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The soluble 14-kDa β-galactoside-binding lectin from bovine heart muscle is a member of a family of proteins related antigenically and structurally which are widely distributed in animal tissues. They are suggested to be involved in cellular recognition events such as differentiation, development and proliferation. Although the precise physiological function and natural ligands of these lectins are not yet known, these appear related to their ability to bind to specific cellular glycoconjugates. Binding specificity studies of the 14-kDa lectin have shown a preference for Galβ(1,4)Glc(GlcNAc) sequences. In order to gain insight into the molecular recognition of this carbohydrate motif, we have investigated the binding of the bovine lectin to the different monodeoxy, *O*-methyl and fluorodeoxy derivatives of methyl β-lactoside. The affinity of the lectin for the different structures provides valuable information on the relative strengths and donor/acceptor relationships of hydrogen bonds between the hydroxyl groups of methyl β-lactoside and the combining site of the lectin. Also, the nature of the groups in the protein involved in hydrogen-bonding can be predicted on the basis of the binding data. According to the results, the strongest hydrogen bonds involve the hydroxyl groups at positions 4 and 6 of the β-D-galactopyranose moiety as donors to charged groups of the lectin. In addition, the C-3 hydroxyl group of the β-D-glucopyranose moiety participates in a weaker hydrogen bond with a neutral group of the combining site. These results are compared with similar data obtained for the galactose-specific lectins from *Ricinus communis*. The greater affinity of the *Ricinus communis* lectins for methyl β-lactoside appears to derive from the fact that every hydroxyl group of the galactopyranose unit is engaged in hydrogen bonding.

We are grateful to Dr. T. Feizi for kindly providing the 14-kDa lectin.

S8.17

Moleculer Cloning of a cDNA Encoding C-Type Lectin from Human Tumoricidal Macrophages

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Macrophages express a variety of carbohydrate binding proteins (lectins) dependent on their organization or on their activation status. Previously, we reported the purification and cDNA cloning of a galactose/*N*-acetyl galactosamine(Gal/GalNAc) specific lectin from murine peritoneal exudate macrophages. This lectin apparently mediated the binding of tumoricidal macrophages to tumor cells. In the present study, macrophage lectin cDNA was cloned from a cDNA library of human IL-2-activated tumoricidal macrophages based on the sequence homology with human asialoglycoprotein receptor (ASGP-R). The human macrophage lectin cDNA consisted of 1451 bp that contained a coding sequence for a 319-amino acid protein (M_r. 35,800). This protein had a single transmembrane domain and a consensus C-type lectin motif. The amino acid sequence was homologous to Gal/GalNAc-specific mouse and rat macrophage lectins and human ASGP-R especially in its carbohydrate recognition domain (60 – 68% homology). Macrophage lectins from mice, rats and humans shared a common feature of having unique 24 amino acid residues that are not found in ASGP-R. Human macrophage lectin had two leucine zipper-like domains, instead of three such domains seen in mouse and rat macrophage lectins. Human macrophage lectin had 35 – 36% amino acid sequence homology with FcεRII and with NKG2-D.

S8.18

Fibronectin in Human Placenta Binds to 14K Beta-Galactoside Binding Lectin through Carbohydrate-Protein Interaction

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14K β-Galactoside binding lectin (14K-lectin) is ubiquitously present in many animal tissues. Endogenous ligands for 14K-lectin, however, have not yet clearly been identified except for laminin (LN). In order to identify other ligands for 14K-lectin, we surveyed 14K-lectin binding proteins in human placenta. The tissue extract with 2 M urea was applied to a Sepharose 4B column conjugated with 14K-lectin purified from frog *(R. catesbeiana)* eggs. The eluate from the column with 100 mM lactose solution was subjected to SDS-PAGE under reducing conditions, which showed a major 220 kDa band and some minor bands of 180 and 40 kDa. Western blotting analysis indicated that 220 and 180 kDa bands were fibronectin (FN) and LN, respectively. Three types of FNs from plasma, placenta and amniotic fluid were examined for the binding capacity to a 14K-lectin column. Placental and amniotic FNs showed significantly higher affinity to the 14K-lectin column than plasma FN (1). Since these FNs have different carbohydrate structures, the sugar chain moiety of FN might be attributable to the difference in affinity. Our results suggest that tissue FN also may function as an endogenous ligand for 14K-lectin as well as a ligand for integrins, and that 14K-lectin may play a role in cell adhesion by interacting with such extracellular matrix molecules as FN and LN through protein-carbohydrate interaction (2).

1) Ozeki *et al.* (1993) *Exp. Cell Res.* submitted;
2) Ozeki *et al.* (1991) *FEBS Lett.*, **289**, 145 – 147

S8.19

Secretion of the Baby Hamster Kidney Carbohydrate-Binding Protein 30 KDa (CBP30) from Polarized and Non-Polarized Cells

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CBP30, originally found in baby hamster kidney (BHK) cells, is expressed in embryonic and new born hamster kidney and disappears shortly after birth [1]. The lectin binds polylactosamine glycans present prominently on extracellular matrix glycoproteins of onco-fetal origin such as EHS tumour laminin and amniotic fluid fibronectin, and inhibits attachment and spreading of BHK cells to EHS laminin substrata mediated by integrin(s) suggesting an extracellular function for the lectin [2,3]. Here we show that CBP30 shares