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DETECTION OF VIRAL ANTIGENS ON THE SURFACE OF CELLS INFECTED WITH JAPANESE ENCEPHALITIS VIRUS BY MODIFIED IMMUNOFLUORESCENT TECHNIQUE

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Summary. - A modified immunofluorescent method employing anti-Japanese encephalitis (JE) virus monoclonal (MoAbs) and polyclonal (immune PF) antibodies was evaluated for the detection of viral antigens expressed on the surface of porcine stable kidney cells infected with JE virus (733913, India). The infected cells showed granular immunofluorescence on the surface with both the MoAbs, Hs-1 and Hx-3 and immune PF 24 hr and 48 hr post virus infection. Interestingly, two strains of JE virus *viz.* Yoken (Japan) and 755468 (India) which did not react with the MoAb Hs-1 in the standard indirect fluorescent antibody (FA) technique, were positive for surface immunofluorescence (IF) with the same MoAb. Thus, the modified technique will be useful for the detection of more labile and conformational-dependent epitopes which might get lost or denatured by prior fixation of infected cells with acetone.

Key words: immunofluorescence; Japanese encephalitis virus; monoclonal antibodies; polyclonal antibodies; porcine stable kidney cells

JE virus is one of the mosquito-borne flaviviruses which often produces epidemics of viral encephalitis in South-East Asian and Western Pacific regions (Rodrigues, 1984). It is an enveloped RNA virus which multiplies in the cytoplasm of infected cells and is released probably by the budding process (White and Fenner, 1986). Recently, Mason (1989) showed that both the structural (E) and nonstructural proteins (NS-1 and NS-1') are released slowly into the culture medium from JE virus-infected Vero cells, suggesting that the viral antigens might be expressed on the cell surface prior to their release into the culture medium. We report a modified immunofluorescent technique employing anti-JE virus (E glycoprotein) MoAbs and immune PF for the detection of viral antigens expressed on the surface of JE virus infected porcine stable kidney (PS) cells.

PS cells were grown in Earle's minimum essential medium (MEM) containing 10 % goat serum, almost to confluency. They were infected with an Indian strain

of JE virus, 733913, belonging to the group I (strain retaining haemagglutination-inhibiting (HI) activity against the Hs group of MoAbs) as described earlier (Ghosh *et al.*, 1989; Gupta *et al.*, 1991). The cells were also infected with four strains of JE virus of group II which have lost HI activity against Hs group of MoAbs (Ghosh *et al.*, 1989). Two of these strains (Yoken and 755468) were earlier reported to be negative against the MoAb Hs-1 in the standard indirect FA (Ghosh *et al.*, 1989).

Surface IF (modified method). The culture medium from Leighton tubes was discarded after 24 hr and 48 hr of virus infection and the coverslips were washed once in cold medium containing 40 mmol/l sodium azide (MSA), without disturbing the cell monolayers. The cells were treated with anti-JE virus MoAbs Hs-1 (JE virus specific) or Hx-3 (flavivirus cross-reactive) (Kedarnath *et al.*, 1986) and immune PF, diluted 1:2 and 1:50, respectively, in MSA for 1 hr at 37 °C, followed by washing thrice with cold MSA. The coverslips were removed and fixed in chilled acetone for 30 min. The fixed cells were treated with FITC-conjugated rabbit anti-mouse gammaglobulins (Nordic Immunologicals) similarly as described earlier for the standard indirect FA (Ghosh *et al.*, 1989; Gupta *et al.*, 1991). The experiments on surface IF were also carried out with unfixed infected cells treated with antibody in the presence or absence of sodium azide at 4 °C. The results obtained with and without acetone-fixation of the cells treated with antibody, were also compared.

Standard indirect FA. The coverslips were removed after 24 hr and 48 hr of virus infection and were fixed in chilled acetone for 30 min. The fixed cells were subjected to indirect FA against undiluted MoAbs Hs-1 and Hx-3 and diluted immune PF (1:50 in 0.85% saline) as described previously (Ghosh *et al.*, 1989; Gupta *et al.*, 1991).

The infected cells treated with MoAbs Hs-1, Hx-3 or immune PF before fixation with acetone (the modified method), showed granular IF only on the surface at 24 hr which was more intense by 48 hr. Surface IF detected after acetone-fixation of antibody-treated infected cells was intense and prominent compared to that observed either with unfixed antibody-treated cells or the cells treated with antibody in the absence of sodium azide. Also, the IF on the surface was more intense in infected cells treated with antibody in MSA at 37 °C rather than at 4 °C. In contrast, the infected cells stained by standard indirect FA showed cytoplasmic IF by 24 hr which was more intense by 48 hr (Gupta *et al.* 1990). Infected PS cells which showed IF (surface or intracytoplasmic) with MoAbs or immune PF by 24 hr and 48 hr were negative when stained with control PF.

Table 1 shows the results obtained with four strains of JE virus belonging to the group II. Interestingly, Yoken (Japan) and 755468 (India) strain of JE virus which were negative for IF by standard indirect FA with the MoAb Hs-1, reacted strongly (surface IF) with this MoAb in the modified method. However, both the strains were positive against the MoAb Hx-3 and immune PF by either method. In contrast, the other two Indian strains of JE virus, *viz.* G9473 and 641686 were positive against the MoAbs Hs-1 and Hx-3 and immune PF by both the methods.

Acetone-fixation of the infected cells has been shown to damage some viral antigens present in the cytoplasm (Buckley and Gould, 1988). Also, some of the MoAbs to human spermatozoa were found to react only by the surface IF but were negative in ELISA and standard indirect FA (Hancock and Faruki, 1985). This probably might lead to the non-detection of some MoAbs due to their non-

Table 1. Results of the surface IF standard indirect FA tests with the group II strains of JE virus

MoAbs and immune PF	Yoken (Japan)		755468 (India)		G9473 (India)		641686 (India)	
	sIF	Std.FA	sIF	Std.FA	sIF	Std.FA	sIF	Std.FA
Hs-1	P	N	P	N	P	P	P	P
Hx-3	P	P	P	P	P	P	P	P
Immune PF	P	P	P	P	P	P	P	P

P - positive for IF (+ to +++), N - negative for IF
sIF: surface IF
Std.FA: standard FA

reactivity against JE virus in either of the techniques. Therefore surface IF should be included in screening of hybridoma culture supernatants for antibody production with both the techniques. Also the demonstration of surface IF with the MoAb Hs-1 by the two strains of JE virus (755468 and Yoken) belonging to the group II indicates that the fixation of infected cells with acetone seems to be responsible for the negative results in the standard indirect FA. However, such an adverse effect of acetone was not observed on cells fixed after the treatment with antibody (surface IF).

In the present study, the IF intensity on the cell surface was found to be enhanced both by incorporation of sodium azide (in MSA) and subsequent fixation with acetone which prevented the loss of antigen-antibody complexes from the unfixed cell surface (Hudson and Hay, 1976). Moreover, the observed IF was more intense on the cells incubated with antibody (in MSA) at 37 °C (rather than at 4 °C) presumably due to better interaction between antigen and antibody on the cell surface.

The present technique is simple and will be useful in understanding the process of viral replication and release of both the structural and nonstructural proteins of the virus from infected cell surface. Also, the detection of more labile and conformational-dependent epitopes might be more efficient, thus leading to better understanding the JE pathogenesis.

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