

Confluent monolayers of 2.5×10^5 cells/cm² in 96-well, flat-bottomed plastic plates (Falcon 3072, Becton Dickinson) were inoculated with a volume of 0.1 ml/well of virus suspension at a multiplicity of infection (m.o.i) of 1. After 30 min of incubation at 37°C under 5.5% CO₂, the inoculum in each well was removed, washed twice with phosphate buffered saline (PBS) and replaced with 0.1 ml/well of EMEM containing 5% heat-inactivated FCS. The infected cells were incubated at 37°C under 5.5% CO₂ for an additional

15 h. The uninfected, TGEV-infected cells served as controls.

Monoclonal and polyclonal antibodies

The MAbs were directed against M (25–22), S (51–13) and N (22–6) determinants of TGEV and had been prepared and used as ascitic fluids following injection of BALB/c mice with the antibody-producing hybridomas (Laude et al., 1986). The anti-TGEV PABs (53–12) obtained from the serum of a sow orally immunized with vaccine containing the Nouzilly strain of TGEV (Aynaud et al., 1985), and having a neutralizing titer of 1:65,000, were used for comparative purposes.

Fixation of infected cells by aldehyde fixatives

Fixatives Paraformaldehyde (PFA) powder (Pro-labo-France) was dissolved in PBS by heating at 80°C. Formaldehyde (FA) solutions were prepared from a 35% stock solution (RPL, Leuven, Belgium). Glutaraldehyde (GA) solutions were prepared from a 25% stock solution (Serva, 23114, research grade). All fixative solutions prepared by dilution in PBS were freshly made just before each experiment.

Fixation procedure The TGEV-infected cells were gently washed twice with PBS and the cells were fixed with the appropriate concentration of different fixatives at 4°C for 30 min (0.2 ml per well). After washing twice with PBS, 0.1 ml/well of blocking solution (5% skimmed milk in PBS) was added to each well and the plates were incubated for 15 min at room temperature (RT).

Fixation of infected cells by dehydrating fixatives

Fixatives or mixtures of fixatives The fixatives tested were acetone (RPL, Leuven, Belgium) at different concentrations, absolute methanol (Pro-labo, France), absolute ethanol (Gruppo Montedison, Milan) and other chemical mixtures containing fixatives, e.g., 6 vols. of ethanol and 4 vols. of ether (RPL, Leuven, Belgium) (Ternynck and Avrameas, 1987), 3 vols. of ethanol and 1 vol. of acetic acid (RPL, Leuven, Belgium) (Ternynck and Avrameas, 1987), 66% acetone and 34% methanol; 66% acetone, 34% methanol plus 0.75% FA (Pauli et al., 1984), 60% acetone, 25% ethanol and 15% methanol (Pauli et al., 1984),

70% acetone and 30% ethanol (Laude et al., 1986).

Fixation procedure The TGEV-infected cells were gently washed twice with PBS and the cells were fixed at -20°C for 30 min with the appropriate fixative (0.2 ml per well) which was previously frozen at -20°C unless otherwise stated. The fixed cells were washed twice with PBS before addition of 0.1 ml/well of blocking solution and incubation for 15 min at RT.

Treatment of aldehyde-fixed cells with permeabilizing agents

Detergents The detergents tested were Triton X-100 (Touzart & Matignon, France) at different concentrations, saponin (Touzart & Matignon, France) at different concentrations, 1% NP-40 (Sigma), 1% emipgen BB (*N*-dodecyl-*N,N*-dimethylglycine) (Calbiochem, Boehringer), Chaps (Calbiochem, Boehringer) at different concentrations, 10 mM *N*-lauroylsarcosine (Sigma), 1% zwittergent 3-12 and 1% zwittergent 3-16 (Calbiochem). The desired concentrations of these detergents were prepared by dilution in PBS.

Treatment procedure The TGEV-infected cells were first fixed with aldehyde fixative (either 0.05% GA or 0.1% PFA) at 4°C for 30 min. The cells were washed twice with PBS and then incubated with detergent at appropriate concentration (0.2 ml per well) for 15 min at RT. After this permeabilization step, the cells were washed twice with PBS and incubated with 0.1 ml/well of blocking solution for 15 min at RT.

