Carbohydrate specificity of probe binding was determined with the help of inhibition by mono- and oligosaccharides. It was shown also that binding of some probes was Ca^{2+} dependent.

1. Abramenko, I.V. *et al.*, *FEBS Lett.*, **307**, 283 – 287 (1992).
2. Gabius, H.-J. *et al.*, *J. Histochem. Cytochem.*, **38**, 1625 – 1631 (1990).

S8.10

Group V of the C-Type Lectins Family in Recognition and Transmembrane Signaling by NK Cells

G. Vlahas[a], O. Horváth[b], *K. Bezouška*[a], A. Fišerová[b], M. Pospíšil[b]
[a]*Inst. Biotechnology, Charles University, Prague;* [b]*Dept. Immunol., Inst. Microbiol., Czech Acad. Sci., Prague, Czech Republic.*

Although NK cells and their activated forms represent one of the most important components of cellular immunity, the nature of their cell surface receptors remains controversial. Recently, several members of the evolutionary group V of C-type lectin family (1) emerged as strong candidates for this function (2,3). We have expressed extracellular soluble portions of rat NKR-P1 and human NKG2 proteins in bacterial expression vectors pMALc/p2 and pIN-III-ompA2. Purified proteins containing coiled-coil regions and extracellular carbohydrate-recognition (lectin) domains have been used to probe possible physiological (endogenous) ligands for these molecules. The ability to recognize both carbohydrate and peptide determinants as a part of complex target structure seems to be characteristic for these C-type lectins. Two glycoproteins (Mr 42 and 130 kDa) and several oligosaccharide components of glycoproteins and glycolipids have been identified as possible ligands; L-fucose and acidic sugars seem to constitute determinants important for recognition. Transfection of genetic deletion mutants of NK cell lectin receptors into eukaryotic cell lines in combination with cellular plate adhesion assays and biochemical activation assays have been also employed to assess the role of these molecules during individual stages of NK cell cytolysis.

(1) Weis, W. *et al.* (1993) *Cold Spring Harbor Symp. Quant. Biol.*, in press
(2) Giorda, R. *et al.* (1990) *Science,* **249**, 1298 – 1300
(3) Hofer, E. *et al.* (1992) *Immunol. Today,* **13**, 429 – 430

S8.11

Demonstration of the Interaction Between the CD23 Molecule and the Galactose Residue of a Glycoprotein using EBV-Transformed Human B-cell Lymphoma

S. K.-Ochiai, E. Horimoto, Y. Takahashi and T. Uede
Section of Immunopathogenesis, Institute of Immunological Science, Hokkaido University, Sapporo 060 Japan.

The CD23 molecule or FcεRII is a low affinity receptor for IgE. EBV-transformed human B-cell lines, L-KT9 and DH3 cells expressed CD23 antigen and these cells grow in an aggregated state. We tested whether the CD23 antigen on those cells interacts with carbohydrates.

The cells were separated into an aggregated-cell-rich fraction and a single-cell-rich fraction. Aggregated cells became disaggregated by removing galactose by β-galactosidase treatment, while single cells became aggregated by exposing galactose by neuraminidase treatment. Interestingly anti-CD23 antibody strongly inhibited this aggregation.

L-KT9 and DH3 cells bound to asialo-fetuin-coupled-Sepharose (ASF-Sepharose) but not to fetuin-coupled-Sepharose beads, suggesting that this binding involves carbohydrates (terminal galactose). Pretreatment of these cells with anti-CD23 monoclonal antibody abrogated this binding.

Furthermore, cell lysate from L-KT9 cell membrane was incubated with ASF-Sepharose, and bound proteins were most effectively eluted by 0.3 M lactose among the competitive sugars tested. In this eluate, CD23 molecule was detected by an immunoblotting technique. These results collectively demonstrate that the CD23 molecule can, indeed, interact with the galactose residue of the terminal sugar chain of glycoproteins.

S8.12

Isolation and Characterization of a Blood-Group P Binding Adhesin from *Streptococcus suis*

K. Tikkanen[a], S. Haataja[b,c] and J. Finne[b]
The [a]*Department of Biochemistry and Biotechnology, University of Kuopio, SF-70211 Kuopio, Finland; the* [b]*Department of Medical Biochemistry; and the* [c]*Department of Chemistry, Institute of Biomedicine, University of Turku, SF-20520 Turku, Finland.*

Binding to cell surfaces is the main primary event in the establishment of bacterial disease. Bacteria attach to specific receptors on host cells with lectin-like bacterial adhesins. *Streptococcus suis* is an important gram positive pathogen of pigs which causes meningitis, septicemia and pneumonia and is also accociated with meningitis in humans. Some *S. suis* strains bind sialylated poly-*N*-acetyllactosamine glycans (Liukkonen, J., Haataja, S., Tikkanen, K., Kelm, S. and Finne, J., *J. Biol. Chem.*, 267, 21105 – 21111) but in most of the *S. suis* strains the cell adhesion specificity is based on binding to the disaccharide galactosyl-α1—4-galactose (Haataja, S., Tikkanen, K., Liukkonen, J., Francois-Gerard, C. and Finne, J., *J. Biol. Chem.*, in press). We have isolated the galactose recognizing adhesin protein from *S. suis* strain after solubilizing it from bacterial cell surface. The adhesin was purified by ammonium sulphate precipitation and preparative native gel electrophoresis. Purification was controlled by testing the binding activity of adhesin to pigeon ovomucoid, which contains Galα1—4Galβ1—4GlcNAc terminals. The molecular weight of the adhesin was 18 000 and isoelectric point 6.4. Polyclonal adhesin antibodies raised in mice recognized the adhesin protein in several *S. suis* strains in western blot analysis.

S8.13

Pharmacokinetic and Biodistribution Analysis of *N*-linked Oligosaccharides

M. Chiu, T. Tamura, M. S. Wadhwa and *K. G. Rice*
Division of Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University, Columbus, Ohio, 43210, USA.

N-linked oligosaccharides were isolated from bovine fetuin