

Immuno-gold replica technique for ultrastructural localization of antigens

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Abstract: We developed a simple method for immunolabelling viral glycoproteins on surface of infected cell monolayers. Cells 9 hours post-infected with Sendai virus were fixed and treated with polyclonal antibody anti HN glycoprotein (Rabbit IgG), labelled with goat immunoglobulin anti rabbit IgG conjugated with colloidal gold particles. Then, the cells were shadowed with platinum-carbon, covered with nitrocellulose glue, and digested with domestic bleach, washed, and replica fragments were caught with uncovered grid.

Key words: Sendai virus, immunolabelling, immunoelectronmicroscopy, replica, ultrastructure.

Replica techniques have been successful at showing three-dimensional (3D) images at transmission electron microscope (TEM), that resemble those of scanning electron microscope (SEM); moreover, they have the resolution of TEM. There have been many variations of this kind of technique, ranging from those in which soft biological specimens, such as animal or plant tissues, are cryostabilized using quick-freezing procedures, to those in which cellulose acetate is used to replicate hard materials (Stolinski & Breathnach 1975). An easy method developed by Akahori (Akahori *et al.* 1986, Hernández and Akahori 1988) uses critical-point-dried specimens, whose surface is shadowed with high-melting-point metal and carbon backed. Then the surface of the specimen is covered with a drop of nitrocellulose glue (Cementidine R) to prevent fragmentation of the replica during the digestion of the organic matter, which is done with hypochloride. When the tissue is digested the remaining replica is washed with distilled water and the nitrocellulose cap is dissolved in acetone.

On the other hand, the immuno-localization of antigens at TEM using immunoglobulins or protein A or G, conjugated with colloidal gold

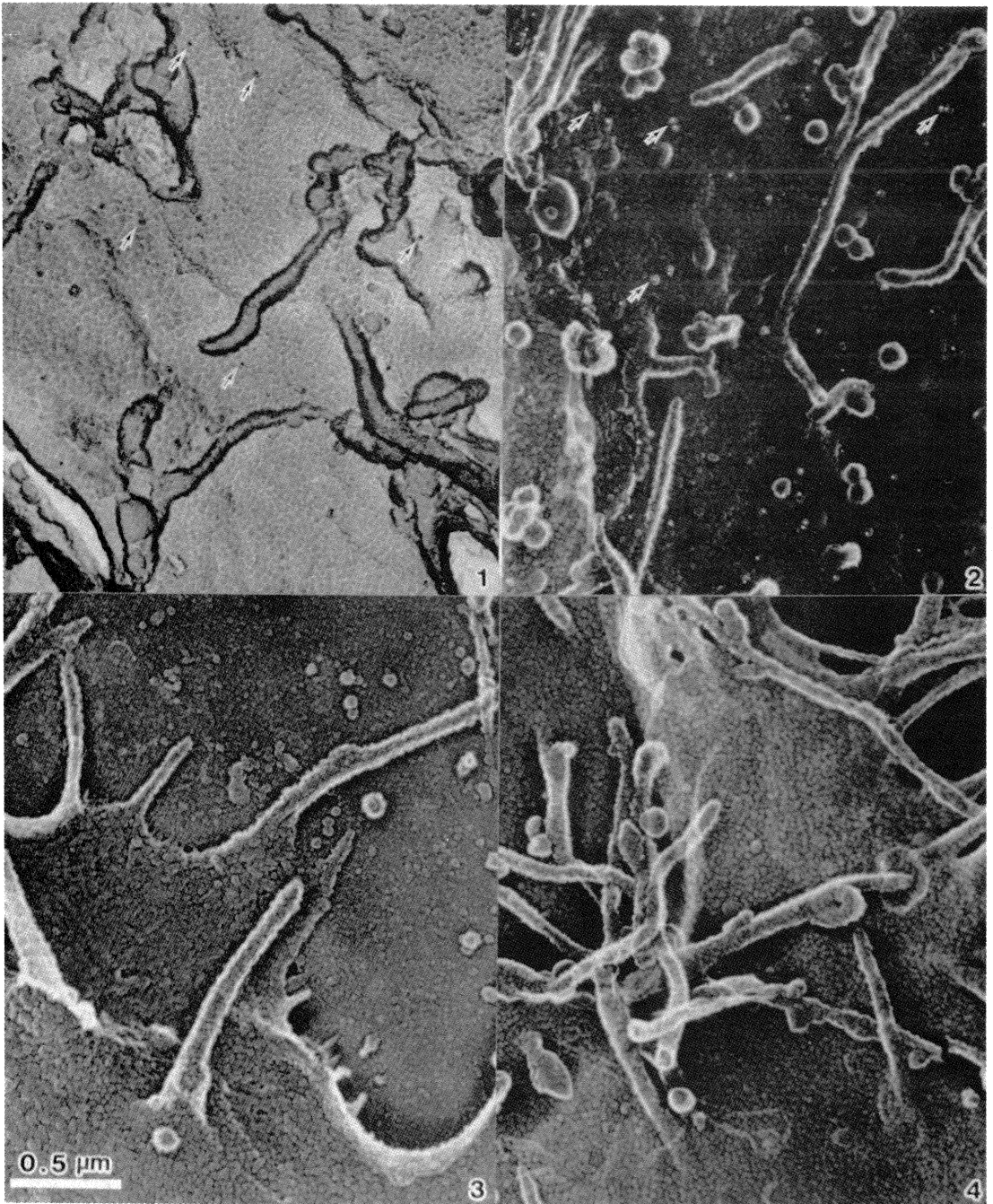
particles as immunomarkers has been extensively used, especially in thin sections (Bendayan 1984, Hodges *et al.* 1987). The application of immuno-localization of antigens at SEM to obtain 3D images has problems related to the resolution of SEM and the size of gold particles, which require backscattered electron detector to visualize the immunomarker on the surface of samples revealed by secondary electrons.

