Principal Investigator Kurt Drickamer

Grant Title Prolectin: a novel glycan-binding protein on B lymphocytes

Abstract

1. Objectives

The goal of this project was to undertake key initial studies on the biological function of prolectin, a novel sugar-binding receptor that we have identified on B cells in germinal centers, and thus to open a new window on the role of glycosylation in the immune system. The aim was to develop tools to investigate two hypotheses: that prolectin functions in activation of B cells upon binding of glycan ligands, possibly accounting for the ability of carbohydrate-associated antigens to stimulate B cells in a T-cell independent manner, or that prolectin plays a role in cell adhesion on B cells as they migrate in or out of the germinal centers, providing a novel example of cell-cell interactions based on sugar recognition.

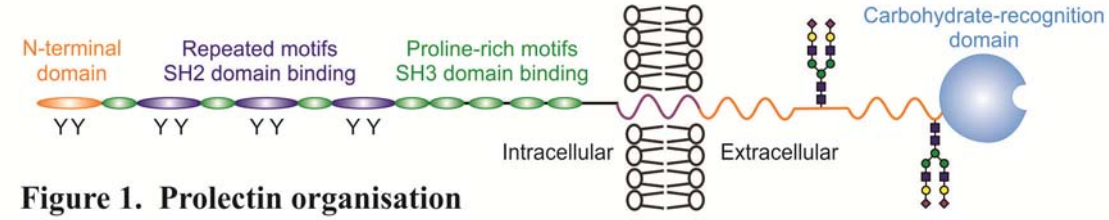

Figure 1. Prolectin organisation

2. Methods

Two experimental approaches have been used: (i) Monoclonal antibodies against prolectin have been characterized as tools for determination of sites of expression of prolectin on different cell types and at specific sites on the cell surface. (ii) Novel affinity resins and cell lines expressing modified prolectin have been developed to test the hypothesis that prolectin acts as a signaling receptor by identifying binding partners for prolectin and examining the ability of prolectin binding to sugar ligands to stimulate signaling pathways.

3. Results

Aim 1 – Characterization of monoclonal antibodies and sites of prolectin expression

A panel of monoclonal antibodies has been characterized using ELISA assays on immobilized prolectin, immunofluorescence on transfected fibroblasts, western blotting of cell extracts, immunohistochemistry on fixed tissues containing germinal centers and flow cytometry of transfected fibroblasts as well as B cell lines. The suitability of the antibodies for ELISA assays, flow cytometry and immunofluorescence was demonstrated. The antibodies have been used to demonstrate expression in a subset of B cell lines and to show that prolectin expression occurs predominantly at points on the cell surfaces which make contact with other cells and with the extracellular matrix. The antibodies will be submitted to the workshops of the Human Cell Differentiation Molecules Council to define a CD designation for the receptor.

Aim 2 – Analysis of prolectin as a signaling receptor

A panel of affinity matrices has been developed to display the cytoplasmic domain of prolectin in which tyrosine residues have been mutated or deleted. These resins have been used to demonstrate phosphorylation-dependent binding of Grb2 as well as phosphorylation-independent binding of Nck1. A novel high affinity, multivalent ligand capable of cross-linking prolectin molecules at the cell surface has also been created by tagging a high mannose oligosaccharide with biotin and complexing it with streptavidin. The ligand has been characterized by gel filtration and *in vitro* binding assays.