Quantification of antibody binding to viral determinants in fixed cells by immunoperoxidase technique

An IPT previously developed for the detection of surface viral antigens induced by the Purdue-115 strain in infected ST cells (Tô et al., 1991) was used. Briefly, the fixed cells were washed gently with PBS and were incubated for 90 min at 4°C with 0.1 ml/well of a predetermined dilution of anti-M, anti-S, anti-N MAb or anti-TGEV PABs. The reagents were removed from the plates by two rinses with tap-water and two washes with PBS containing 0.05% Tween 20 (Serva) and then replaced by 0.1 ml/well of a working dilution of peroxidase-labelled goat anti-mouse Fc serum (ICN ImmunoBiologicals, Israel) or peroxidase-

labelled rabbit anti-swine (heavy and light chain specific) serum (Cappel, Organon Technica, France). After incubation at 4°C for 90 min, the plates were washed as before and the enzymatic reaction was developed by incubation at 37°C for 1 h with 2,2'-azino-bis (3-ethyl benzthiazoline-6 sulphonic acid) (ABTS (Boehringer, Mannheim, Germany))/ H_2O_2 chromogen/substrate solution. The supernatants were transferred to another plate containing 0.02 ml of 10% sodium dodecyl sulphate (SDS) (Serva) to stop the enzymic reaction and to permit the reading of the plate. The optical density (OD) was measured at 415 nm by the ELISA reader (Titertek Multiskan) coupled to a microcomputer for data storage and statistical processing.

The OD signals obtained from the IPT are composed of three components: (a) a specific OD signal generated by the second-step conjugate specifically bound to the antigen-bound monoclonal antibody; (b) non-specific OD generated by non-specifically bound second-step conjugate; and (c) nonspecific spontaneous OD due to other reasons, e.g., spontaneous decay of the substrate or the intrinsic OD of the cells. The first component is the one of interest. The other two contribute non-specifically to the OD signal and therefore should be corrected in order to determine the specific signal. The quantity of each antigen, tested in quadruplicate, was expressed as the difference between the OD at 415 nm of virus-infected and uninfected cells using the formula:

$$\text{Specific OD} = (\text{OD of virus-infected cells} - \text{OD background of virus-infected cells}) - (\text{OD of uninfected cells} - \text{OD background of uninfected cells})$$

For comparative purposes the degree of relative antibody binding to a specified determinant was expressed as a percentage of antibody binding to unfixed infected cells.

Results

Effects of aldehyde fixation on antibody binding to viral determinants

The effects of cell fixation with FA, GA and PFA at various concentrations on antibody bind-

ing to viral determinants in the TGEV-infected cells are shown in Fig. 1. Antibody binding to the N determinant was close to zero when cells were fixed with all these three aldehyde fixatives. Fixation with FA at different concentrations led to irregular patterns for antibody binding to M and S determinants (Fig. 1a). FA at high concentrations caused a decrease of antibody binding to S determinant but an increase of the M determinant up to 175% of controls at a FA concentration of 0.5%. The levels of antibody binding to M and S determinants were very low using fixation with GA at different concentrations (Fig. 1b). The M and S determinants were best detected in

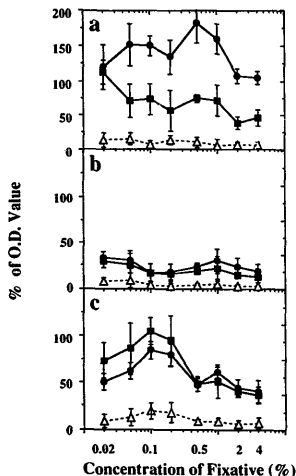


Fig. 1. The comparative effect of aldehyde fixation on antibody binding to viral determinants. Optical density data obtained with TGEV-infected cells fixed with various concentrations of formaldehyde (a), glutaraldehyde (b) and paraformaldehyde (c) and incubated with MABs having specificity for M (25–22) (●), S (51–13) (■) and N (22–6) (△) determinants; compared with the optical density obtained with unfixed, TGEV-infected cells. The OD of unfixed, mock-infected cells (2.5×10^5 cells) was 0.064 ± 0.017 for M (25–22) determinant; 0.066 ± 0.028 for S (51–13) determinant and 0.075 ± 0.011 for N (22–6) determinant.

