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THURSDAY 6 SEPTEMBER POSTERS

CELL MIGRATION

B1

The role of Rho GTPases in astrocyte polarity and migration after injury

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Small Rho GTPases have been implicated in astrocyte polarity and migration by the use of dominant negative or constitutive active constructs or pharmacological inhibitors that may affect several members of the family. To overcome these limitations we used a genetic approach, taking advantage of conditional alleles of Cdc42, Rac1 and RhoA (Brakebusch, 2005 and 2006) t elucidate the distinct functions of these proteins in astrocytes in vitro and in vivo. First we used the in vitro scratch wound assay, a culture model for astrocyte polarity and migration. Astrocytes from postnatal cortices of mice containing the Cdc42, Rac1 or RhoA genes flanked by loxP sites were infected with lentiviruses containing Cre-IRES-GFP or GFP as control. The scratch assay was performed 2 weeks after infection and reorientation of the centrosome or the formation of protrusions were quantified. Only 22% of Cre- infected Cdc42 fl/fl astrocytes formed protrusions towards the scratch, while this was observed in 55% of all control-infected astrocytes. Similar defects were seen in centrosome reorientation in Cdc42 deficient astrocytes. Interestingly, deletion of Rac1 resulted in random formation of protrusions while the reorientation of the centrosome appeared less affected. Next we examined astrocyte polarity and migration in vivo after stab wound injury. We demonstrated an increase in the number of astrocyte near the lesion site (Buffo *et al.*, 2005) and are currently testing the contribution of directed astrocyte migration to this phenomenon by viral injections at varying distances from the lesion site. Recent data on the effect of Cdc42 and Rac1 deletion on astrocyte migration towards the injury site will be presented.

B2

Novel, radial glial dependent and independent aspects of tangential neuronal migration in cerebral cortex

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Interneurons originating from the ganglionic eminence migrate tangentially into the developing cerebral wall as they navigate to their distinct areal and laminar positions in the cerebral cortex. Compromised interneuronal migration disrupts the appropriate positioning of interneurons and the resultant changes in the connectivity of interneurons are thought to be an underlying cause in the emergence of neurodevelopmental disorders such as schizophrenia. Previously, it was suggested that tangential migration of interneurons occurs in a radial glia independent manner. Here, using simultaneous imaging of genetically defined populations of interneurons and radial glia, we demonstrate that dynamic interactions with radial glia regulate the trajectory of interneuronal migration and thus the positioning of interneurons in cerebral cortex. Furthermore, there is extensive interneuronal migration in tangential direction opposite to that of pallial orientation all across the cerebral wall. This counter migration of interneurons may be essential to locally position interneurons once they invade the developing cerebral wall from the ganglionic eminence. Together, these observations suggest that interactions with radial glial scaffold and localized migration within the expanding cerebral wall are essential for the guidance and placement of interneurons in the developing cerebral cortex.

B3

Nedd9 makes multipotent cells motile: a key role in delamination and migration in vivo

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Cell migration is a fundamental process during nervous system development but the underlying molecular mechanisms are unclear. The neural crest is one of the largest populations of highly migratory cells. Nedd9 is an intracellular adaptor in the integrin signaling pathway which may be involved in vivo in cell migration, but this has not been addressed. Nedd9 was found to be present in multipotent Sox10+ neural crest cells (NCCs) and in Sox2+ central nervous system (CNS) progenitor cells of neurogenin-2 (ngn2)+/Nurr1+ domains. Loss of Nedd9 activity in chick NCCs resulted in alterations of the actin cytoskeleton and focal complexes, a disruption of epithelial to mesenchymal transition (EMT) and cell migratory properties, while forced expression increased cell motility. Our results suggest an intracellular mechanism regulating competence for EMT and migration of multipotent progenitor cells in vivo by linking the extracellular matrix (ECM) to cell migratory properties. Because Nedd9 acts in a concentration-dependent manner, regulation of its expression by local changes in retinoic acid (RA) may influence cell migratory behavior of progenitor cell populations of diverse tissues in a graded manner.

B4

Acetylcholine-mediated axon-glia communication triggers calcium signaling and migration of glial cells in the developing insect olfactory system

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The vertebrate olfactory bulb and the insect antennal lobe, the primary olfactory relay stations in the CNS, share analogous morphology and function. Because insect development can easily be studied and manipulated during metamorphosis, the antennal lobe has been used as a model for olfactory system development. The migration of glial cells and the formation of glomeruli in the developing insect antennal lobe is triggered by ingrowing olfactory receptor axons. It is not known, however, how receptor axons and glial cells communicate and whether calcium signaling is involved in this communication. We studied calcium increases in glial cells in vivo and in situ, evoked by electrical stimulation of olfactory receptor axons in pupae and by odor stimulation of receptor neurons in adults of the sphinx moths Manduca sexta. Axonal activity led to calcium increases in glial cells that were blocked by nicotinic acetylcholine receptor (nAChR) antagonists and could be mimicked by acetylcholine and carbachol application. In addition, calcium transients were abolished by removal of external calcium and blockage of voltage-gated calcium channels. During development, calcium signaling evoked by both nAChRs and voltage-gated calcium channels could first be elicited at stage 6, the time when glial cells start to migrate. Glial migration was reduced and glomeruli formation failed after injection of either nicotinic antagonists, calcium channel blockers, the calcium chelator BAPTA-AM, or the CaM kinase inhibitor KN93 into pupae. The results show that calcium signaling can be induced by acetylcholine release from olfactory receptor axons, which activates nAChRs and leads to voltage-gated calcium influx. The developmental up-regulation of the calcium signaling temporally correlates with the onset of glial cell migration. The results further suggest that cholinergic signaling and CaM kinase activity in the olfactory system are required for glial cell migration and glomerulus formation in Manduca.

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B5

Effects of neuropeptide galanin on microglial migration

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Galanin (GAL) is 29/30-amino-acid neuropeptide and is up-regulated following neuronal axotomy or inflammation, therefore may affect the brain immune system. GAL has three types of receptors; GalR1, GalR2 and GalR3. They are widely distributed in the central and peripheral nervous system. Activation of GalR1 and GalR3 couples to inhibition of adenylyl cyclase, while activation of GalR2 primarily couples to activation of phospholipase C and formation of inositol-1,4,5-triphosphate. It was reported that rat cultured microglia express mRNA for GalR2 but not for GalR1 and Gal3. However the function of GalR2 in microglia is poorly understood. In the present study, we found that GAL increased motility and chemotaxis of rat cultured microglia using time lapse video microscopy system and Boyden chamber. GAL-induced motility was mimicked by galanin-like peptide (GALP), a selective GalR2 agonist. GAL-induced motility and chemotxis were blocked by charybdotoxin (CTX), a blocker of Ca2+-dependent K+ channels, but not by pertussis toxin (PTX), suggesting that Gi protein-independent Ca2+ increase and subsequent activation of K+ channels is required. We also found that GALP-induced increase in motility was cancelled by inhibitors of protein kinase C (PKC), phosphoinositide 3-kinase (PI3k) and mitogen-activated protein kinase kinase (MEK). To see whether or not GAL activates microglia, expression of major histocompatibility complex class 2 (MHC-2) and inducible NO synthase (iNOS) was observed immunocytochemically. The expression of OX-6 but not iNOS was significantly increased by GAL and GALP. Our results may help to understand the functional importance of interplay between GAL and microglia in neuronal injury or inflammation in the brain.

