Recent work in *T. cruzi* has identified a novel cell-surface trans-sialidase that directly transfers host sialic acids from the host glycoconjugates to the surface of the parasite as it emerges from infected cells. The transsialylation appears to play an essential role in the adhesion of the parasite to the host cells. The enzyme is specific for $\alpha$2-3 linked sialic acids and requires a $\beta$-linked penultimate Gal residue. An overview of the state of our knowledge in these two areas will be presented. (Supported by NIH AI21334 & AI32966 & MacArthur Fdn.)

S10.2

## Enzymatic Characterization and Possible Biological Significance of Trans-Sialidase from *Trypanosoma brucei*

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*Trypanosoma brucei*, the causative agent of African sleeping sickness, expresses developmentally regulated sialidase (1,2) and trans-sialidase activities. These activities are only present in the procyclic life stages, which reside within the midgut of tsetse-flies and not in the mammalian bloodstream forms. The enzymes of sialic acid metabolism sialylate lyase, CMP-Neu5Ac synthase and sialyltransferase are absent from procyclic *T. brucei*. Trans-sialidase, however, enables the parasites to transfer sialic acids from various glycoconjugates into new $\alpha$(2-3)glycosidic linkages without the requirement for CMP-Neu5Ac. The enzyme displays many characteristics in common with *T. brucei* sialidase (1) and thus both enzymic activities may be catalyzed by the same protein. 2-Deoxy-2,3-didehydro-*N*-acetylneuraminic acid and *N*-(4-nitrophenyl)-oxamic acid are only poor inhibitors for both enzymic activities. *T. brucei* sialidase/trans-sialidase is anchored to the outer plasma membrane by a GPI-PLC resistant GPI-anchor. A back and forth sialic acid transfer between intact erythrocytes and surface epitopes of live trypanosomes is possible. One of the trans-sialylated epitopes is the GPI-anchor of procyclic acidic repetitive protein (PARP) (3); also structures within a variant surface glycoprotein can be trans-sialylated *in vitro*. Thus, the expression of trans-sialidase alone may control the presence of sialic acids on the trypanosomal surface. A detailed characterization of *T. brucei* trans-sialidase is presented and possible biological roles of sialidases and trans-sialidases in African trypanosomes are discussed.

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(2) M. Engstler and R. Schauer (1993) *Parasitol. Today* (in press).
(3) M. A. J. Ferguson, P. Murray, H. Rutherford and M. J. McConville (1993) *Biochemical J.* (in press).

S10.3

## Studies on GPI-Specific Phosphodiesterases in the Unicellular Parasite *Trypanosoma brucei brucei*

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*Trypanosoma brucei brucei* is covered by a dense layer of $10^7$ identical variant surface glycoproteins (VSG). This cell surface protein is anchored in the outer leaflet of the plasma membrane by a glycosylphosphatidylinositol molecule. During normal protein turnover and during transformation a part of the VSG can be recovered in the cell supernatant in its soluble form (sVSG) without its dimyristoyl glycerol membrane anchor. The appearance of sVSG implies the action of a phospholipase C.

We have obtained biochemical and immunological evidence for the occurrence of two different GPI-specific enzymes in *T.b.brucei*, the GPI-specific phospholipase C (1) (GPI-PLC) and the GPI-phosphodiesterase (2) (GPI-PDE). GPI-PLC located on the cytoplasmic face of intracellular vesicles is found in the cytosol after stimulation of trypanosomes with phorbol esters. The GPI-PDE on the other hand, not cross-reacting with polyclonal antibodies against GPI-PLC is located within intracellular vesicles as indicated by immunelectron microscopy. Our results indicate that this enzyme is associated with compartments of the endocytotic route. Under culture conditions which allow preadaptation of bloodstream forms to transformation to procyclic insect forms, a process connected with shedding of sVSG, GPI-PDE is also detectable on the cell surface by biotinylation. We therefore propose that the GPI-PDE is involved in the selective release of variant surface glycoproteins.

(1) Bülow and Overath, *J. Biol. Chem.*, **261**, 11918 – 11923, 1986. (2) Schuler *et al.*, submitted

S10.4

## Specific Interactions of HIV-1 and HIV-2 Envelope Glycoproteins with Mannosyl/N-Acetylglucosaminyl Derivatives, Sulfated Polysaccharides and Mannose-6-Phosphate

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The aim of this study was to test whether HIV's glycoproteins have carbohydrate binding properties. Using soluble synthetic or natural carbohydrates or carbohydrate derivatives and carbohydrate substituted affinity matrices, we demonstrate that HIV-1 and HIV-2 recombinant envelope glycoproteins (HIV-1 rgp160 and rgp120, HIV-2 rgp140) specifically interact with high-mannose and with the mannosyl core of complex-type *N*-linked glycans as well as with the *N*-acetylglucosaminyl core of oligosaccharidic structures[1,2,3]. In addition HIV's glycoproteins specifically interact with sulfated polysaccharides such as dextran sulfate and heparin, and with mannose-6-phosphate, either in soluble form or coupled to a matrix[4,5,6]. That rgp160, rgp 120, rgp 140 specifically bind in a saturable and mannose-6-phosphate inhibitable manner to bovine liver $\beta$ glucuronidase leads to the hypothesis of their possible interference with lysosomal enzyme targeting in the host's cells[6]. These carbohydrate binding properties, which are conserved among related lentiviruses certainly play a role in the pathogenesis of HIV's infection and the virus life cycle.

[1]L. Gattegno *et al.*, *Carbohyd. Res.*, **213**, 1991, 79. [2]M. Haïdar *et al.*, *Glycobiology*, **2**, 1992, 429. [3]M. Haïdar *et al.*,