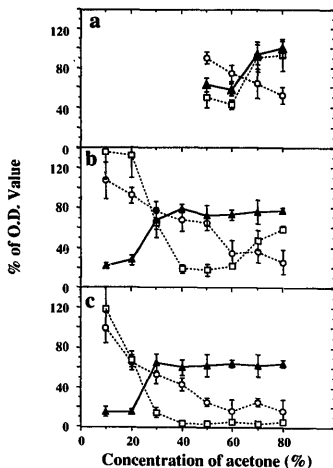


Fig. 2. The effect of cell fixation with various concentrations of acetone at different temperatures on antibody binding to viral determinants. Optical density data obtained with TGEV-infected cells fixed with acetone at -20°C (a), at $+4^\circ\text{C}$ (b) and at room temperature (c) and incubated with MABs having specificity for M (25–22) (●), S (51–13) (○) and N (22–6) (△) determinants. The degree of antibody binding to M (25–22) and S (51–13) determinants were expressed as percentages compared with the value obtained with 0.1% paraformaldehyde-fixed, TGEV-infected cells. The degree of antibody binding to the N (22–6) determinant was expressed as a percentage compared with that obtained with 80% acetone-fixed, TGEV-infected cells.

infected cells which were fixed with 0.1% PFA and antibody binding profiles for these two determinants agreed well (Fig. 1c).

It is clear that, among the aldehydes tested, the M and S determinants were readily detected in TGEV-infected cells which were fixed with 0.1% PFA.

Effect of acetone fixation on antibody binding to viral determinants

The effects of cell fixation on antibody binding to viral determinants in the TGEV-infected cells and at different temperatures are shown in Fig. 2.

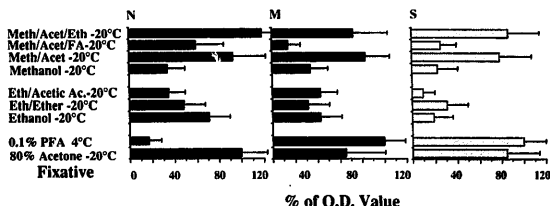


Fig. 3. The comparative effect of cell fixation with various dehydrating fixatives or with the fixative mixture on antibody binding to viral determinants. The degree of antibody binding to the N (22–6) determinant was expressed as the optical density value obtained with TGEV-infected cells which were fixed with the indicated fixative preparation and incubated with the anti-N (22–6) determinant MAb (block 1), relative to the optical density obtained with 80% acetone-fixed TGEV-infected cells. The degree of antibody binding to the M (25–22) and S (51–13) determinants was expressed as the optical density value of cells which were fixed with the indicated fixative preparation, incubated with the anti-M (25–22) determinant (block 2), or anti-S (51–13) determinant (block 3) MAb, relative to the optical densities of 0.1% paraformaldehyde-fixed, TGEV-infected cells. Abbreviations: methanol (Meth), ethanol (Eth), acetone (Acet), acetic acid (acetic Ac), formaldehyde (FA), paraformaldehyde (PFA).

Antibody binding to M determinant was decreased whereas binding to S and N determinants increased in cells fixed with increasing acetone concentrations at -20°C (Fig. 2a). When cell fixation occurred at 4°C , an increase in acetone concentration led to poor detection of M and S determinants although the latter could be detected in cells which were fixed with 70% or 80% acetone (Fig. 2b). In contrast, the antibody binding to the N (22–6) determinant reached a plateau

at 40%. The antibody binding profiles to the M, S and N determinants in cells fixed with acetone at RT showed similarities with the profiles obtained in cell fixation at 4°C although their OD values were lower (Fig. 2c).

In conclusion, M and S determinants were not well detected when cell fixation occurred at 4°C and at RT while the N determinant was well detected in cells fixed with high acetone concentrations regardless of the fixation temperature.

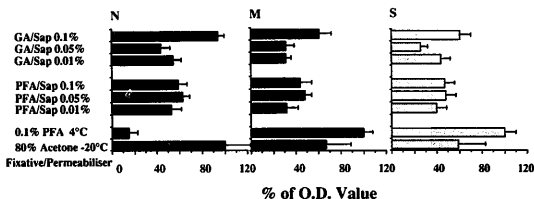


Fig. 4. The effect of treating cells with 0.05% glutaraldehyde or 0.1% paraformaldehyde and then various concentrations of saponin on antibody binding to viral determinants. The degree of antibody binding to the N (22–6) determinant was expressed as the optical density value obtained with TGEV-infected cells prefixed with the above-mentioned fixative preparation, treated with saponin to permeate the cell membrane and then incubated with anti-N (22–6) MAb (block 1), relative to the optical density obtained with 80% acetone-fixed, TGEV-infected cells. The degree of antibody binding to the M (25–22) and S (51–13) determinants was expressed as the optical density value obtained with TGEV-infected cells prefixed with above-mentioned fixative preparation, treated with saponin and then incubated with MABs having specificity for the M (25–22) (block 2) and S (51–13) determinants (block 3) relative to the optical densities of 0.1% paraformaldehyde-fixed, TGEV-infected cells.