B6

Analysis of NIK function in neuronal migration during mouse brain development

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The Nck-interacting kinase (NIK) was identified in our lab as a partner for the β1 integrin cytoplasmic domain. NIK is a serine-threonine kinase of the STE20/GCK family with an N-terminal kinase domain and a C-terminal CNH (Citron Homology) regulatory domain. We have shown previously that the interaction between β1 integrin and NIK is conserved and essential for commissural axon navigation in C. elegans and that NIK genetically interacts with the Rho GTPase Rac (Poinat P. *et al* 2002). In order to understand NIK function in neuronal migration during mouse brain development, we have used in utero electroporation. This procedure allowed to introduce transgenes into the ventricular zone of developing brains and to visualize cortical migration. Expression of a kinase-dead version of NIK (NIK-KD) leads to abnormal radial migration, with accumulation of cells into the subventricular zone and intermediate zone. These experiments show the importance of NIK during cortical neuronal migration. The phenotype is very similar to the one observed after expression of a dominant-negative form (N17Rac1) or a constitutively-active form of Rac1 (V12Rac1) in brain, suggesting a common pathway for Rac and NIK. To analyse a possible link between NIK and Rac, coelectroporations were performed. Coexpression of NIK-KD and N17Rac1 rescued the migration defects whereas coelectroporation of NIK-KD and V12Rac1 increased the inhibition of migration. These results suggest that NIK may act on growth cone cytoskeleton dynamics via the regulation of Rac activity. Studies in progress will clarify how NIK regulates Rac activity.

B7

The role of FGF signaling in the development of Drosophila eye-disc glia

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Interactions between neurons and glia are important at many stages of nervous system development. An attractive model system to study these interactions is the Drosophila eye-imaginal disc. Here differentiating photoreceptor neurons project their axons to the visual center of the brain. Glial cells are born in the optic stalk and migrate onto the eye disc guided by still unknown signals. As soon as the glial cells contact axons they undergo differentiation to become wrapping glia. The molecular mechanisms triggering the transition from the migratory to the differentiated state are also unknown. In a search for molecules that might control glial development we determined the role of the FGF-receptor Heartless. Using in situ hybridization and newly generated antibodies we could show that Heartless is specifically expressed by the eye disc glia, especially in the cells at the migrating front. Block of FGF-receptor function by RNAi or expression of a dominant negative receptor demonstrates its requirement in glial cell proliferation and migration, whereas expression of an activated form of Heartless induces glial proliferation but not glial migration. In the eye disc Heartless appears to be activated by the FGF8-like ligand Thisbe that is expressed in photoreceptor neurons. To further characterize the role of FGF signaling in the developing glia we are currently analyzing clonal patches of Heartless mutant cells as well as studying further components of the FGF-signaling pathway.

B8

Characterisation of the role of individual FGF-receptors in cortical development

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Distinct members of the FGF family, together with the IIIc isoforms of three FGF receptors (FGFR1–3) are expressed in dynamic patterns in the developing rodent cerebral cortex (Hajihosseini & Dickson 1999 MCN 14: 468–85; Hasegawa *et al.* 2004 J. Neurosci 24: 8711–19). FGFs can modulate cortical cell proliferation and differentiation, but the role(s) played by each receptor in the set of processes that regulate cortical development, which also includes mode and rate of precursor cell division, migration of differentiated progeny etc. have remained elusive. This is in part due to embryonic lethality of FGFR-deficient mice prior to corticogenesis and/or the apparent functional redundancy between receptors, observed in single and compound FGFR mutant mice. An alternative to generating double- and triple-receptor knockout mice is combinatorial silencing of FGFRs using small inhibitory RNA interference. We have thus generated lentiviruses that carry one or a combination of siRNAs against FGFRs 1–3, in addition to a GFP marker to enable fate analysis of transduced cells and their progeny. Application of these viruses to primary cortical cultures and developing cortex in utero will allow fate analysis at both a single cell and clonal level. These studies are complimented by our gain-of-function studies in vivo, using mice that carry hyperactive FGFR1 and FGFR2 mutations (Hajihosseini *et al.* 2001 PNAS 98; 3855–60; Hajihosseini *et al.* 2004 Development 131: 325–35). Together, these studies will enable us to dissect the precise function of each FGFR during cortical development.

B9

Polyunsaturated fatty acids (PUFA) influence astrocytes morphology and motility

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Docosahexaenoic acid (DHA) and arachidonic acid (AA) are major n-3 and n-6 PUFA in brain cell membranes. Their ratio partly depends on the of n-3/n-6 balance of PUFA dietary intakes. The very low n-3/n-6 ratio in western diet may induce a low DHA/AA ratio in brain cells. This DHA deficiency causes cognitive impairment in animal models and is associated to various neurodegenerative and psychiatric disorders in humans. The underlying cellular mechanisms, related to membrane properties and lipid signalling in brain cells, are currently investigated. Given the high morphological plasticity of astrocytes, the influence of DHA/AA ratio in membrane and cytoskeleton re-arrangements may be of great importance in their physiology. We recently showed that it influences astrocyte gap junction communication (Champeil-Potokar *et al*, Eur J Neurosci, 2006). To explore the role of DHA and AA in astrocyte morphology and motility, we have used a model of cultured rat astrocytes with high or low DHA/AA ratio in their membrane. Cells were grown in medium supplemented with DHA (resulting in a high DHA/AA membrane ratio, as found in brain of rats fed n-3 balanced diet) or AA (very low DHA/AA membrane ratio) or without supplement (low DHA/AA ratio, as found in n-3 deficient rat brain). PUFA supplemented cells showed specific morphological changes, as compared to polygonal unsupplemented cells : DHA-cells exhibited oriented long processes containing dense GFAP labelling, rounded by thin ezrin filopodia; AA-cells exhibited stellate non oriented GFAP processes without ezrin filopodia. PUFA supplementation slowed down astrocytes motility measured by scratch-wound-assay. The decreased motility was associated with changes in phosphotyrosine signalling on focal adhesion complex proteins, as shown by immunocytochemistry and western blotting. Our results indicate that PUFA supplementation has profound effects on astrocyte morphology and motility by acting on cytoskeletal proteins. Part of the effect is specific to n-3 PUFA.

B10

Implication of megalin in the Shh-dependent oligodendrocyte precursor migration during optic nerve development

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Sonic hedgehog (Shh), a well studied morphogen during CNS development, has fundamental roles in developmental processes such as early regionalization and ventralization, cell fate specification, cell proliferation and axonal guidance. Previous results from our group showed that Shh has a chemoattractant effect on oligodendrocyte precursors

(OPCs) of the optic nerve. This effect is completely blocked by the Shh blocking antibody 5E1, but not in the presence of the Ptc antagonist cyclopamine, suggesting that new receptors could be involved in the control of OPCs migration. Megalin is a multiligand endocytic receptor of the LDLR gene family. Members of this family have already been shown to interact with morphogens, such as Lrp5 involved in Wnt signalling. Megalin inactivation leads to holoprosencephaly, a feature often associated to defective Shh signalling. Analysis of the megalin knockout mice revealed absence of Shh expression in the ventral forebrain leadind to the loss of ventrally derived oligodendroglial and interneuronal cell populations. Subsequently in vitro experiments suggested that Shh was a megalin ligand but no in vivo evidence has yet been provided. In this work we used an ex-vivo approach to investigate the role of megalin in Shh signalling at various developmental stages. We first show that megalin is expressed at early stages (E12.5–E14.5 mouse embryo) in some oligodendrogenic domains, closely related to Shh expression. We also show that, at E16.5, an important number of the cells within the optic nerve express megalin. With chemotaxis assays on optic nerve explants, we show that the inhibition of megalin by megalin-blocking antibodies and/or RAP significantly reduces the Shh-mediated chemoattraction on the OPCs of the optic nerve. In conclusion, megalin expressed by the cells in the optic nerve is essential for the Shh-induced OPCs migration.

