Abstract

Carcinoembryonic cell adhesion molecule 1 (CEACAM1) is human membrane glycoprotein. The purpose of the first part of this thesis was to isolate CEACAM1 glycoprotein from bile and characterise its purity and recovery that have not been described before. Affinity chromatography of CEACAM1 on hydrazide-activated cellulose with immobilized monoclonal anti-CEA F34-187 antibodies is described. The method is based on the oxidation of the immunoglobulin carbohydrate component located on the Fc fragment of antibody by periodate and oriented bounding to hydrazide-activated matrix. Crude protein fraction from bile was applied onto the affinity column and CEACAM1 was eluted with 6 M guanidine-HCl. A single immunopositive 85 kDa band of CEACAM1 was detected on the western blot with anti-CEA antibody. Probably due to the high glycosylation of CEACAM1 any common method of protein staining was not applicable except staining with lectin.

Results were published at: Muchová, L., Jirsa, M., Kuroki, M., Dudková, L., Beneš, M. J., Mareček, Z. and Šmíd, F.: Immunoaffinity isolation of CEACAM1 on hydrazide-derivated cellulose with immobilized monoclonal anti-CEA antibody. *Biomed. Chromatogr.* (2001), **15**: 418-422.

The second part describes an effect of exogenous gangliosides on signalling of mast cells. Gangliosides released from tumour cells, as well as administered exogenously, suppress the immune responses by largely unknown mechanisms. We show here that a pretreatment of rat basophilic leukemia cells with isolated brain gangliosides inhibited the release of preformed secretory mediators from cells activated via FcεRI but not Thy-1 glycoprotein. Exogenously administered gangliosides also affected the cell-substrate adhesion and the levels of polymeric filamentous actin in Ag-activated cells. Although the production of phosphoinositides was also decreased, enzymatic activity of phosphatidylinositol 3-kinase was not inhibited. Gangliosides had no or only marginal effect on the association of aggregated FcεRI with glycosphingolipid-enriched membranes (GEM) and on tyrosine phosphorylation of FcεRI and the linker for activation of T cells (LAT). Though pretreatment with gangliosides did not inhibit the association of LAT with phospholipase C (PLCγ1 and PLCγ2), tyrosine phosphorylation of these enzymes, as well as their enzymatic activities and association with detergent-insoluble signaling assemblies were reduced. This resulted in a decreased production of inositol 1,4,5-trisphosphate and an inhibition of Ca^{2+} mobilization. The combined data support the concept that exogenously

administered gangliosides interfere with those properties of GEM that are important for the formation of plasma membrane-associated signaling assemblies containing PLCγ but not for initial tyrosine phosphorylation of FcεRI subunits.

Results were published at: Dráberová, L., Dudková, L., Boubelík, M., Tolarová, H., Šmíd, F. and Dráber, P.: Exogenous administration of gangliosides inhibits FcεRI-mediated mast cell degranulation by decreasing the activity of phospholipase Cγ. *J. Immunol.* (2003), **171**: 3585-3593.