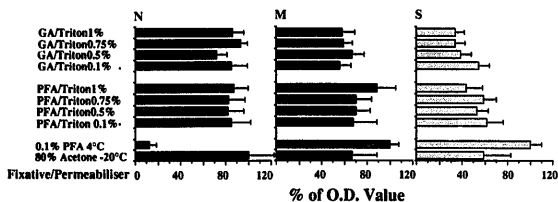


Fig. 5. The comparative effect of treating cells with 0.05% glutaraldehyde or 0.1% paraformaldehyde and then with various concentrations of Triton X-100 on antibody binding to viral determinants. The degree of antibody binding to N (the 22-6) determinant was expressed as the optical density value obtained with TGEV-infected cells prefixed with the above-mentioned fixative preparation, treated with Triton X-100 to permeate the cell membrane and then incubated with a MAb having specificity for the N (22-6) determinant (block 1), relative to the optical density obtained with 80% acetone-fixed, TGEV-infected cells. The degree of antibody binding to the M (25-22) and S (51-13) determinants was expressed as the optical density value obtained with TGEV-infected cells prefixed with above-mentioned fixative preparation, treated with Triton X-100 and then incubated with MAbs having specificity for the M (25-22) determinant (block 2) and S (51-13) determinants (block 3) relative to the optical densities of 0.1% paraformaldehyde-fixed, TGEV-infected cells.

Comparative effect of dehydrating fixatives on antibody binding to viral determinants

As shown in Fig. 3, the degree of antibody binding to M, S and N determinants in methanol/acetone/ethanol- and methanol/acetone-fixed cells showed similarities with the results obtained in 80% acetone-fixed cells. The extent of antibody binding to these determinants in

methanol/acetone/FA-, absolute methanol-, ethanol/acetic acid-, ethanol/ether-, absolute ethanol-fixed cells was lower than that detected in 80% acetone-fixed cells. It can be noted that antibody binding to the M and S determinants in 0.1% PFA-fixed, TGEV-infected cells was greater than those of cells fixed with any of these dehydrating fixatives.

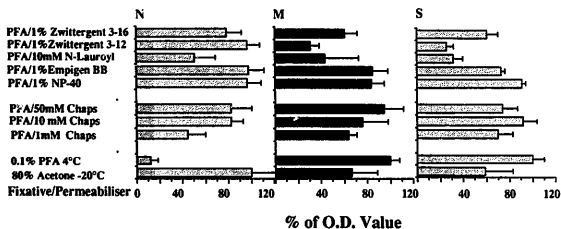


Fig. 6. The comparative effect of treating cells with 0.1% paraformaldehyde and then with different detergents on antibody binding to the viral determinants. The degree of antibody binding to the N (22-6) determinant was expressed as the optical density value obtained with TGEV-infected cells prefixed with above-mentioned fixative preparation, treated with indicated detergent to permeate the cell membrane and then incubated with a MAb having specificity for the N (22-6) determinant (block 1), relative to the optical density obtained with 80% acetone-fixed, TGEV-infected cells. The degree of antibody binding to the M (25-22) and S (51-13) determinants was expressed as the optical density value obtained with TGEV-infected cells prefixed with above-mentioned fixative preparation, treated with detergent and then incubated with MAbs having specificity for the M (25-22) (block 2) and S (51-13) determinants (block 3) relative to the optical densities of 0.1% paraformaldehyde-fixed, TGEV-infected cells.

Effect of cell treatment with detergent on antibody binding to viral determinants

The degrees of antibody binding to viral determinants in cells which were fixed with the aldehyde fixative, e.g., 0.05% GA or 0.1% PFA and then permeated with different concentrations of saponin are shown in Fig. 4. The degree of antibody binding to the M, S and N determinants in 0.05% GA-fixed cells which were permeated with 0.1% saponin was similar to that obtained in 80% acetone-fixed cells. With the exception of the 0.1% PFA control the degree of antibody binding to all other samples was lower than that detected in 80% acetone-fixed cells.