B11

Organization of the radial glia in the caudal subpalium and migration patterns

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It has been demonstrated that radial glial processes are potential guides for neuronal migration, and their orientation in the distinct areas of the embryonic brain may provide further information about the origin of the cells in each one of them. The anterior preoptic area (POA) has been considered as part of the anterior hypothalamus or as a rostral extension of it, but also as a telencephalic subpalial area. This area participates in regulatiing many homeostatic and sex-dependent functions in adults. Rostral to it, an other subpalial area has been defined by its gene expression pattern, the anterior entopeduncular area (AEP), wich is a source of the forebrain oligodendrocytes. However, the radial glia pattern of this area is not known enough and is not clear the extension of the corresponding ventricular zone. In a previous research in which chick/quail chimeras were used, we demonstrated that cells from thalamic eminence (TE) reach this area. Other studies state both the lateral ventricles and the third ventricle as cell sources for this region. In the current study we have examined the radial glia distribution in chick embryos brain using intraventricular injection of the lipophilic dye DiI. We examine the distribution of radial glial processes in the AEP and adjacent histogenetic areas and we state the ventricular zones of each one of them.

B12

AQP4 plasma membrane localization increases migration in astrocyte primary cultures

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An altered astrocyte migration is a common event of different CNS pathological states. We have previously shown that the glial water channel protein, Aquaporin-4 (AQP4), has a key role for astrocyte morphological changes and more recently its role in astroglial cell migration has been also demonstrated. To study if AQP4 modulation can affect astrocyte migration, we performed two migration assays: the Boyden Transwell (BT) and the wound-healing assay. The experiments were performed on rat astrocytes untreated and treated with 1 mM dibutyryl-cAMP and 50 μM Lovastatin, both able to induce astrocyte stellation and AQP4 plasma membrane targeting. In the BT assay, the number of astrocytes in 1 % FBS that migrated towards 10 % FBS, across 8 μm diameter pores over 12 hours, was assessed. Results show that the migration was significantly faster in astrocytes treated with cAMP and Lovastatin since the number of migrated cells increased by 25 +/− 1 % (cAMP) and 35 +/− 2 % (Lovastatin) compared with untreated astrocytes. Wound healing was quantified after 24 h as the linear speed of the wound edges. Our results show that this speed was remarkably faster in astrocytes treated with db-cAMP (4.48+/− 0.38 micron/hour) and Lovastatin (5.38+/−1.38 micron/hour) compared with untreated astrocytes (2.14+/−0.47 micron/hour). The wound healing assay was performed also on AQP4 knockdown astrocytes obtained by RNA interference. AQP4 inhibition slowed the speed of the wound edge by 57+/−3%. All together, the results show that AQP4 plasma membrane expression raises the speed of astrocyte migration and that cAMP and Lovastatin can be used to increase the cell motility through AQP4 membrane expression. Thus, modulation of AQP4 expression/function in the CNS can be considered a novel strategy to control glial scar formation and for the treatment of the CNS pathologies in which an altered astrocyte migration occurs.

B13

Effects of neurotrophins on neural crest and Schwann cell migration

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The neural crest is a migratory population of cells that gives rise to a wide range of cell types in the peripheral nervous system of vertebrate embryos. It has been shown that neural crest cells migrate along very specific pathways throughout the embryo. The reason for such specificity is not fully known. During the last years, some known axon pathfinding repellants (ephrinB2, SemaIIIa, Slit2, etc) have been shown to repel neural crest cells as well during their migration through the somites. However, we know very little about the migratory clues that guide the neural crest for the rest of their path and as little regarding Schwann cells. The goal of this study was to find which other molecules are capable of guiding the neural crest and its derivative the Schwann cell

precursor (SCP). For this purpose we had set out to screen a group of neurotrophic factors that are expressed at the same time that the crest is migrating through the embryo. Our results suggest that neural crest and SCPs are attracted to Heregulin, MIF and GDNF. We also tested the ability of neurotrophins to stimulate the migration of these cells, neural crest and SCP. Our results suggest that Heregulin and NGF enhance SCP motility, while Heregulin did not stimulate mature Schwann cells motility. These preliminary data suggests that neural crest and SCP use a variety of neurotrophic factors as guiding clues during their extensive migration in the embryo. This work was supported by a grant from NIH SCORE grant 2-SO6-GM048680-12A1 and a NMSS fellowship to FA1383-A-1.

MYELIN

B14

Targeted transport of stealth immunoliposomes to Schwann cells

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Manufacturing of systems which would be able to specifically transfer pharmacological agents to Schwann cells from systemic circulation is a topic problem of modern neuropharmacology [Partridge WM, 2003; Torchilin VP, 2006]. In the present study, we attempted to synthesize stealth immunoliposomes directed to Schwann cells. In our previous works, we had prepared monoclonal antibodies specific to myelin basic protein (anti-MBP-antibodies) which could bind to Schwann cells; later they were used as a vector for targeted transport of pharmacological agents [Chekhonin VP, 2001]. Stealth liposomes, consisting of cholesterol (38.5 mol%), lecithin (55.3 mol%), PEG-2000-bound phosphatidyl ethanolamine, and fluorescent label Dil (1%), were conjugated with thiolated anti-MBP-antibodies via a maleimide reaction [Lasic D.D, 1995]. A culture of the Schwann cells was prepared from 19-day rat embryonic spinal ganglia [Brockes JP, 1980; Brook GA, 1993]. The absorption degree was assessed in vitro. It was established that immunoliposomes specifically bound to myelin basic protein on the surface of Schwann cells. A preincubation of the cells with free anti-MBP-antibodies competitively inhibited specific binding of immunoliposomes (supposedly, because of the blockade of MBP). Control experiments performed with liposomes conjugated with nonspecific mouse immunoglobulines and with antibodies to neuron-specific enolase (nonspecific for Schwann cells), as well as additional tests performed with cultured rat fibroblasts had shown that this transport system was highly specific to myelin basic protein and, hence, to the Schwann cells. We concluded that the conjugation of monoclonal anti-MBP-antibodies with stealth liposomes allowed to make a highly specific container system for targeted transport of diagnostic and pharmacological agents to Schwann cells. Those systems may be useful for diagnostics and treatment of demyelinating diseases.

B15

The functional role of the raft protein MAL in axon-glia interaction

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The myelin and lymphocyte protein MAL is a raft-associated membrane protein expressed by oligodendrocytes and Schwann cells. We used genetically transformed MAL-expressing mice for studying it's functional role in myelinogenesis. In a recent study, we have shown that genetic ablation of MAL resulted in cytoplasmic inclusions within compact myelin, paranodal loops that are everted away from the axon and disorganized transverse bands at the axoglial junction in the adult CNS (Schaeren-Wiemers *et al.*, 2004). These structural changes were accompanied by a marked reduction of the paranodal proteins Caspr and NF155. Biochemical analysis revealed that MAG, MBP and NF155 protein levels were reduced in myelin and in myelin-derived rafts. These results demonstrate a critical role for MAL in the maintenance of the CNS axoglial junction and the nodal environment. In contrast to the CNS, the adult peripheral nerve structure did not disclose a qualitative difference in its morphology. Since in the PNS MAL is already expressed before myelination we have investigated its functional role during postnatal development in these mice. Our results show that MAL plays a role in the onset of myelination, and it influences the mean diameter of myelinated fibers during early development. We further investigated the myelin proteinsisolated from sciatic nerve tissues during development and in the adult by quantitative Western blot analysis. Our results suggest a functional role of MAL in myelin formation of the PNS, probably by influencing the trafficking of particular membrane components important in axon-glia interaction during the process of myelin initiation and sheath formation. Altogether, we suppose that MAL is involved in the assembly and targeting of particular transport vesicles, and in the stabilization and maintenance of particular glycosphingolipid-enriched membrane domains.

