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Post Embedding Immunogold Labeling for Transmission Electron Microscopy, to Confirm Light Chain Restriction in Renal Diseases

Edward Roberts[1], Lois Anderson[1] and Rajib Gupta[1]

[1.] Department of Pathology, Johns Hopkins University, Baltimore, USA

Ultrastructural immunolabeling or immunoelectron microscopy (IEM) is an exquisitely sensitive technique that helps resolve diagnostic dilemmas in many areas of histopathology, in both non-neoplastic and neoplastic diseases. Ultramicrotomy followed by immunogold labeling of subcellular structures is of great help in the field of nephropathology where on occasion, conventional or immunofluorescent microscopy fails to provide a definitive diagnosis. One such area is confirming the presence and monoclonality of light chain by IEM when all other modalities have failed. Chronic B-cell lymphoproliferative disease including plasma cell dyscrasias usually cause deposition of monotypic light chains in the kidney in various forms. Confirmation of monoclonality in these hematolymphoid malignancies is extremely important before starting any treatment. However, often such deposits are subtle in the early phase or present in unusual locations within the kidney as a result of which they are not picked up easily on light microscopy. Although immunofluorescence is more sensitive in demonstrating these monoclonal deposits, sometimes this method also fails as the light chains are composed of abnormal truncated proteins that are not recognized by commercial antibodies. In such circumstances, standard transmission electron microscopy (TEM) can usually demonstrate these light chains in different renal compartments such as casts in tubules, fine powdery deposits along the glomerular and tubular basement membranes, crystals within glomeruli or tubular epithelial cells or fibrils of varying diameters in the glomeruli. However in a few cases, even conventional TEM is not enough. This is because either EM resolution is not sensitive enough to pick up minute deposits, or other entities like injury-related casts, drug-related crystals or casts (for example, casts caused by certain HIV drugs like Indianavir), or immune-complex deposits in various glomerulonephritides can render TEM diagnosis non-specific. In such circumstances, IEM serves as the gold standard in confirming not only the presence of light chain proteins but also monoclonality.

Two cases of interest were selected, one demonstrating a light chain cast nephropathy with lambda light chain restriction and the second demonstrating a light chain deposition disease, positive for kappa light chains and essentially negative for lambda light chains. Post embedding IEM on glutaraldehyde fixed tissue, was used to confirm the presence of the expected monoclonal light chain deposits in each case.
Renal tissue obtained by percutaneous needle biopsy from patients was fixed with 3 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 overnight at 4^{o}C. The following day, tissues were washed in the same buffer for 15 minutes, 3 times, prior to secondary fixation with 1% osmium tetroxide in 0.1M phosphate buffer for 1 hour at room temperature. The tissues were dehydrated using a graded ethanol series, transitioned through toluene, infiltrated and embedded with Poly/Bed 812 Embedding Media and polymerized overnight at 60^{o}C. Areas of interest for IEM were selected from thick sections (1 micron) stained with Toluidine Blue. Thin sections (100 nanometer) were cut with a diamond knife and placed on 200 mesh nickel grids for post-embed immunogold staining.
Prior to immunostaining, antibody binding sites were unmasked by treating the sections with 4% sodium (meta) periodate (Sigma-Aldrich, MO, USA) for 30 minutes at room temperature [1]. After 3 rinses in deionized water for 1 minute each, non-specific binding sites were blocked by treating with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 10 minutes at room temperature.

In indirect antibody labeling experiments, rabbit polyclonal antibodies (Immunoglobulin G, IgG) against human kappa (Dako North America Inc, CA, USA) or lambda (Bio-Rad, CA, USA) immunoglobulin light chains were used. The grids were floated on drops of primary antibody diluted in 0.5% BSA/PBS and incubated overnight at 4^{o}C. Omission of the primary antibody served as negative control. Control grids were floated on drops of 0.5% BSA/PBS only. The next day, after rinsing sections in 0.5% BSA/PBS for 5 minutes, 3 times, the sections were incubated with goat anti-rabbit IgG (H+L) conjugated to 10 nm gold nanoparticles (Cytodiagnostics Inc, Ontario, Canada) for 1 hour at room temperature. The sections were washed in PBS for 5 minutes, 3 times, stabilized by post fixation in 3% glutaraldehyde in PBS for 3 minutes, rinsed in deionized water and air-dried on filter paper. Some grids were post-stained with uranyl acetate and lead citrate to correlate gold labeling with ultrastructure.

The grids were placed into a JEOL1400 *Plus* transmission electron microscope (JEOL USA Inc, MA, USA) equipped with a lanthanum hexaboride cathode operating at 80 kV accelerating voltage. The acquisition of electron micrographs at various magnifications was performed using a QUEMESA 11 megapixel bottom-mounted CCD TEM camera system (Olympus Soft Imaging Solutions, Germany).

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