AN IMMUNOELECTRONMICROSCOPIC STUDY ON THE PANCREATIC B-CELLS OF MOUSE

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SUMMARY : Recently developed protein A-gold technique for detection of intracellular antigenic sites on thin sections was used to localize a secretory protein, insulin in the pancreatic B-cells of mouse. Treatment of thin sections of glutaraldehyde-fixed, osmium tetroxide-postfixed pancreatic tissue with saturated aqueous solution of sodium metaperiodate, followed by incubation with anti-insulin antibody and protein A-gold complex, restored the labeling without altering the structural preservation. The good ultrastructural preservation obtained led to superior resolution in the labeling. Pancreatic secretory protein, insulin was observed over perfectly preserved secretory granules of the pancreatic B-cells. The protein A-gold technique is proposed as general method for visualization of antigenic sites on thin sections.

Key Words : Protein A-gold, pancreatic B-cell.

INTRODUCTION

In recent years, several proteins having property of binding immunoglobulins have been identified and isolated from different strains of bacteria : Protein A from staphylococci (8), Protein G from streptococci (4), and protein L from peptococcus (5). Among these proteins, the most common and most completely studied is protein A. The most relevant property of protein A consist in its affinity toward immunoglobulin of type G through binding to the Fc fragment (8). An interaction of lower affinity has also been reported between protein A and the fab region of the IgGs and IgEs (6,12,13,21). Protein A, interacts with immunoglobulin type G of several mammalian species, with represents additional advantage to this protein (15). The interaction occurs between the protein A molecule and Fc fragments of the immunoglobulins without interfering with binding of the antibody to its antigen. Owing to this unique property, protein A is being used extensively in several immunocytochemical techniques. It is also display moderate affinity to certain Ig A, IgE and IgM molecules (10,11,13).

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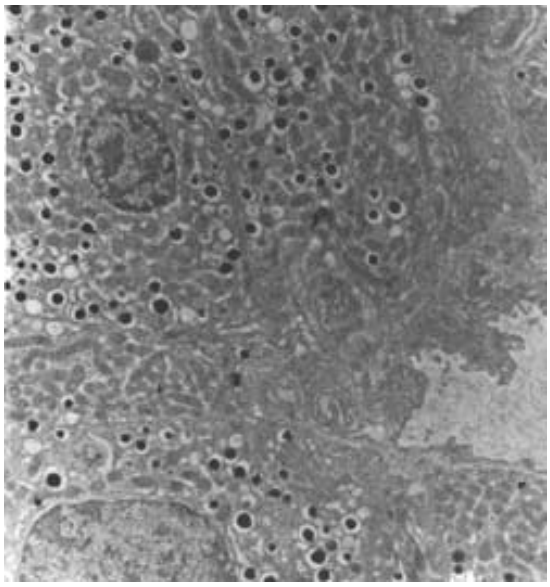
Figure 1: An electron micrograph of lower magnification of mouse pancreatic B-cell located around a capillary. They were incubated with anti-insulin antibody and protein-A gold complex. The gold particles are seen over the secretory granules. X 13500.

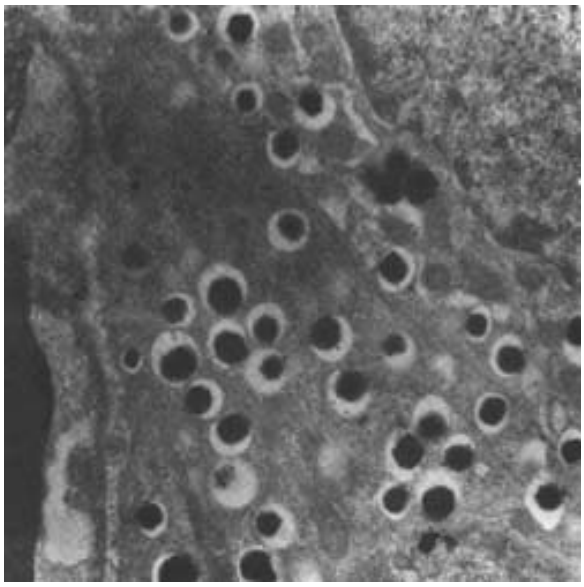


Protein A molecule is low molecular weight, cannot be visualized directly, and must be tagged an electron dense marker for its detection in microscopy. Among the different electron-dense marker, colloidal gold has many advantages when compared to others and has been extensively used in this field. One tagged with colloidal gold particles, the protein A form a complex, protein A-gold complex (19,20) which can be applied in immunocytochemistry at the light microscope, transmission electron microscope and scanning electron microscope levels.

Since its introduction in immunoelectronmicroscopy by Faulk and Taylor (7) colloidal gold has proven to be one of the best electron-dense markers in cytochemistry, displaying several major advantages when compared to other markers such as, ferritin and peroxidase. Because of its particulate nature, very accurate identification and delineation of the labeled structure is possible without masking them. Being one of the smallest markers (down to 3 nm), it allows for the best resolution in cytochemistry. Furthermore quantitative evaluation of the intensity as well as spatial distribution of the labeling can be performed. Since it can be easily prepared in different sizes from 3 to 100 nm (9), one can perform multiple labeling of various binding sites in the same section.

The first application of protein A-gold complex was reported by Romano and Romano (19) for the pre-embedding labeling of surface antigens on red blood cells. Roth *et. al.* (20) adapted this approach for the post-embedding detection of tissue and intracellular antigens. In contrast to ferritin, colloidal gold has no spontaneous affinity to the various resins used in electron microscopy, which result in negligible non-specific adsorption to tissue sections and makes it a suitable marker for post embedding labeling.

