IMMUNOLABELING EFFICIENCY OF PROTEIN A - GOLD COMPLEXES. Lucian Ghitescu* and Moise Bendayan, Department of Anatomy, Université de Montréal, Montreal, Quebec, Canada.

A systematic study of the adsorbtion of protein A on colloidal gold particles varrying in size from 5 to 16 nm was performed at different protein concentrations. The number of protein A molecules bound per colloid particle was evaluated for each experimental condition, and the Scatchard analysis of the adsorption parameters was applied for each size of the colloid. The binding of the protein A to the colloidal gold surface exhibited the same affinity pattern for any of the particle size. At low concentratrions of stabilizing protein the adsorbtion took place with high affinity (Kd=1.7-3.0 nM) and the maximum number of protein A molecules attached with this affinity correlated with the surface of the particle. At higher concentrations of protein A, the adsorbtion exhibited a significantly lower affinity (Kd= 630-800 nM), and no saturation was recorded. Competition of protein A binding by albumin did not reveal a preferrential removal of the "low affinity" bound protein A molecules, contradicting the model of successive shells of stabilizing protein around the colloid particle. The immunolabeling efficiency of conjugates of similar size but carrying different numbers of protein A molecules was comparatively investigated by quantitative post-embedding immunocytochemistry. For each size of colloid particles, the maximum intensity of labeling was obtained with those complexes carrying the maximum number of protein A molecules which could be adsorbed with high affinity. Overloading as well as underloading of the complexes resulted in a significant decrease of their efficiency.

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The Use of Immunohistochemistry for Analysis of the Lesions of Atherosclerosis. A. M. Gown, Department of Pathology, University of Washington, Seattle, Washington 98195.

Immunohistochemistry is a technique which can yield important information regarding the composition of human atherosclerotic lesions, both with respect to cell type and cell state. We have developed and acquired a series of monoclonal antibodies to assist in the analysis of human atherosclerotic lesions, and applied single and double labeling techniques to study critical questions regarding the nature of the cells comprising lesions. Monoclonal antibodies specific for muscle cells, macrophages and lymphocytes have identified these cell types in all stages of human atherosclerotic lesions, and have been particularly important in identifying the nature of the foam cell population. In more advanced (complicated) lesions of the aorta and coronary arteries, the vast majority of these cells have been identified as macrophage in origin, while in early fatty streak lesions, the vast majority appear to be muscle cell-derived. The heterogeneity of lesions, as well as a complex substructure within individual lesions, is also revealed by these studies. By also applying a series of monoclonal antibodies to growth factors (e.g., the PDGF B chain) and proliferation-related antigens (e.g., PCNA/cyclin), it is possible to identify growth factor-positive macrophages in all stages of lesions, as well as to identify the proliferating cell compartment of lesions at various stages; the vast majority of the latter in early as well as more mature lesions, for example, appear to be macrophages. Finally, by employing these same methods on lesions of animal models of atherosclerosis, such as the fat-fed monkey, it is possible to directly test hypotheses regarding the applicability of these models for the study of human disease. (Supported by grants #HL-29873 and CA-36250.)

Signal Transduction by IL-2 Receptor β Chain: An Immunocytochemical Study. K. Goto, S. Ueda, H. Akaishi, T. Takeshita,* K. Sugamura* and H. Nagura. Dept. Pathology, Tohoku Univ. Sch. Medicine, *Dept. Bacteriology, Sendai, Japan.

IL-2 is a well-characterized lymphokine which is responsible for growth or differentiation of lymphocytes. The cell growth signal induced by IL-2 is transduced via the surface IL-2R with high affinity to IL-2, but little information is available about the mechanism of signal transmission from the IL-2R.

In the present study, we examined the IL-2 binding to IL-2R and IL-2R-mediated internalization of IL-2 in Human T cell leukemia cell lines by the immuno-electron microscopic technique (immuno-gold, ferritin and emzyme labeled antibody methods).

Results Surface staining: The cell surface of ILT-Mat (with IL-2 dependent growth) and MT-2 (with IL-2 independent growth) in the absence of IL-2 was positively stained with anti-α chain—H31, anti-β chain—TU-27 (competetive to IL-2), and anti-β chain—TU-11 (incompetetive to IL-2). In the presence of IL-2 these cell surfaces was positive for TU-11 but negative for TU-27, and the cell surface of MT-1 with very few high-affinity receptors had H-31, but not TU-27 and TU-11. Par 19-14, anti-parvo virus as a control antibody could not bind to the cell surface of MT-2, ILT-Mat and MT-1. Internalization: The first internalized vesicles positive for TU-11 were observed with 2 min. of incubation at 37°C by the enzyme-labeled antibody method. A rapid increase in these vesicles was detected during the first 30 min. of incubation.

A novel combined light and electron microscopic immunocytochemical procedure to study antigen distribution in the same cells at both histologic and ultrastructural levels.
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In biomedical research, it is often necessary to examine the same cell or a tiny defined structure at both light and electron microscopic levels. Electron microscopic (EM) localization of positive cells identified by light microscopy (LM) is extremely time consuming and often impossible. We developed a procedure which allows a direct correlation between light and electron microscopic observations into the same cells using immunogold silver staining techniques. Atrial natriuretic peptide in rat cardiac atrium was used as a model. The fixed tissues were embedded in paraffin as for LM and 20 micron thick sections were cut and mounted on an poly-L-lysine coated Epon disk (1 cm in diameter and 0.2 cm thick). After dewaxing, the indirect immunogold silver method was performed on the sections. The immunostaining was examined without counterstain and cover-slip by a light microscope. The positive cells of interest were identified and the rest of the section was trimmed away with a fine dissecting blade under a microscope. The section and the disk were dehydrated in increasing concentrations of alcohol to propylene oxide. A drop of Epon was placed and cured to cover the tissue section on the disk. The Epon disk was then cured onto a Epon beem capsule and cut into electron microscopic sections with glass and diamond knives. Depending on the penetration of the antibodies and the plan of the cut surface, electron microscopic immunostaining may not be necessary. When needed it could be repeated with the same antibodies. After counterstain, the same cells were viewed under an electron microscope. The morphology, antigen preservation and labelling adequacy were sufficient to both LM and EM. This procedure has good potential in the study of antigen distribution in a very defined region at both LM and EM levels.