When using Triton X-100 (Fig. 5), antibody binding to the M and N determinants in 0.05% GA- or 0.1% PFA-fixed cells which were then permeated with Triton X-100 at various concentrations showed similarities with the results obtained in 80% acetone-fixed cells. The degree of antibody binding to the S determinant in 0.05% GA-fixed cells which were then permeated with Triton X-100 at 1.0, 0.75 and 0.5% was lower than that obtained with 80% acetone-fixed, infected cells. The degree of antibody binding to the S determinant in 0.1% PFA-fixed cells which were permeated with different concentrations of Triton X-100 was similar to that detected in 80% acetone-fixed cells. The OD of cells incubated with anti-M MAb was equal to that obtained from 0.1% PFA-fixed, infected cells while the OD of cells incubated with anti-S was significantly lower than that of 0.1% PFA-fixed, infected cells.

For some other permeabilizing agents (Fig. 6), antibody binding to the N determinant in 0.1% PFA-fixed cells which were permeated with either 1% zwittergent, 1% empigen BB, 1% NP-40, 10 mM and 50 mM Chaps showed similarities with the results obtained in 80% acetone-fixed cells while antibody binding to this determinant in cells permeated with 10 mM *N*-lauroyl and 1 mM Chaps was lower than that of 80% acetone-fixed, infected cells. The degree of antibody binding to the S and M determinants in cells which were permeated with 1% zwittergent 3-12 or 10 mM *N*-lauroyl was lower than that of 80% acetone-fixed, infected cells. The degree of antibody binding to these determinants in cells permeated

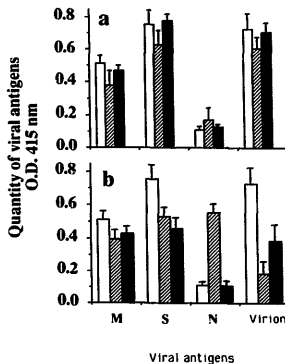


Fig. 7. Use of paraformaldehyde as a fixative for surface virus antigens (a) and acetone as fixative for cellular virus antigens (b) in the immunoperoxidase test. Comparison of the quantities of virus-induced antigens detected by this test performed on unfixed, TGEV-infected cells (open bars), on TGEV-infected cells fixed with fixative prior to their incubation (hatched bars) and on TGEV-infected cells fixed with fixative after their incubation (grey bars) with MAbs having specificity for the M (25-22) (block 1), S (51-13) (block 2) and N (22-6) determinants (block 3) and with PABs (block 4). The OD of unfixed, mock-infected cells (2.5×10^5 cells) was 0.051 ± 0.04 for E1, 0.053 ± 0.03 for E2 and 0.054 ± 0.002 for NP.

with 1% zwittergent 3-16, or 1% empigen BB, 1% NP-40 or Chaps was similar to that obtained in 80% acetone-fixed, infected cells.

Differences in localisation of antigens detected in infected cells according to aldehyde and dehydrating fixation

The following experiments were carried out to identify differences in localisation of antigens detected in infected cells which were fixed with either 0.1% PFA at 4°C (Fig. 7a) or with 80% acetone at -20°C (Fig. 7b) using two different fixation procedures. The degree of antibody binding to viral determinants in unfixed, infected control cells was compared with that measured in infected cells which were fixed (i) before their incubation with antibody or (ii) after their incubation with antibody. The antibodies used in this

experiment were MAbs and PABs as mentioned in the materials and methods section. Whichever PFA fixation procedure was used, the degree of antibody binding to the M, S, N determinants and to virions in TGEV-infected cells was not significantly different from that of controls. However, antibody binding to viral determinants varied between cells fixed with 80% acetone at -20°C and the control infected cells. The degree of antibody binding to the M determinant in cells fixed according to the two different fixation procedures was similar to that obtained in the controls. The degree of antibody binding to the S determinant was lower than that with the controls suggesting that after acetone fixation the accessibility of this determinant to the MAb was affected. The degree of antibody binding to the N determinant in cells fixed with 80% acetone after their incubation with MAb was equal to that of controls but considerably lower than that of infected cells which were fixed with 80% acetone prior to their incubation with MAb. The anti-TGEV polyclonal binding profiles to viral antigens were similar to those obtained with the M and S determinants.