B16

Gene expression profile in CMT1B mice with activated unfolded protein response

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Myelin Protein Zero (MPZ, Po) is the most abundant glycoprotein in peripheral nerve myelin. In humans, diverse mutations in Po cause different hereditary neuropathies, suggesting gain of function mechanisms. For example, deletion (S63del) or conversion of serine 63 to cysteine (S63C) results in Charcot Marie Tooth disease 1B or Dejerine-Sottas syndrome, respectively. We showed that when expressed in mouse together with wild type Po, either mutant Po produces a demyelinating neuropathy that mimics the corresponding human disease (Wrabetz *et al.*, 2006).

While S63C is delivered to the myelin where it causes a packing defect, S63del never reaches the myelin sheath and is instead retained in the endoplasmic reticulum (ER)-Golgi complex. Accumulation of Ser63del in the ER triggers an unfolded protein response (UPR) in a dose-dependent fashion, indicating a toxic gain of function. Ablation of the transcription factor CHOP, a know mediator of the UPR, rescues the motor deficit in Ser63del mice suggesting that the UPR is pathogenetic. Gene expression profiling of Ser63del and Ser63del//CHOP −/− mice followed by bioinformatic analysis suggests that ER associated degradation (ERAD) and the control of protein translation could be involved in the motor rescue in Ser63del//CHOP −/− mice.

B17

Endocytic recycling is common to myelin proteins and promotes myelin subdomain morphogenesis

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The central nervous system myelin sheath is a multilayered specialized membrane, which is characterized by a highly organized subdomain structure. How these membrane domains evolve during myelin formation is unknown. Neuronal cells control the endocytic recycling of the major myelin protein proteolipid protein (PLP). We asked if endocytic trafficking is common to myelin proteins and analyzed the endocytic fates of proteins with distinct myelin subdomain localization. Interestingly, we found that PLP, myelin-associated glycoprotein (MAG), and myelin-oligodendrocyte glycoprotein (MOG), which localize to compact myelin, periaxonal loops, and abaxonal loops respectively, exhibit distinct endocytic fates. PLP was internalized via clathrin-independent endocytosis. MAG and MOG were both endocytosed by a clathrin-dependent pathway, however each reached a distinct endocytic compartment. Following endocytic sorting, PLP, MAG, and MOG recycled to distinct oligodendroglial membrane domains, which mimicked the biochemical characteristics of myelin subdomains. Thus, endocytic sorting and recycling is common to myelin proteins and promotes oligodendroglial plasma membrane remodeling necessary for myelin subdomain morphogenesis.

B18

Analysis of SNARE proteins involved in myelin formation

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Biogenesis and maintenance of the myelin membrane depends on the control of membrane trafficking pathways. Targeted vesicle fusion is mediated by SNARE-Proteins, thus we are investigating the role of SNAREs in myelin formation. Initially, we performed an expression profiling of post-Golgi SNAREs in oligodendroglial cells and found an expression of the t-SNAREs Syntaxin 2, 3, 4, 6, 7, 8, 13, 16, SNAP23, and SNAP29 as well as the v-SNAREs VAMP 2, 3, 4, and 7. Among these, Syn 3, 4, SNAP23, and VAMP3 are upregulated during differentiation whereas SNAP29 is downregulated. The major myelin protein PLP is enriched in the late endosomal / lysosomal compartment where it co-localises with Syntaxin 8 and VAMP7. In addition, a subpopulation of PLP co-localises with the v-SNARE VAMP3, which associates with the transferrin receptor-positive recycling endosome. To investigate the role of VAMP7 and VAMP3 in PLP trafficking, we overexpressed a dominant-negative VAMP7 protein or performed siRNA experiments for VAMP3 and measured the translocation of PLP to the plasma membrane. Overexpression of the N-terminal fragment of VAMP7 resulted in abnormal cell morphology and reduced PLP incorporation in the plasma membrane. Upon VAMP3 silencing, we observed a selective reduction of the db-cAMP dependent transport of PLP. These results suggest that the v-SNAREs VAMP7 and VAMP3 control the transport of the major myelin protein, PLP to the myelin membrane. Funding: ELA (to E.-M. K.-A.), EU FP6 ''Signalling and traffic'' (to J.T.)

B19

Maturation block of oligodendroglial progenitor cells in chronic MS lesions

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Multiple sclerosis (MS) is the most frequent demyelinating disease of young adults. MS lesions are characterized by demyelination, inflammation, axonal loss and reactive gliosis. Although remyelination is extensive in a subset of patients, in the majority of cases remyelination is absent or limited to the plaque border. Possible explanations for limited remyelination in MS lesions include lack of oligodendroglial progenitor cells (OPCs), axons, or growth factors or the presence of inhibitory molecules within the lesion. Olig2 and Nkx2.2 are transcription factors expressed by OPCs and mature oligodendrocytes. Recent studies have revealed the importance of these two factors for the differentiation of neural progenitor cells into the oligodendroglial lineage and the development to mature oligodendrocytes. In this report we demonstrate that the transcription factors Olig2 and Nkx2.2 identify oligodendroglial progenitors in adult human CNS. In the periplaque white matter and remyelinating areas of early MS lesions, increased numbers of oligodendroglial progenitor cells strongly expressing Olig2 were observed. A subpopulation of these progenitor cells also expressed Nogo-A, a marker of mature oligodendrocytes suggesting that these cells were recently recruited from the progenitor pool. OPCs were still present in chronic MS lesions, but their maturation to myelinating oligodendrocytes appeared to be impaired. Our data indicate that a maturation block of OPCs rather than a lack of OPCs contributes to the limited remyelination observed in chronic MS lesions.

B20

Imaging in vivo myelination in zebrafish

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We decided to study in vivo myelination in zebrafish because these organisms have proven to be extremely useful for imaging due to its transparency. Also, the CNS of fish retains the capacity to re-grow severed axons and restore functional connections with target areas into adulthood. This feature makes it an attractive model to study remyelination after injury of the spinal cord. In vivo time-lapse studies were

performed in transgenic zebrafish expressing GFP under the control of the PLP promoter (Yoshida and Macklin, JNR'05). GFP is expressed in oligodendrocytes (OLs) and Schwann cells (SCs) at the time that the myelination program starts, enabling analysis of the forming myelinating structures in vivo. For imaging, embryos were anesthetized, embedded in low-melting-point agarose, and mounted on a glass-bottom dish. Myelinating cells were visualized through the transparent skin with an inverted confocal laser-scanning microscope. Myelination by OLs in the spinal cord can be detected as early as 2 days post fertilization (dpf). At this developmental stage SCs can be seen migrating along the posterior lateral line (PLL); by 6 dpf SCs exhibit the typical morphology of myelinating cells and are engaging axons of the PLL nerve. The membrane of these cells exhibits a ribbon-like structure similar to the one observed in rat SCs. We have been able to targeted single OL for laser ablation without damage to the neighbor OLs along the spinal cord, neither to the axon that was engaged by it. Laser ablation of the OL cell bodies that myelinate a determinate axonal segment permits to create a very controlled area of demyelination. The arrival of OPCs to the damaged area, or neighbor OLs trying to repair the damaged myelin will be assessed by time-lapse.

