involved in fundamental biological processes like cell – cell interaction and control of cell growth and differentiation. Different types of proteoglycans were found on cell surfaces, in basement membranes and in extracellular matrix. Whereas the importance of growth factors and colony-stimulating factors in the control of growth and development in the haematopoetic system has been shown by numerous studies, only recently the role of the microenvironment of the haematopoetic tissue, e.g. the bone marrow stromal cells and their extracellular matrix was realized. It has become clear that stromal cells play a critical role in haematopoesis. Furthermore it has recently been shown, that proteoglycans might be involved in the interaction of haematopoetic stem and stromal cells (1,2).

Here we report on the isolation and characterization of proteoglycans from a series of mouse and human haematopoetic stem cell and stromal cell lines. Proteoglycans were isolated from biosynthetically labelled cells and purified by several chromatographic steps including anion-exchange chromatography and size exclusion chromatography. Biochemical characterization of the isolated proteoglycans was done by electrophoresis and blot prior and after digestion with glycan-specific enzymes using autoradiography or immunochemical detection with proteoglycan-specific mAbs. The possible role of these proteoglycans in the control of growth and differentiation of haematopoetic cells will now be investigated.

(1) Gordon, M. Y., Riley, G. P., Watt, S. M. and Greaves, M. F. (1987), *Nature*, **326**, 403 – 405.
(2) Roberts, R., Gallagher, J., Spooncer, T., Allen, D., Bloomfield, F. and Dexter, T. M. (1988), *Nature*, **332**, 376 – 378.

S5.13

Minimal Sequence in Heparin/Heparan Sulfate Required for Binding of Basic Fibroblast Growth Factor

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Heparin and heparan sulfate are glycosaminoglycans composed of alternating units of hexuronic acid (D-GlcA or L-IdoA) and D-glucosamine, with sulfate substituents in various positions. Binding of basic fibroblast growth factor (bFGF) to heparan sulfate is important for storage of intact growth factor in the extracellular matrix and is further required for efficient interaction between bFGF and its high-affinity cell-surface receptor(s) (Gallagher, J. T. and Turnbull, J. E. (1992) *Glycobiology* **2**, 523 – 528). The present studies of saccharide-bFGF interactions in free solution, using a nitrocellulose-filter binding assay, show that the growth factor binds to heparin and to selectively GlcN 6-*O*-desulfated heparin, but poorly to IdoA 2-*O*-desulfated heparin. The 2-*O*-sulfate groups thus are essential to the interaction, whereas 6-*O*-sulfates are not required, nor do they interfere with bFGF binding.

Radiolabeled oligosaccharides derived from heparin and heparan sulfate were separated into bFGF-bound and -unbound fractions, which were further separated by anion-exchange HPLC. Analysis of the products implicated a minimal pentasaccharide sequence for bFGF binding, with the structure: hexuronic acid — glucosamine *N*-sulfate — hexuronic acid — glucosamine *N*-sulfate — iduronic acid 2-*O*-sulfate- (reducing terminus). This structure is abundant in heparin, albeit heavily obscured by additional (nonessential, noninterfering) *O*-sulfate groups, and also occurs, although to a lesser extent, in various heparan sulfates. The higher apparent affinity of polysaccharides, compared with oligosaccharides, for bFGF is due to the occurrence of multiple consecutive repeats of the pentasaccharide motif. Additional structures may be required to promote binding of the growth factor to its high-affinity receptor and to elicit a biological response *in vivo*.

S5.14

Spaciotemporal Expression of a Brain Specific Species of Chondroitin Sulfate Proteoglycan, Neurocan, in the Rat Cerebrum

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The mammalian brain contains many species of proteoglycan. To identify each proteoglycan species, we have raised monoclonal antibodies (MAbs) against proteoglycans purified from 10-day-old rat brains (1). One MAb, named 1G2 recognized two chondroitin sulfate proteoglycans (CSPGs) with 220 and 150 kDa core glycoproteins (CSPG-220 and CSPG-150). Partial amino acid sequences of *N*-termini of their core proteins coincided with those of neurocan, a brain-unique CSPG species, whose complete coding sequence was recently reported (2). Western blots revealed that the level of CSPG-220 was extremely high in the cerebrum during the first 10 days after birth, and it disappeared from the brain around postnatal day 30 (P30). In contrast, a fairly large amount of CSPG-150 remained in the mature brain. Immunohistochemical studies revealed that 1G2 antigen was first localized diffusely in the preplate zone, then both in the marginal zone and in the subplate of the rat cerebrum at early developmental stages. By embryonic day 20, immunolabeling with MAb 1G2 spread in the developing cortical plate. On P10, the neuropil of the cerebral cortex, except for the barrel field, was diffusely stained with the antibody. The barrel hollows were negative to immunostaining at this stage, but became weakly positive with maturation of the brain. The 1G2 antigen was immunohistochemically associated largely with glial fibrillary acidic protein-positive cells in primary cultures of the neonatal rat cerebrum, suggesting that the 1G2 antigen is produced mainly by astrocytes in the brain. From these observations, neurocan can be considered to play some roles in cortical development.

(1) N. Maeda *et al.* (1992) *Dev. Biol.*, **151**: 564 – 574.
(2) U. Rauch *et al.* (1992) *J. Biol. Chem.*, **267**: 19536 – 19547.

S5.15

Rat Ovarian Granulosa Cells Synthesize Anticoagulant Heparan Sulfate Proteoglycans

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Antithrombin (AT) binding heparan sulfate proteoglycans