Discussion

The present study has shown that GA fixation alters the accessibility of TGEV determinants to anti-M, anti-S and anti-N MAbs and that FA fixation has the opposite effect on the detection of M and S viral determinants compared with PFA fixation. In another study Smith et al. (1988) has shown that formalin fixation blocks, alters or destroys a specific antigen expressed in bovine viral disease virus (BVDV)-infected cells. Similar results were also obtained by Saunders (1977) for the detection of hog cholera virus antigen after GA fixation at different concentrations.

To investigate the influence of fixation temperature on viral antigen detection, the fixation step was carried out at -20°C , 4°C or at RT. The detectable quantity of M antigen decreased with an increase in acetone concentration at all three test temperatures. The increase in acetone concentration paralleled the increase of detectable N antigen. Our results suggest that the acetone concentration in fixative preparation and the fix-

ation temperature are two important criteria for a fixation procedure. Similar results were also obtained (Groen et al., 1989) by comparing the use of ethanol (-70°C) or acetone ($+20^{\circ}\text{C}$) fixation for the detection of Hantaan virus. In his study on the development and evaluation of an enzyme labelled antibody test for the rapid detection of hog cholera antibodies, Saunders (1977) showed that this test gave a good result only when the fixative solution contained less than 30% acetone.

It should be noted that 100% acetone is a routinely used fixative which gives excellent results in immunohistochemistry. In our experiments, cell fixation with a high concentration of acetone (80%) resulted in adequate detection of TGEV antigens. However, we could not use higher acetone concentrations to fix the cells because the microtiter plates would be distorted with acetone concentration higher than 80% or would melt with 90–100% acetone (personal observation). Therefore, we do not know if acetone concentrations higher than 80% would give the same results in fixed-cell immunoperoxidase.

A comparison of cell fixation with dehydrating fixatives and mixtures of these fixatives (Fig. 3) suggested that the highest quantity of antigen detected was in TGEV-infected cells which were fixed with either 80% acetone or mixtures of act/meth or act/meth/eth. It is possible that adequate detection of TGEV antigens in act/meth- and act/meth/eth-fixed cells is due to the acetone in the fixative preparations since viral antigens are not so readily detected in infected cells which are fixed with methanol or ethanol alone. Similarly, comparisons of cell treatment with permeabilizing agents (Figs. 4, 5 and 6) lead to the conclusion that the TGEV antigens were readily detected in PFA-fixed cells which were then permeated with 1% NP-40 and in GA-fixed cells which were then permeated with 0.1% saponin.

Several authors have compared the effects of aldehyde fixative and dehydrating fixative in the fixed-cell ELISA. Cannon (1987) found that both methanol containing 0.5% H_2O_2 and 4% FA were suitable fixatives for HEp-2 cells infected with respiratory syncytial virus (RSV), although methanol/ H_2O_2 was considered to be the fixative of choice. The author also found that where

a MAb specific for virus NP was used in IPT, staining was only seen following FA fixation but this was not the case for MAb specific for RSV envelope glycoproteins. The latter generally gave good results with either fixative. Similarly, FA fixation seemed to preserve Borna virus-specific antigens better than acetone/methanol or acetone fixation and the labelling was pronounced when using MAb (Pauli et al., 1984). Smith et al. (1988) also noted that immunoperoxidase staining of BVDV-infected cells fixed with formaldehyde was less intense in cells fixed in acetone.

Our results (Fig. 7) showed that only viral antigens which are present on the membrane of infected cells may be detected after aldehyde fixation and that the accessibility of these antigenic determinants to the appropriate antibodies was not changed after PFA fixation. However, both surface and cytoplasmic antigens were detected after acetone fixation. This is due to the fact that after PFA fixation the cell membrane was still intact but became permeable after acetone fixation. Thus MAbs also bind to cytoplasmic viral antigens (Tô et al. 1991). It seems that acetone fixation at -20°C had no effect on the accessibility of the N determinant to the MAb. The same results (data not shown) were also obtained in TGEV-infected cells which were fixed with either act/meth or act/meth/eth using two different fixation procedures. It would seem that the stronger the permeabilizing capacity of the fixative, the more N antigen but less M and S antigens were detected. Consequently, the interpretation of results comparing aldehyde and dehydrating fixatives should take this observation into consideration.

In conclusion 0.1% PFA appears to be a suitable fixative for the detection of TGEV antigens on the membrane of infected cells. For cellular viral antigens; 80% acetone, a mixture of act/meth or act/meth/eth are the fixatives of choice.

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