nitrocellulose. After mild acid hydrolysis, blotted glycoproteins showed PNA-binding bands in the range of 58 – 200 kDa. One of these bands with apparent molecular weight of 190 kDa was characteristic for TGr III cells. TGr II cells were characterized by the presence of two glycoproteins with apparent molecular weight over 200 kDa.

These results indicate for altered expression of cell surface carbohydrate chains possessing terminal and subterminal galactose, particularly PNA-binding glycoproteins, in urinary bladder cells with tumorigenic properties.

S4.8

Expression of the Lewis^x and Sialyl Lewis^x Antigens on Mouse Lewis Lung Carcinoma LL2 Cell Line and its Aleuria Aurantia Agglutinin-Resistant High-Metastatic Variant

D. Duś[4], E. Marcinkowska[1], S. Delescluse[2], J. Montreuil[2] and H. Debray[2]
Department of Tumor Immunology[1], *Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, and Laboratoire de Chimie Biologique*[2], *U.M.R. du C.N.R.S. No 111, U.S.T. Lille, Villeneuve d'Ascq, France.*

Fucosylated type-2 chain antigens such as Lewis^x (Le^x) and its sialylated form the sialyl Lewis^x (sle^x) are present in various human cancer tissues, including lung cancer cells (Zenita *et al.*, 1988, Hakomori, 1991).

The presence of these antigens in mouse Lewis lung carcinoma LL2 cell line and its Aleuria aurantia agglutinin-selected variant (LL2AAA) with changed metastatic properties (Duś *et al.*, 1990) was studied using anti-Le^x, and anti-sLe^x monoclonal antibodies. Screening was performed by indirect fluorescence labelling, evaluated by flow cytometry, of living native and neuraminidase-pretreated cells.

Our results indicate that the fucosylated type-2 chain antigens are present on both cell lines. The positive incidences order of these antigens in LL2 cells was: sLe^x>Le^x. LL2AAA high metastatic variant presents less sLe^x and more Le^x antigens than the LL2 parent line. Total sialylated and non-sialylated Lewis antigens content, calculated as a difference between relative fluorescence intensities, was 18% lower in LL2AAA cell line. This can be correlated with a 25% decrease in the content of α-fucosyl residues associated with a decreased activity of the α-1-3/1-4 fucosyltransferase found in Aleuria aurantia agglutinin-resistant variant.

Investigations of the glycan structures expressing Le^x and sLe^x which can be present in *N*- and *O*-linked glycans or on glycolipids of both cell lines are now in progress.

Zenita K. *et al.*, *Int. J. Cancer*, 1988, **41**, 344; Hakomori, S., *Current Opinion in Immunol.*, 1991. **3**, 646; Duś, D. *et al.*, *Clin Expl. Metastasis*, 1990, **8**, 277.

S4.9

Developmental Changes in Intestinal Glycoprotein Glycosylation

M. C. Biol, D. Lenoir and P. Louisot
Unité INSERM-CNRS U 189, Faculté de Médecine Lyon-Sud, BP 12, 69600 – Oullins, France.

In the rat small intestine, during postnatal development, the activity of a microsomal sialyl-transferase was high after birth and decreased rapidly until weaning. This activity was essentially due to an α(2-6)-sialyl-transferase, whose activity was high and predominant in *N*-glycans in the suckling rats but low and present both on *N*- and *O*-glycans in the weaned rats. An α(2-3)-sialyl-transferase activity could also be detected but at a lower level whatever the age may be. The decrease in sialyl-transferase activity was accompanied by a large increase in the fucosyl-transferase activity just after weaning.

The study of sialo- and fuco-glycoproteins in the intestinal brush-border membranes was performed by specific lectin immunoblotting after SDS-PAGE and transfer of proteins on cellulose nitrate membranes. After coupling of glycoproteins with MAA or SNA conjugated to digoxigenin followed by reaction of an antidigoxigenin antibody conjugated with phosphatase alkaline, α(2-3) or α(2-6) sialic acid residues were revealed in several glycoproteins of suckling rats, whereas only one glycoprotein with α(2-6) sialic acid residues was revealed in weaned rats. On the contrary, fucose residues were revealed in four glycoproteins of the brush-border membranes only in weaned rats, after coupling glycoproteins with Ulex conjugated to biotin, followed by reaction with streptavidin conjugated with alkaline phosphatase.

This study extends previous works indicating a developmental shift between sialylation and fucosylation in the rat small intestine and gives new information concerning the linkage specificity of sialic acid in the small intestinal mucosa.

S4.10

Galα4Gal is not Present in Glycoproteins in Normal Human Tissues but is Present in some Tumor Tissues

J. Bergström, Z. Yang and K. Karlsson
University of Göteborg, Department of Medical Biochemistry, Medicinaregatan 9, S-413 90 Göteborg, Sweden.

In order to detect the presence or absence of Galα4Gal sequence in oligosaccharides of human glycoproteins, extracts from different tissues were tested. Saccharide structures in glycoproteins on replicas of SDS-PAGE gels (blots) were analysed by Galα4Gal specific reagents. The reagents used were *Escherichia coli* expressing *Pap* or *Prs* genes or different antibodies against Galα4Gal. (Galα4Galβ4Glcβ)-BSA and pigeon ovomucoid were used as positive controls for binding.

The normal human samples tested were erythrocytes (P_1+ and P_1-), kidney, ventricle, small intestine, meconium, granulocytes and brain. No binding with our reagents to the glycoproteins was detected. There have been reports in the literature about Galα4Gal-containing glycoproteins in human P_1+ erythrocyte [1], but we could not find any binding to band 4.5 as was reported to contain P_1 activity.

However, when human tumor tissues were tested some showed a clear binding of *Pap* or *Prs E. coli*. The Galα4Gal-expressing tumors were Burkitt lymphoma, gall bladder cancer, lung cancer, colon cancer, liver cancer and melanoma.The antibodies did not bind to any glycoproteins from those tumors indicating that the Galα4Gal-sequence is internal and not terminal in the oligosaccharide chain.

This study clearly indicates that Galα4Gal sequences are only expressed in glycolipids in normal human tissues and not in glycoproteins. However, if the cell is transformed and the compartmentalization has broken down the Galα4Gal sequence could be found also in glycoproteins.