B21

Myelin-associated glycoprotein, galactosylcerebroside and Caspr expression during Schwann cell differentiation and myelination

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The temporo-spatial expression profiles of the two myelin-associated glycoprotein isoforms (L-MAG and S-MAG), the galactosylcerebrosides (Gal-CBs), and Caspr were analysed by immuno-fluorescence confocal microscopy during Schwann cell differentiation and myelination in rat Schwann cell cultures and in dorsal root ganglion/Schwann cell cocultures. The temporal expression profiles demonstrate that the mechanisms triggering and up-regulating the expression MAG and Gal-CBs are different and independent. They also provide further support for a role of MAG, but not Gal-CBs or Caspr, in the establishment of the Schwann cell/axon interaction and in the formation of the primary mesaxon, while both S-MAG and Gal-CBs, but not Caspr, may participate in the further maturation of the mesaxon. L-MAG expression is observed already in Schwann cells that have not yet contacted an axon, and persists in the mature sheaths, where it is restricted to the periaxonal Schwann cell membrane. Very little, if any, L-MAG is detected in the paranodal regions and Schmidt-Lanterman incisures. The spatial expression profiles demonstrate that, while the Gal-CBs are present in regions from which MAG is excluded, they are highly concentrated in the same structures (mesaxonal spirals, paranodes and Schmidt-Lanterman incisures) as S-MAG in both developing and mature sheaths, where it is therefore possible that they could have a complementary functional relationship.

B22

The role of Septins in myelinating cells

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During myelination oligodendrocytes and Schwann cells are challenged to build up and maintain a highly complex multilaminar plasma membrane structure. It is well-known that myelin membranes are divided into subdomains with distinct protein and lipid composition. Recently, more insights into the sorting and trafficking mechanism of particular myelin proteins in their compartment are emerging. Polarized trafficking and sorting mechanisms require cytoskeletal components and in line with this, we have identified members of the Septin family of cytoskeleton proteins being incorporated into different myelin structures. Here, we show the coordinated regulation of multiple Septin isoforms during differentiation and myelination in Schwann cells and oligodendrocytes. On the protein level we identified that particular Septin isoforms were enriched in myelin membranes. There, they form distinct complexes both in CNS and in PNS as shown by immunoprecipation experiments. To identify their specific cellular localization, we analysed the protein distribution of Septins in teased peripheral myelinated fibers. As expected for soluble proteins, they can be localized in the Schwann cell cytoplasm namely the Cajal bands. Besides this the Septins are intriguingly concentrated in the microvilli and paranodal regions at the Node of Ranvier. Currently, we are investigating interaction partners of the Septin complexes with special focus on myelin components. To test the functional implication of Septins in formation and maintenance of the myelin sheath, we are currently using retroviral RNAi constructs selectively down-regulating individual Septin isoforms in in vitro myelinating DRG/Schwann cell co-cultures. We believe that distinct Septin-myelin membrane complexes play a role in establishing and maintaining cell polarity. In addition, Septins may have a strucural function at the Nodes of Ranvier and they might be involved in vesicle targeting in myelinating cells.

B23

NRG1/ErbB signaling in CNS myelination

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Oligodendrocytes and Schwann cells enwrap axons with a multilayered myelin sheath. A striking feature of myelin in the adult CNS and PNS is the finely tuned numerical ratio between the diameter of each axon, when viewed in cross section, and the thickness of the myelinated fiber (termed g-ratio). In contrast to Schwann cells, oligodendrocytes myelinate up to 40 axons that can differ in caliber. The neuregulin-1 gene (NRG1) encodes a family of EGF-like growth factors with multiple functions in the nervous system. We have previously shown that NRG1 type III is essential for myelination in the peripheral nervous system, and regulates the amount of myelin that is assembled by Schwann cells (Michailov *et al.* Science 304, 701–703, 2004). To better understand CNS myelination and human white matter diseases, it is important to determine whether oligodendrocytes respond to the same axonal signals. To investigate the consequences of altered NRG1/erbB signaling on brain development, and specifically on the myelination of CNS fiber tracts, we generated and analyzed a battery of mice with either reduced NRG1 gene dosage, neuronal NRG1 overexpression, as well as various conditional NRG1 null

mutations, as defined by Cre-recombination at different steps of development. Surprisingly, oligodendrocyte development and CNS myelination in conditional mouse mutants proceeds normally even in the complete absence of NRG1. In contrast, neuronal NRG1 overexpression caused many axons to become hypermyelinated, even in response to the soluble isoform NRG1 type I. Collectively, these data demonstrate that the regulation of myelination by NRG1/erbB signaling is substantially different in the central and peripheral nervous systems.

B24

Multiple roles of cholesterol in PNS myelination

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Cholesterol is a major structural component of the mammalian cell membrane. It is also involved in a variety of intracellular processes, including signal transduction and the formation of lipid rafts. In the brain up to 70% of cholesterol is associated with myelin. To investigate the function of cholesterol in myelin, conditional mouse mutants lacking functional cholesterol biosynthesis specifically in myelin-forming glia cells were generated using the loxP/Cre recombinase system. These mice show a severely hypomyelinated phenotype, ataxia, tremors and approximately 30% mortality by one month of age. Myelination in the CNS slowly normalizes, leading to an improvement of the shaking phenotype with age. In the PNS, however, hypomyelination is persistent and has still not reached wildtype levels by one year of age, resulting in progressive hindlimb paralysis. Nevertheless, axonal integrity is preserved. In addition to the severe delay in myelination, lipid and protein composition of peripheral myelin is changed, and frequently stretches of uncompacted myelin can be observed. This suggests that cholesterol plays a role in myelin compaction. Finally, many mutant Schwann cells fail to terminally mature and continue to express the promyelinating marker Oct6. The differences in pathology between PNS and CNS might be explained by a lack of cholesterol supply from non-targeted cells to mutant Schwann cells.

B25

Lovastatin induces abnormal myelin sheet formation by oligodendrocytes

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Statins, inhibitors of HMG-CoA reductase, are widely used to block cholesterol synthesis and protein isoprenylation. Recently, statins have been proposed as novel therapeutic drugs for multiple sclerosis, and lovastatin and simvastatin are currently tested in human trials. Although statins are thought to act predominantly via their immunomodulatory properties, both lovastatin and simvastatin can cross the blood-brain barrier and may therefore affect cellular processes in the central nervous system. This is especially relevant in respect to remyelination as a proposed therapeutic treatment for MS, since cholesterol is a major myelin component. Interfering with the cholesterol synthesis in oligodendrocytes may therefore be detrimental for the intrinsic repair mechanisms in MS tissue. Here we show that primary oligodendrocytes, treated with lovastatin, form extensive membrane sheets, which contain galactosphingolipids. Surprisingly, however, these membrane sheets are devoid of the major myelin specific proteins, MBP and PLP. Reduced MBP protein expression was confirmed by SDS-PAGE and Western Blotting. In situ hybridization experiments revealed that localization of MBP mRNA in oligodendrocyte processes was impaired suggesting that lovastatin blocks MBP mRNA transport. In addition, localization of PLP, which is predominantly localized in myelin sheets of control oligodendrocytes, was restricted to cell body and primary processes after treatment with lovastatin. Hence, the data demonstrate that expression and/or transport of the two main myelin proteins is inhibited by lovastatin. Interestingly, another inhibitor of cholesterol synthesis (Ro 48-8071), which does not interfere with isoprenylation, had a similar effect on the localization of PLP, but did not affect MBP expression. These results suggest that lovastatin impairs PLP transport due to inhibition of cholesterol synthesis, whereas reduced MBP expression is caused by impaired isoprenylation. In general, our results demonstrate that it is essential to carefully monitor the effect of statins on myelin formation prior to their use in demyelinating diseases.

