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Protein Kinase A Phosphorylation on Serine of LRP Cytoplasmic Tail Contributes to Receptor-mediated Endocytosis

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The LDL receptor-related protein (LRP) is a multi-ligands endocytic receptor which participates in a variety of distinct physiological and pathological processes. In the present study we found that LRP phosphorylation in human glioblastoma U87 cells was significantly decreased by inhibitors of cAMP-dependent protein kinase (PKA) and was enhanced by the cAMP/PKA activator forskolin. The role of PKA in LRP phosphorylation was further confirmed by *in vitro* phosphorylation of GST-LRP-tail fusion protein with PKA. Using site-directed mutagenesis and LRP minireceptor constructs, we further identified the predominant LRP phosphorylation site at serine 76 of its cytoplasmic tail. The two neighboring serine residues (serine 73 and serine 79) also contribute although less to LRP phosphorylation. Using LRP minireceptors stably expressed in CHO cells that lack endogenous LRP, we found that the initial endocytosis rate of LRP decreases from $t_{1/2}$ ~ 22 seconds for the wild type to $t_{1/2}$ ~ 40 seconds for the phosphorylation mutant. Finally, we demonstrated that "classical endocytosis motifs" that are present within LRP tail are responsible for rapid endocytosis of LRP, whereas serine phosphorylation of the receptor further enhances internalization. Thus, the role of PKA phosphorylation of LRP in receptor-mediated endocytosis may provide a mechanism by which the endocytic function of LRP can be regulated by external signals.

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Smooth muscle foam cell formation: Influence of macrophages and cytokines.

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One of the early changes in the arterial intima during atherosclerosis is the accumulation of both lipid-laden macrophages (MΦ) and foamy smooth muscle cells (SMC). The aim of our studies is to investigate the mechanisms leading to foam cell formation in SMC and the role of scavenger receptors in this process. We established direct and indirect co-culture systems to test the influence of MΦ on the uptake of DiI-Ac-LDL in SMC. Our experiments showed that the uptake of DiI-Ac-LDL by SMC is elevated when the cells were directly co-cultured with increasing numbers of lipid-laden MΦ. We found a moderate basal expression of scavenger receptors in SMC that was slightly enhanced after stimulation with phorbol ester. The uptake of DiI-Ac-LDL in SMC was increased very efficiently after treatment of the cells with mixtures of calcimycin and phorbol ester. Competition experiments showed that the uptake of DiI-Ac-LDL was considerably decreased in the presence of a twentyfold surplus of Ac-LDL, so uptake of DiI-Ac-LDL was very specific. At present we are investigating whether the uptake of DiI-Ac-LDL in SMC can be blocked by an antibody against type I and type II scavenger receptors in order to verify that transformation of SMC into foam cells is due to the uptake of modified lipoproteins via scavenger receptors.

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Elucidation of Ganglioside-binding Motif in Cholera Toxin B-subunit

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The receptor recognition domain of cholera toxin B-subunit (CTB) involved in the binding of the cell surface receptor gangliosides G_{M1}, a sialic acid-containing glycosphinolipid, was studied by using affinity labeling methods. We found that the sodium periodate-oxidized form of ganglioside G_{M1} could bind to CTB and formed a covalent adduct upon reduction by [^{3}H]NaBH$_4$. Analysis of the adduct by a novel thin layer chromatography technique revealed that at least 25% of CTB could be affinity labeled. The binding of oxidized-G_{M1} to CTB was specific because only preincubation of CTB with G_{M1} could abolish the subsequent binding of oxidized G_{M1}, other gangliosides such as G_{T1b}, G_{D1a}, and G_{M3} were of no effect. Analysis of the adduct by using SDS-polyacrylamide gel electrophoresis revealed a protein band with an apparent M_r corresponding to 13.8 kDa. This result suggests that only one molecule of G_{M1} (M_r=1,600) was bound to one molecule of CTB (M_r=11,600). We are currently purifying the tritium-labeled G_{M1}-CTB adduct by using various methods. Subsequently, peptide fragment of the isolated adduct will be generated after enzymatic and/or chemical cleavages. The amino acid sequence of the G_{M1}-labeled peptide will be determined by using automated sequencing techniques and confirmed by using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. These investigations should provide better insights into the molecular motif involved in binding of ganglioside G_{M1} to protein such as CTB.

Leukocytes (1835 - 1836)

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ULTRASTRUCTURAL LOCALIZATION OF ANIONIC SITES, SECONDARY LYSOSOMES AND LECTIN BINDING SITES IN IDIOPHATIC PULMONARY FIBROSIS HUMAN NEUTROPHILS

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The bronchoalveolar lavage (BAL) of patients with IFP is characterized by the presence of an enhanced number of neutrophils. These cells express cell-surface receptors that mediate interactions with soluble and particulate ligands in their microenvironment. In order to analize the pattern of neutrophil lectin-binding sites these cells obtained from BAL of patients with IPF were incubated with colloidal gold complexed lectins (Peroxidase, RCA_I, RCA_{II}, BSL-1) during 30 minutes at 4°C. For the study of neutrophil surface charge and the observation of secondary lysosomes we incubated the cells either with cationized ferritin (CF) during 30 minutes at 4°C or with gold-peroxidase at 37°C during 2 hours. After incubation they were processed to routine transmission electron microscopy. We found an enhanced binding of BSL-1 by neutrophils. Gold-RCA_I (β-D-gal) was forming a sparce labeling at the plasma membrane while RCA_{II} (D-galNAc and β-D-gal) and peroxidase (α-D-mannose) were not observed at the cellular surface of these cells. At ultrastructural level we observed clusters of CF particles concentrated in small areas of neutrophil surface. The gold-peroxidase particles were poorly taken up by neutrophils and were observed inside small vacuoles. The results suggest that α-D-galNAc residues labeled with gold-BSL-1 may be involved in the inflammatory process.

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Accelerated Recruitment of Inflammatory Cells to Dermal Wounds by the Thrombin Peptide TP508.

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TP508 is a synthetic peptide corresponding to amino acids 508 through 530 of human prothrombin. A single topical application of TP508 to full-dermal excisional wounds at the time of injury accelerates their closure in both normal and healing-impaired animals. The present study tested the hypothesis that TP508 acts by enhancing the rate of recruitment of inflammatory cells to the injured site during the early phase of healing. Full-thickness, 2-cm excisions on the backs of normal rats were treated with a single dose of saline +/- TP508. After 24-72h, wound tissue was prepared for histological analysis. Sections stained for nonspecific esterase activity revealed a dense layer of mainly neutrophils, together with macrophages and lymphocytes, extending along the length of the wound. The area occupied by the inflammatory cell layer (ICL) was quantitated along the margin of injured dermis between the epidermis and paniculus carnosus. At 24h post-injury, the ICL in TP508-treated wounds was twice as large as in saline controls; by 48 and 72h, the ICL formed the wound crust and its size was equivalent in control and treated wounds. Accelerated recruitment of inflammatory cells by TP508 during the first 24h may help prevent infection and promote the release of cytokines and growth factors to enhance wound healing. (AI38153, DK53580 and Chrysalis BioTechnology)

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