possible role of Gb3/CD77 molecule in the process of apoptosis, we have used one of its recently described natural ligand, a bacterial toxin named Verotoxin (VT) or Shiga-like toxin. We have shown that VT is able to kill BL cells, not only by inhibiting protein synthesis as classically described, but also by inducing apoptosis. Moreover a recombinant B subunit of Verotoxin (VT-B), which carries only the binding property of the holotoxin, also induces apoptosis in Gb3/CD77(+) cells. Indeed, Gb3/CD77(+) BL cells, treated with VT or VT-B subunits, exhibited the morphological features associated with programmed cell death (condensation of the nucleus with formation of crescent-shaped aggregates of chromatin lining the nuclear membrane, marked vacuolisation of the cytoplasm and membrane blebbing) as well as the typical cleavage of the nuclear DNA, revealed on agarose gel electrophoresis as a ladder of approximately 180 base pair multimer fragments. By contrast, Gb3/CD77(-) BL cells, treated with VT or VT-B subunits in the same conditions, remained unaltered. Gb3/CD77 could thus represent the first example of a glycolipid antigen able to transduce a signal leading to apoptosis.

S3.6

Effects of Ganglioside Sialidase Inhibitors on Proliferation and Differentiation of Human Neuroblastoma Cells

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Gangliosides act as modulators of signal transduction pathways that regulate important cell functions including proliferation and differentiation. In cultured human neuroblastoma cells (SK-N-MC), two different ganglioside GM3-sialidase activities, one located in the lysosomes and the other on the external side of the plasma membrane, were detected [1]. In order to investigate the role of the plasma membrane-bound sialidase activity, we studied potential sialidase inhibitors and their influence on growth kinetics and on differentiation markers in SK-N-MC cells.

In homogenates of SK-N-MC cells 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid, heparin and heparan sulphate were potent inhibitors, the plasma membrane-bound activity being much more strongly affected than the lysosomal one. When these agents were added to the culture medium of SK-N-MC cells, a profound change in proliferation kinetics was observed, indicating a release from density-dependent control of cell division. In controls without inhibitor, density-dependent inhibition of cell growth was accompanied by the appearance of morphological and biochemical differentiation markers, whereas in the presence of sialidase inhibitors no such differentiation was observed.

The results suggest a participation of the plasma membrane ganglioside sialidase in the processes of proliferation and differentiation in human neuroblastoma cells.

(1) Kopitz, J., von Reitzenstein, C., Mühl, C., Sinz, K. and Cantz, M. (1992) *Biol. Chem. Hoppe-Seyler* **373** 846.

S3.7

Lysosomal and Plasma Membrane-Bound Ganglioside GM3-Sialidases of Human Neuroblastoma Cells During Cell Proliferation

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Gangliosides of the plasma membrane are modulators of transmembrane signaling, influencing cell proliferation and differentiation. The potential role of ganglioside-specific sialidases (neuraminidases; EC 3.2.1.18) in these processes is poorly understood.

We developed specific radiometric assays to distinguish between plasma membrane-bound and lysosomal GM3-sialidase activities. The assays are based on the specific activation of the lysosomal sialidase by sodium glycodeoxycholate (GDC) and of the plasma membrane-bound sialidase by Triton X-100 [1]. In cultured human neuroblastoma cells (SK-N-MC) both activities were detectable. When we measured the growth kinetics of SK-N-MC cells and correlated it with lysosomal and plasma membrane-bound activities during different growth phases, specific activities of the Triton-activated sialidase increased about 15-fold during logarithmic growth of the cells. Lysosomal sialidase activity and activities of the marker enzymes alkaline phosphodiesterase (plasma membrane, EC 3.1.4.1.) and β-hexosaminidase (lysosomes, EC 3.2.1.30) remained unchanged during proliferation of SK-N-MC cells. Interestingly the changes in plasma membrane sialidase activity differed clearly from the kinetics of ornithine decarboxylase (EC 4.1.1.17), an early marker for cell proliferation.

The two different sialidase activities were further characterized by analyzing their potential substrate specificities towards the most abundant gangliosides of neural tissues. Ganglioside composition of the cells during different growth phases was also determined.

(1) Schneider-Jakob, H. R. and Cantz, M. (1991) *Biol. Chem. Hoppe-Seyler* **372** 443 – 450.

S3.8

Possible Involvement of β-Galactoside in Hepatocyte Proliferation

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We have previously reported that DNA synthesis, as measured by [^{3}H]-Thymidine incorporation, increased in response to the addition of EGF(epidermal growth factor) and insulin to cultured *rat* hepatocytes. The increment depends on not only density of the cells but inclusion of liver plasma membranes into the culture. The rate of DNA synthesis in a low-cell density decreased progressively as either cell density was increased or more liver membranes were added. However, prior treatment of the membranes by β-galactosidase abolished the decrease. It is likely that β-galactoside of *N*-linked sugar chains might be involved in hepatocyte proliferation. This assumption was further supported by the following observations. (1) The decreased rate of insulin and EGF-stimulated DNA synthesis in the presence of liver membrane was restored to an original level by simultaneous addition of asialofetuin or lactose in a concentration-dependent manner. (2) Elute by lactose from the lactose- or asialofetuin-Sepharose column, onto which solubilized rat