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S15.2

## Signal Transduction in Atherosclerosis: A Possible Role of Glycosphingolipids

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We have investigated the levels of various glycosphingolipids in plaques, streaks, and unaffected aortic specimens from ten patients who died of coronary artery disease at the Johns Hopkins Hospital. The most marked and consistent difference among these tissues was the abnormal accumulation of lactosylceramide (LacCer) in the fatty streaks (.044 fold – .43 fold) and plaques (2 fold – 10 fold) compared to control. In advanced plaques, the level of (LacCer) was increased about 13 fold compared to the normal tissue from the same patient. The levels of glucosylceramide, globotriosylceramide, globotetrasosylceramide, and sulfatides were also increased in plaques in a descending order, but not to the same extent as LacCer.

Subsequently, we have investigated the effects of LacCer on aortic smooth muscle cell (SMC) proliferation employing viable cell counting, (3H) thymidine incorporation into DNA and the release of lactate dehydrogenase and proliferating cell nuclear antigen (PCNA). Assays for UDP Gal: GlcCer, B1-4 galactosyltransferase (GalT-2) in control and treated cells was pursued simultaneously.

Lactosylceramide stimulated cell proliferation in the order of 2–5 fold. (1) It also increased the cellular levels of PCNA as a function of concentration and time of incubation. Antibody against LacCer, but not GbOse3Ce, blocked the proliferative effects of LacCer in these cells. This phenomenon was specific for SMC as LacCer decreased cell viability of aortic endothelial cells and had no effects on pulmonary endothelial cells. D-PDMP stimulated the activity of GalT-2 in SMC and markedly prevented cell proliferation. The level of PCNA in cells incubated with D-PDMP was also decreased. In contrast, L-PDMP stimulated the activity of GalT-2 in smooth muscle cells, and stimulated cell proliferation. It also increased the cellular levels of PCNA. Antibody against GalT-2 inhibited cell proliferation.

In summary, our findings suggest that the activation of GalT-2 leads to increased LacCer levels, which in turn, may be involved in aortic smooth muscle cell proliferation. Our studies in atherectomy samples suggest that the abnormal accumulation of LacCer may be a non-traditional risk factor in coronary artery disease in man.

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S15.3

## Sialyl Sugar Chain-Mediated Recognition of Influenza Viruses

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Detailed sialyl sugar chain-mediated recognitions of human and animal influenza viruses were determined to elucidate the mechanism of viral molecular evolution and host tropism. An improved receptor binding assay systems were used to determine the binding specificity to sialyl sugar chains including different molecular species of silaic acid (Neu5Ac, Neu5Gc, Neu5,9Ac) and different sialyl-linkage between terminal Neu5Ac and Gal ($\alpha$2-3, $\alpha$2-6, $\alpha$2-8, $\alpha$2-9) in native and synthetic sugar chains. The most potent sugar chains commonly recognized by influenza A and B viruses were sialyllacto-series type I (Neu5Ac$\alpha$2-3(6)Gal$\beta$1-3GlcNAc$\beta$1-) and Type II (Neu5Ac$\alpha$2-3(6)Gal$\beta$1-4GlcNAc$\beta$1-) chains. The change of the binding specificity during the variation of HA molecule appeared as a change of the sialyl linkage recognition ($\alpha$2-3; $\alpha$2-6). Most influenza virus receptor destroying enzyme (neuraminidase) preferentially hydrolyzed $\alpha$2-3 linkage and the linkage specificity between HA and NA was not always the same. Drift of the receptor binding specificity to $\alpha$2-3 and $\alpha$2-6 linkage during the isolation year (1934–1990) was found in human influenza A viruses. Drift of the sialyl-linkage specific recognition of the neuraminidase was also found in human influenza B virus isolates (1940–1990).

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S15.4

## The Biomedical Importance of Carbohydrates as Attachment Sites for Microbes on Animal Cells

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The knowledge is far from complete for a statement in general terms that carbohydrate analogues may be used as antiadhesion agents for therapy of infections. However, a large volume of results obtained during the last ten years shows a promising direction (1, 2). The old concept of adhesion to target cells as essential for colonization and infection has been substantiated through identification of a number of carbohydrate-binding specificities for viruses and bacteria and bacterial toxins, and information is also appearing for eukaryotic parasites. Carbohydrate-binding proteins, adhesins, have been genetically cloned and expressed for detailed binding studies. In case of influenza virus, complexes of hemagglutinin and various oligosaccharides and analogues have been analyzed by NMR and by X-ray crystallography, with information of conceptual interest (3 with references).

Only one report exists so far on a clinically relevant situation where carbohydrate has been successfully used to treat an infection (4). Colostrum-deprived new-born calves were infected with lethal doses of *E. coli* K99, a bacterium producing a toxin-induced diarrhoea. When symptoms of disease appeared, the animals were given in the drinking water