B26

Distinct roles of syntaxins 3 and 4 in the biogenesis of myelin membranes in oligodendrocytes

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The myelin membrane is a sheet-like extension of the plasma membrane of oligodendrocytes that can be considered as a specialized domain consisting of myelin-specific lipids and proteins, and its biogenesis and maintenance presumably requires specialized sorting and targeting devices. As part of the SNARE machinery regulating intravesicular transport and membrane docking at target membranes, we demonstrate that in oligodendrocytes syntaxin-3 and -4 are distributed in a polarized manner, the former being enriched in the cell body and the latter in the myelin sheet. Whereas syntaxin-3 is present throughout development, syntaxin-4 becomes significantly expressed when oligodendrocytes differentiate concomitant with myelin sheet biogenesis. Interestingly, overexpression of syntaxin-3, shown to impede syntaxin-3-mediated fusion, caused an accumulation of the myelin protein proteolipid PLP in the cell body, which was accompanied by a shift in PLP membrane localization, i.e., from a Triton-X100 soluble to a Triton-X100-insoluble localization. These data suggest that PLP transport involves a syntaxin-3 dependent transport step to the plasma membrane, after which a transcytotic mechanism mediates transport to the sheet. Interestingly, this transport step apparently does not depend on sheet-localized syntaxin-4, as its overexpression was without any effect on PLP transport. Surprisingly, syntaxin-4 overexpression completely inhibited 'non-vesicular' MBP-mRNA transport, thereby preventing MBP expression in the sheet, but not its biogenesis. Thus, as part of the mechanism to establish membrane polarity,

oligodendrocytes exploit a polarized distribution of syntaxins 3 and 4, and, moreover, syntaxin-3 and -4 differentially regulate sheet-directed transport of the two major myelin-specific proteins, PLP and MBP.

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B27

Role of Lgi4 in nerve development and myelination

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Development of the peripheral nerves depends on the interactions between neurons, Schwann cells and the surrounding mesenchymal tissue. While the neurons transmit information from one part of the body to the other, the Schwann cells assist and support the neurons. Myelination of the axons of neurons by Schwann cells provides faster conduction of nerve impulses. The cellular processes that govern the development of peripheral nervous system (PNS) and myelination have been extensively studied, but many of the molecular mechanisms involved are yet to be identified. The claw paw (clp) mutant mice display delayed axonal sorting and hypomyelination in the PNS; therefore this model provides a useful tool for the identification of molecules and the study of mechanisms involved in PNS development. The clp mutation has been identified as an insertion in the Lgi4 gene. Lgi4 protein contains two repeat elements (leucine-rich repeat and epilepsy associated repeat) that are implicated in protein-protein interactions. We have identified Lgi4 as a secreted and glycosylated protein, which is expressed throughout the PNS, mainly in Schwann cells. On the other hand, the clp mutation results in a mutant Lgi4 protein that is retained inside the cell. Exogenous wild type Lgi4 protein restores the myelination defect in clp/clp neuron-Schwann cell co-cultures, indicating that the PNS abnormalities in clp mice arise from the loss of Lgi4 function. Further characterization of Lgi4 in a spatial and temporal context, and the identification of its interaction partners would thus be an important step in the elucidation of the mechanisms controlling PNS development and myelination.

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B28

Development and characterization of a mouse model of Charcot-Marie-Tooth disease 4C

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Charcot-Marie-Tooth disease (CMT4C, OMIM #601596) is a rare form of autosomal recessive demyelinating neuropathy with an early onset. The clinical manifestations include delayed age of walking, distal weakness, muscle atrophy, reduced nerve conduction velocity and progressive scoliosis. Histologically, thin myelin, "giant axons", basal lamina "onion bulb" formation and extended Schwann cell processes are observed on a nerve biopsy. The gene mutated in CMT4C disease, KIAA1985 was recently identified (Senderek *et al*, 2003) however the function of the protein it encodes remains unknown. In order to obtain an insight into the role of KIAA1985 we have decided to characterize the function of its mouse orthologue, the mKIAA1985. We have generated mKIAA1985 knockout mice where we have replaced the first exon of the mKIAA1985 gene with an eGFP cassette. The homozygous mKIAA1985−/− animals develop early onset peripheral neuropathy manifested by decreased motor nerve conduction velocity and decreased myelin thickness. Transcriptional analysis performed on the endoneurial compartment of developing peripheral nerve isolated from control and mKIAA1985−/− animals revealed a concordant decrease in all tested myelin markers confirming the presence of peripheral neuropathy in the knockout animals. The successful creation of an animal model of CMT4C disease should now allow us to study in detail the role of KIAA1985 in peripheral nerve, the pathological mechanisms leading to the neuropathy in CMT4C patients, and may also provide a model for the development and evaluation of potential therapeutical approaches leading to the treatment of neuropathy.

B29

Lipin1-regulated lipid metabolism is essential for Schwann cell myelination

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Lipin1 knockout animals ($Lpin1^{fld/fld}$) are characterized by a reduced adipose tissue mass (lipodystrophy), insulin resistance, and a progressive peripheral neuropathy. Sciatic nerve analyses in these mice revealed severe demyelination, poorly compacted myelin sheaths, active myelin breakdown, and hypertrophic Schwann cells. In order to study the role of Lipin1 in the development and maintenance of the peripheral nervous system, we have used a conditional knockout approach to inactivate its function selectively in Schwann cells. We have generated mice in which the coding region of Lipin1 is flanked by loxP sites ($Lipin1^{floxEx2–3/floxEx2–3}$) and crossed it with the myelin protein zero-Cre transgenic mice (Mpz-Cre). While epineurial fat deposits are not affected in $Lipin1^{\Delta Ex2–3/\Delta Ex2–3}$/Mpz-Cre mice, the endoneurial compartment shows a demyelinating phenotype and an accumulation of lipid debris similar to the complete Lipin1 knockout mice. In addition, we have observed a significant decrease of Mg2+-dependent phosphatidate phosphatase (PAP1) activity in endoneurium of both complete and Schwann cell specific Lipin1 knockout mice, consistent with the recently suggested function of Lipin1 as PAP1 in liver, WAT and kidney. These results demonstrate that the pronounced peripheral neuropathy evident in the mutants is a direct consequence of the absence of Lipin1 within Schwann cells and strongly suggests that during peripheral nerve myelination, the Lipin1 mediated PAP1 activity plays a crucial role in the in situ production of diacylglycerol

needed for the synthesis of triacylglycerol and phospholipids by the Schwann cells.

