its exit from medium Golgi to cell surface (Sialidase resistance acquisition) (iii) altered the conformation of gp120 expressed on uncleaved gp160 (iv) abolished gp41 mediated membrane fusion (5).

In contrast, **after biosynthesis**, CHO present on mature viral **gp120 and gp41** are involved neither in their bioactivity nor in their immunoreactivity. Binding to CD4 of enzymatically deglycosylated gp120 was not modified (1) and the ability of deglycosylated virus to bind and to infect CD4+ cells was reduced by only 10 fold (2).

CHO are then necessary to create but not to maintain the functional conformation of HIV *env* products.

(1) E. F. *et al.*, *J. Exp. Med.*, 1989, **169**: 807–23.
(2) E. F. *et al.*, *J. Virol*, 1990, **64**: 2841–8.
(3) E. F. *et al.*, *J. Gen. Virol*, 1991, **72**: 1919–26.
(4) E. F. *et al.*, *Virology*, 1992, **187**: 825–8.
(5) E. F. *et al.*, *J. Virol*, 1993, **67**: in press.

S7.4

Structure and Function of the *N*-linked Oligosaccharides of the Human Transferrin Receptor

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The transferrin receptor (TfR) mediates cellular iron uptake via binding and internalization of diferric transferrin. Human TfR is a glycosylated class II membrane protein composed of two identical subunits of 90-95 kd that are linked by two disulfide bonds. Each subunit has three potential *N*-glycosylation sites. Neither the structure nor the function of the *N*-glycans of the human TfR are exactly known. In the present study structures of the *N*-linked oligosaccharides of human TfR purified from placenta and from HepG2 cells were analyzed. Furthermore, the impact of *N*-glycosylation on receptor function was assessed.

After tryptic digestion *N*-glycans were released from the glycopeptides by endo H or PNGase F, separated from remaining peptides by reversed phase HPLC and characterized by anion-exchange HPLC and HPAE-chromatography. Glycans from placental TfR were further characterized by methylation analysis and, in part, by liquid secondary mass spectrometry. Sialylation was examined by lectin affinity blotting with Sambucus nigra agglutinin and Maackia amurensis agglutinin. The data show that human TfR from placenta predominantly carries biantennary and triantennary *N*-acetyllactosaminic glycans as well as hybrid-type species almost completely substituted with α(2-3)-linked sialic acid residues at the terminal galactose. By comparison, TfR from HepG2 cells is glycosylated with oligomannosidic glycans with six to nine mannose residues and tetrasialylated complex-type oligosaccharides apart from mono-, di- and trisialylated species.

In order to assess the influence of glycosylation on the binding function of the receptor, purified placental TfR was modified under non-denaturing conditions either by enzymatic desialylation, or by removal of the hybrid-type glycans with endo H, or by complete *N*-deglycosylation with PNGase F. Determination of the thermodynamic constants and of the number of active binding sites showed that none of these modifications had any influence on the ligand binding properties of the receptor. Moreover, as shown by CD spectroscopy, removal of the oligosaccharides had no influence on the overall secondary and tertiary structure of the receptor.

S7.5

The Role of CD45 in NK Cell Recognition and Signaling

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The molecular nature of natural killer (NK) cell receptor(s) remains unknown in spite of considerable research efforts. Lectin-type receptors are currently among few molecules that are expressed specifically on NK cells and have beside the molecule CD16 the potential for transmembrane signaling. Triantenary degalactosylated oligosaccharide has been shown to bind strongly to the surface of purified rat and pig lymphocytes and efficiently inhibit the NK cell mediated cytotoxicity. Analysis of proteins eluted by the ligand from resins by SDS-PAGE and deglycosylation revealed a pattern characteristic for CD45. The specificity of the interaction has been confirmed by immunoblotting with anti-CD45 antibodies and photoaffinity labeling experiment with the azidophenyl derivative of the triantenary ligand strongly suggested the 205 kDa porcine receptor as a novel member of the leucocyte common antigen family. Interestingly, it seemed that carbohydrates bind into the most *N*-terminal extracellular region of the molecule (*O*-linked poly-*N*-acetyllactosamine chains). Mixture of *N*-linked oligosaccharides prepared from the target cell line K562 as well as defined, isolated glycans were added to lymphocytes and the activation evaluated by transmethylation of membrane phospholipids. To prove that the activation signals were really transmitted through CD45 as the predominant activation receptor, we performed inhibition experiments with a series of monoclonal as well as polyclonal antibodies. Additional experiments indicated that mAb against various isoforms of CD45 differ greatly in their potency to inhibit lymphocyte activation.

S7.6

Carbohydrate Deficient Glycoprotein Syndrome is Asn-*N*-linked Oligosaccharide Transfer Deficiency

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The carbohydrate-deficient glycoprotein (CDG) syndrome, which was first reported by Jaeken *et al.* in 1984, is an autosomal recessive congenital disorder with severe nervous system involvement, growth retardation, and hepatopathy during infancy (1). We recently found that serum transferrin of three patients with CDG syndrome has 3 major isoforms, and their abnormal species were missing either and both of two *N*-linked disialylated biantennary sugar chains in combination with SDS-PAGE, chromatofocusing, lectin affinity chromatography, structural analysis of sugar chains,