We propose an alternative method to obtain a 3D view of immunolabelling specimens, using Akahori's replica method on immunolabelled specimens.

MATERIAL AND METHODS

Immunolabelling: Mouse L fibroblast monolayers (grown on glass coverslips) uninfected, or 9 h post infection with Z strain of Sendai Virus, were fixed with 1 % paraformaldehyde plus 0.5 % glutaraldehyde (PFA-GA) in Tris buffer saline (TBS; 25 mM Tris buffer, pH 7.4 and 0.5 M NaCl).

The cell monolayers were washed with TBS, and treated with 3% gelatin in TBS at 37 °C for 1 h. Then, they were treated with 50 µl of 100-fold diluted monoespecific rabbit polyclonal



Figs. 1 and 2: Positive and negative imagen of L cells infected with Sendai virus, treated with polyclonal rabbit antibody anti HN glycoprotein labelled with goat anti rabbit immunoglobulin conjugated with colloidal gold particles. The immunomarker appear on cell surface such as high contrast grains (black in positive imagen or white in inverted image, arrows).
 Fig. 3. Replica of uninfected L cells treated as was indicated above. No gold particles can see on the cell surface.
 Fig. 4. Replica of infected L cells treated as was described for Fig. 1. Except that insted the antibody was used normal rabbit serum. The cell surface does not show immunolabelling.

antibody anti Hemagglutinin-neuraminidase (HN) glycoprotein of Sendai virus (Pab anti HN), or with normal rabbit serum. They were washed and incubated with 25-diluted goat anti rabbit IgG conjugated with 15 nm colloidal gold particles (GAR G15; Janssen Belgium), as previously described (Hernández et al. 1987).

All the incubations periods were 1 h at 37 °C.

Replica: After the immunolabelling process, the specimens were fixed again with 2.5 % GA, post fixed with 1 % Osmium tetroxide, dehydrated and critical-point dried. The replica technique was previously described by Hernández *et al.* (1986).

The specimens were shadowed with platinum (15 mm by 0.3 mm Pt wire) at 45° and carbon backed (90°) in a vacuum evaporator (Hitachi HU-5). Subsequently, the shadowed surfaces were covered with a drop of nitrocellulose glue (Cementine C^R) and kept at room temperature (RT) overnight. The organic matter was digested in sodium hypochloride (domestic bleach) for 2 h at RT, washed with distilled water (DW), dried at RT and carbon backed again, to sandwich the Pt film between two layers of carbon. The shadowed surface was divided into squares with a razor blade. The nitrocellulose cap was dissolved in acetone and the replica fragments were collected with uncovered grids for their analysis under a transmission electron microscope.

RESULTS AND DISCUSSION

The replicas of the infected cell monolayer treated with Pab anti HN and labelled with GAR G15 show a heterogeneous distribution of the gold particles, which appears as spherical high-contrasted bodies on the platinum-carbon shadowed cell surfaces. Some areas of the cells have accumulations of gold particles, which may correspond to clusters of viral antigens. Also, the cells show protruding structures, such as elongated and spherical microvilli. Nevertheless, at 9 h post infection there are no virus-budding structures from the cell surfaces, but the HN antigens are present in the cell membranes, because they are labelled with the immuno-marker (Figs. 1 and 2). The controls, uninfected cells treated with Pab anti HN or infected cells treated with normal rabbit serum,

and treated with GAR G15, did not exhibit gold particles (Figs. 3 and 4), which confirms the specific labelling of the viral antigen.

The specimens after shadow casting can be analyzed under scanning electron microscopy, to take panoramic pictures. Then their surfaces are covered with nitrocellulose glue to follow the replica process. Another application of this technique is the simultaneous localization of two antigens, if they are labelling with a Mab and Pab, respectively, and immunomarked using GAM G15 and GAR G5, respectively; as we described for Sendai virus suspensions (Hernández *et al.* 1993).

RESUMEN

Se describe un método simple para inmunomarcación de glicoproteínas virales en la superficie de células infectadas. Monocapas celulares 9 h post infección con virus Sendai fueron fijadas y tratadas con un anticuerpo policlonal (Conejo IgG) anti la glicoproteína HN, marcado con inmunoglobulina de cabra anti IgG de conejo, conjugada con oro coloidal. Luego, las células fueron sombreadas con platino-carbón, cubiertas con nitrocelulosa y digeridas con blanqueador doméstico, lavadas y los fragmentos de réplicas fueron recuperados sobre rejillas sin membrana soporte.

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