Figure 2: The higher magnification of one of the cytoplasmic part of pancreatic B-cell. The gold particles are intensely observed over dense core of the B-cell granules. X 28500.



The purpose of the present study is to describe a simple and reliable protein A-gold technique for the ultrastructural detection of intracellular antigen (secretory protein, insulin) in the secretory granules of pancreatic B-cells of mouse.

MATERIALS AND METHODS

Small pieces of mouse pancreatic tissue were fixed at room temperature for 2 hrs. in 0.5% glutaraldehyde diluted in phosphate buffered saline (PBS, pH7.4). Then the tissue fragments were rinsed in PBS and post-fixed in 1% osmium tetroxide for 1 hr. After further rinses in PBS, the tissues were dehydrated in increasing ethanol concentration and embedded in Agar Resin 100. Thin sections were cut by glass knives and picked up to 200 mesh, parlodion-carbon coated nicel grids.

Thin sections of pancreatic tissue were pretreated with sodium metaperiodate for 60 min before processing them for the immunocytochemical labeling.

Cytochemical labeling

Sections mounted on nicel grids were labeled as follows:

1. The grids were floated, sections down, on drops of saturated solution of sodium metaperiodate (so-called etching procedure) 60 min at room temperature and were then washed several times in distil water.

2. After transferring on drops of PBS+1% ovalbumin, the gruds were incubated for 5 min.

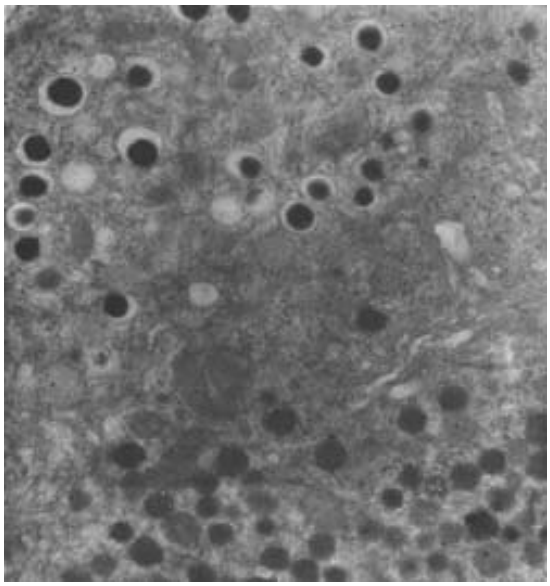
3. They were passed directly on drops of anti-insulin antibody (Sigma) diluted with PBS at 1/200 and incubated for 2 hrs.

4. After five washes in PBS, the grids were then incubated on drops of PBS containing 1% ovalbumin for 5 min.

5. Without rinsing, they were transferred to drops of protein A-gold complex with gold particle 15 nm in diameter (Agar) and incubated for 30 min.

6. Then they were washed with PBS to remove unbound protein A-gold complex and rinsed with distilled water.

Figure 3: The pancreatic B-cell and centroacinar cell are seen side by side. The protein gold labeling is positive in the granules of pancreatic B-cell. X 28500.



7. The grids were dried and stained with uranly acetate for 10 min and washed with distilled water.

8. All sections were examined under the electron microscope, Carl Zeiss EM9S-2.

Cytochemical Controls

In order to demonstrate the specificity of the staining, the following controls were performed:

a. Incubation of the sections with pA-gold complex alone for 1 hr at room temperature.

b. Incubation of the sections with the specific antisera, then exposure of the sections to non-labeled pA 1 hr, followed finally by pA-gold.

RESULTS

Treatment of thin sections of glutaraldehyde-fixed, osmium tetroxide-post fixed pancreatic tissue with a saturated aqueous solution of sodium metaperiodate for 60 min, folled by incubation with anti-insulin antibody and protein A-gold complex, revealed intense labeling over the dense core of the insulin containing granules of the pancreatic B-cells (Figures 1, 2 and 3). The fine structure of pancreatic tissue were also well preserved, after using mild aldehyde fixation, post-osmication, embedding in Agar Resin 100 and pretreatment with sodium metaperiodate.

All control conditions were characterized by a very low amount of gold particles present on thin sections.

DISCUSSION

Fixation of the pancreatic tissue with osmium tetroxide alone, or with a mixture of glutaraldehyde and osmium tetroxide, completely impares labeling with the protein A-gold technique. Pretreatment with sodium metaperiodate is again, under both conditions, able to restore the labeling that is present over well preserved organelles. Pretreatment of thin sec-

tions of post-osmicated tissue with sodium metaperiodate let to a labeling intensity higher than that obtained with non-osmicated non-treated tissue. The restoration of the labeling with sodium metaperiodate is time dependent. Maximal labeling intensity obtains after 60 min (3).

Among the different oxidizing agents used, sodium metaperiodate has been found to be the most suitable, giving a labeling of high intensity and specificity without altering the ultrastructural preservation (1,2,16-18).

In the present study the protein A-gold technique allowed labeling on osmicated pancreatic tissue. Sodium metaperiodate was found capable of unmasking protein antigenic sites on glutaraldehyde-fixed, osmium-post fixed pancreatic tissue, giving in a further step labeling by the protein A-gold technique. The ultra-structure of pancreatic tissue and B-cell secretory protein, insulin, were well preserved. The good ultrastructural preservation obtained led to superior resolution in the labeling.

The protein A-gold technique can be applied to the localization of cellular antigens on aldehyde-fixed, osmium-post fixed tissues and results in high resolution labeling. Furthermore as the technique can be performed even on tissues processed for routine electron microscopy several years ago. It becomes an invaluable tool for localization of antigenic sites and opens new possibilities for reinvestigation specimens from normal, pathologic or experimental situations that are kept in laboratory collections.

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