B30

Yin Yang1, a critical transcription factor for oligodendrocyte progenitor differentiation

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Myelination of the nerves is essential for quick saltatory electronic signal conduction through the axons. In the central nervous system, myelination is carried out by oligodendrocytes. Previous studies in our laboratory have shown that at chromatin level, deacetylation of histones by HDACs is a necessary step. To identify possible molecular links between histone acetylation and oligodendrocyte differentiation, we screened the promoters of genes regulated by HDAC during oligodendrocyte differentiation for the presence of common binding motifs. This analysis revealed the presence of transcription factor Yin Yang1 (YY1) binding motif on 25% of the genes examined. Conditional knockout mice deleting yy1 specifically in oligodendrocyte lineage were generated by crossing yy1 flox/flox line with cnp1-cre mice. The yy1 cko mice developed tremor, ataxia and head wobbling during first 2–3 weeks and progressively deteriorated to paralysis by the age of 2 months. EM studies revealed sever hypomyelination in the central nervous system of yy1 cko mice. This hypomyelination is caused by the arrest of oligodendrocyte progenitor differentiation, while the apoptosis or proliferation is relatively unaffected. In addition, in vitro ablation of YY1 in nestin+ neuronal precursor cells impairs oligodendrocyte differentiation in a cell type-specific manner. At molecular level, this arrest is associated with persistent histone acetylation and high levels of transcriptional inhibitors (Tcf4 and Id4). Therefore YY1 emerges as a critical regulator of oligodendrocyte differentiation.

B31

PtdIns(3,4,5)P3 triggers myelin outgrowth by Schwann cells

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In the PNS, myelin is a spiral extension of the plasma membrane of a Schwann cell that insulates the axon for rapid impulse propagation. We have previously shown that myelin sheath thickness is regulated by axonal NRG1. However, it is unclear how glial erbB receptors signal through compact myelin to the Schwannn cell nucleus. One attractive second messenger is membrane-diffusible PtdIns(3,4,5)P3, synthesized by PI3 kinase. This lipid is normally antagonized by PTEN, a 3-phosphoinositide phosphatase. To test our model, we conditionally inactivated a floxed PTEN gene in Schwann cells hypothesizing that elevated PtdIns(3,4,5)P3 levels should cause hypermyelination similar to axonal NRG1 overexpression. Conditional mutants die prematurely at 4–5 months of age (presumably due to a thyroid hyperplasia). At this time, the PNS exhibits a higher number of Schwann cells and myelinated axons, the latter recruited from the pool of normally unmyelinated C-fibers. In support of our model, small calibre axons (below 2μm in diameter) are significantly hypermyelinated. Unexpectedly, large caliber axons show hypermyelination as tomacula-like focal myelin outgrowth that predominantly arises from regions of non-compact myelin, possibly the sites of normal PTEN localisation. Loss of PTEN from the non-myelinating Schwann cells causes a novel, spiral enwrapping of C-fiber axons, with several axons in a Remak bundle ensheathed by multiple layers of non-compact membranes, reminiscent of early steps in myelination. Occasionally, this ''Remak-myelin'' incorporates the cell adhesion protein Po and becomes compacted. Surprisingly, even collagen fibrils are found to be spirally enwrapped by Remak Schwann cells with multiple layers of membrane. Thus, myelination by glial cells can proceed in principle cell-autonomously, i.e. in the absence of continuous axonal signals.

B32

An in vitro model for specific de- and remyelination using whole brain spheroid culture

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An in vitro model for specific de- and remyelination using whole brain spheroid culture Vereyken E.J.F., Fluitsma D.M., Dijkstra C.D. and Teunissen C.E. Department of Molecular Cell Biology and Immunology, VUMC, FdG, PO Box 7057, 1007 MB Amsterdam, the Netherlands. Tel: +31 20 4448083; Fax: +31 204448081; e-mail: ejf.vereyken@vumc.nl The model most often used to study demyelination in MS is the experimental autoimmune encephalitis (EAE) model. This model has drawbacks: demyelination is limited, large numbers of animals are necessary and involvement of the immune system makes it difficult to differentiate the contributions of different cell types. Therefore, an in vitro model for de- and remyelination is necessary. The aim of this study was to develop such a model using lysophosphatidyl choline (LPC) in whole brain spheroid cultures. The neuronal and glial cell types present form 3D contacts, leading to multilayered myelin. Using the spheroid culture system we investigated the characteristics of repeated exposure to LPC. LPC and various drugs were added 3 times a week, after which medium was replaced and spheroids were left to recover for another week. Decreased 2',3', cyclic nucleotide 3'-phosphodiesterase (CNPase) activity, myelin basic protein (MBP) staining and concentration was observed after 1 week of exposure to LPC. These results are indicative of demyelination, confirmed by EM. CNPase activity and MBP-staining and concentration were normalised when spheroids were treated with LPC and either vitamin E, Simavastatin or cholesterol. After one week of recovery MBP-staining and CNPase activity normalized, suggestive of remyelination. The protective effect of vitamin E, simvastatin and cholesterol against LPC toxicity may be explained by the decline in both LPC induced ROS generation in myelin and loss of membrane integrity. The specific de- and remyelination observed supports the use of this model in research into mechanisms of remyelination and preclinical drug testing in MS.

B33

Dominant-negative β1 integrin transgenic mice reveal differential requirements for β1 integrin in cns and PNS myelination

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Previous work in our lab has shown that integrins, cell surface receptors for extracellular matrix molecules, make an important contribution to oligodendrocyte development. In order to study β1 integrin function in myelinating cells in vivo, we have generated dominant-negative β1 transgenic mice (dnβ1) whereby IL2R-β1 is expressed under the myelin basic protein (MBP) promoter. Myelination was then analysed in the dnβ1 by electron microscopy in optic nerve, cerebellum and sciatic nerve at different ages. In the CNS, no difference in the percentage of unmyelinated axons or in the myelin, as measured by g-ratio, was observed. In the PNS, however, the phenotype resembles the Schwann cell phenotype obtained by Feltri *et al* in the conditional β1 KO, albeit less severe, where a delay in the process of axonal sorting is present at earlier ages. We conclude that there are different requirements for β1 integrin in CNS and PNS myelination.

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B34

Transgenic mouse model of congenital hypomyelination neuropathy due to mutation in human myelin protein zero

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The autosomal dominant Q215X mutation in the MPZ gene causes an extremely severe, early-onset dysmyelinating peripheral neuropathy (congenital hypomyelination) with motor deficits. Po (the MPZ product) is the most abundant protein in nerve and is a trans-membrane glycoprotein with adhesive properties. The Q215X mutation truncates the last 33 residues of the cytoplasmic domain. To characterize pathogenesis, we targeted the Q215X mutation to the mouse Mpz gene, leaving a loxP site in Mpz intron 5. Although transgenic mice manifest no external neuropathic phenotype, rotarod test on P11 mice shows a motor deficit in the mutant mice. Morphological analysis of Q215X heterozygous mice reveals hypomyelination postnatally and at all ages, but in particular, the presence of bundles of unsorted mixed-caliber axons at P11. This phenotype is distinct from that of heterozygous Mpz-null nerves, suggesting gain of function. Accordingly, PoQ215X protein is synthesized, and smaller (~23kD) as expected, but there is a significant reduction (~80%) of MpzQ215X mRNA. We detect no nonsense mediated decay of mutant message. Rather targeted mRNA is reduced in both Q215X mice, and in control mice containing only the loxP site in intron 5, suggesting a LoxP-dependent mechanism. To separate the effect of the Q215X mutation from the general reduction of Po protein present in these mice, we crossed Q215X mice with mice overexpressing wt-Po. These mice showed amelioration of hypomyelination, but maintained the sorting defect previously observed. We conclude that the MpzQ215X mutation in mice causes a transient delay in radial sorting of axons and congenital hypomyelination with an associated motor deficit. The mutation acts through gain of function; accordingly the phenotype in mice is probably ameliorated by limited dosage.

B35

Differential myelin proteome analysis: PLP/DM20 is required for transport of SIRT2 into central nervous system myelin

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Mice lacking the expression of PLP/DM20 in oligodendrocytes provide a genuine model for spastic paraplegia (SPG-2). Their axons are well myelinated but exhibit impaired axonal transport and progressive degeneration, which is difficult to attribute to the absence of a single myelin protein. We have hypothesized that secondary molecular changes in PLP-deficient myelin contribute to the loss of PLP/DM20-dependent neuroprotection and provide more insight into glia-axonal interactions in this disease model. By gel-based proteome analysis, we identified >160 proteins in purified myelin membranes, which allowed us to systematically monitor the CNS myelin proteome of adult PLP-deficient mice, prior to the onset of disease. We identified three proteins of the septin family to be reduced in abundance, but the NAD+/NADH-dependent deacetylase sirtuin 2 (SIRT2) was virtually absent. SIRT2 is expressed throughout the oligodendrocyte lineage, and immuno-electron microscopy revealed its association with myelin. Loss of SIRT2 in PLP-deficient myelin was post-transcriptional, suggesting that PLP/DM20 is required for its transport into the myelin compartment. Since normal SIRT2 activity is controlled by the NAD+/NADH ratio, its function may be coupled to the axo-glial metabolism and the long-term support of axons by oligodendrocytes.

B36

Choline transporter-like proteins in normal human CNS and multiple sclerosis lesions

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Changes in choline metabolism have been observed in the CNS of multiple sclerosis patients using MRI techniques. Among the five CTL (choline transporter-like, also termed SLC44A1–5) genes found in humans, CTL1 and CTL2 are the most highly expressed in the CNS. CTL1 mRNA has been localized to oligodendrocytes and specific groups of neurons throughout the nervous system, while CTL2 expression is more restricted. We have characterized CTL1 and CTL2 distribution using antibodies developed against fusion proteins and peptides representing different domains of the two proteins. Several of these antibodies appear to be specific for CTL protein isoforms which are generated by alternative

splicing and by post-translational modifications. In normal control white and grey matter samples, specific CTL1 and CTL2 antibodies labelled 60–70 kDa MW bands on immunoblots, and oligodendrocyte and neuronal cell bodies were visualized by immunohistochemistry. A striking change in CTL protein labelling occurs in multiple sclerosis lesions, with the appearance of lower MW bands recognized by antibodies to both CTL1 and CTL2. A subpopulation of swollen axons and also macrophages were immunolabelled by some CTL1 antibodies in active multiple sclerosis plaques. Understanding more about the mechanisms inducing CTL1 and CTL2 protein modification will shed light on how and why choline metabolism changes in multiple sclerosis lesions.

B37

Myelin protein expression by olfactory ensheathing glia in vitro: a comparison to Schwann cells

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A crucial aspect of repairing the damaged CNS is the restoration of myelin around axons, so functional conductivity can be restored. Olfactory ensheathing glia is one cell type reported to provide such a role in the damaged CNS. We have examined levels of protein zero (PO), myelin basic protein (MBP), myelin associated glycoprotein (MAG) and 2'3'-cyclic nucleotide 3' phosphodiesterase (CNP) within the olfactory bulb and in primary cultures of OEG. Analysis was carried out in vitro using different growth mediums supplemented with mitogens, neuregulins, and cAMP analogues. Adult Schwann cells (SCs) were used as a comparison, due to OEG producing 'SC-like myelin' within the CNS. Myelin proteins were examined with immunocytochemistry and western blots (WBs). RNA levels were measured by PCR and realtime PCR. Results in vitro indicated OEG showed increased levels of PO protein compared to SCs when cAMP,was present and this was reduced by the addition of serum. PO protein is present in SCs when grown in serum free media supplemented with neuregulin $1 (NRG1), however no PO protein was observed in OEG. In all mediums, no basal MAG or MBP protein was present in OEG with WBs. CNP protein was present in OEG and SCs in all tested mediums.but CNP levels were lower in OEG especially in the presence of cAMP. Basal RNA levels in OEG showed expression of all myelin genes, with these regulated by addition of NRG1 and cAMP. Results suggest that OEG have some non SC-like characteristics of myelin gene expression in vitro. Furthermore, these differences may indicate an alternate regulatory mechanism of myelin expression.

B38

Multiple fates of nascent PLP in a model of Pelizaeus-Merzbacher disease with increased PLP1 gene dosage

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Increased dosage of the proteolipid protein (PLP1) gene is the most common cause of Pelizaeus-Merzbacher disease, resulting in dysmyelination. We have used a transgenic mouse model to study the dynamics of PLP/DM20. Small increases in Plp1 gene dosage present in hemizygous mice do not cause dysmyelination and have no significant effect on the rates of translation, degradation and myelin incorporation of PLP/DM20. In contrast, homozygous mice with increased gene dosage sufficient to cause dysmyelination results in a modest decrease in PLP translation, a markedly enhanced rate of degradation and a severe reduction in incorporation of PLP into myelin. Treatment with MG132 prevented the enhanced degradation, suggesting the involvement of the proteasome. Surprisingly, steady state levels of PLP/DM20 in oligodendrocyte cell bodies, as opposed to myelin fractions, are not reduced and this PLP/DM20 pool was probably associated with autophagic vacuoles and lysosomes. Within the myelin sheath, PLP/DM20 is normally associated with various lipids partitioning in the low-density fractions after extraction by CHAPS detergent. This association is perturbed in the dysmyelinated mice with PLP/DM20 partitioning in both low and high-density fractions. We suggest that the vast majority of PLP/DM20 generated by increased gene dosage is degraded by the proteasome although a small proportion enters the myelin sheath. Whether the presumptive long-lived pool in autophagic vacuoles and lysosomes derives from the cell membrane or from newly synthesised protein has yet to be determined.

B39

Critical role of schwannomin/merlin in mature Schwann cells

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Schwannomin/merlin is the product of a tumor suppressor gene mutated in neurofibromatosis type 2 (NF2). Although the consequences of NF2 mutations on Schwann cells proliferation are well established, the physiological role of schwannomin in differentiated cells is not known. We studied peripheral nerves in mice overexpressing specifically in Schwann cells mutated schwannomin bearing either a deletion occurring in NF2 (SCHD39–121) or a C-terminal deletion. The myelin sheath was preserved in both lines and nodal regions appeared normal. In contrast, paranodes and juxtaparanodes were disorganized and the limits between these regions poorly defined. The electrophysiological properties of sciatic nerves were only marginally altered. To determine whether these abnormalities were secondary to a dominant negative effect or to a gain of function of overexpressed mutated schwannomin, we studied mice with a conditional deletion of the Nf2 gene in Schwann cells. These mice had a phenotype similar to that observed in SCHD39–121 mice, albeit more severe. SCHD39–121 and conditional mutant mice displayed short internodes and a markedly increased number of Schmidt-Lanterman incisures. Thus, schwannomin plays an important role in mature Schwann cells and is necessary for the correct organization of axo-glial contacts. These results also suggest that NF2 mutations may alter nerve functions.

B40

Functional analysis of myelin basic protein gene regulation

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By locating and characterizing the relevant regulatory elements and combinatorial relationships required to drive coordinate expression of the myelin gene family, we hope to learn more of the mechanism/s regulating myelin sheath formation, maintenance and repair. In our initial search for regulatory regions within the mbp gene, four highly conserved non-protein coding modules were encountered in its 5' flanking sequence. In the context of reporter constructs in transgenic mice, these regulatory modules confer distinct cell specificity and developmental expression programs. M1, M2 and M3 drive expression in oligodendrocytes while M4 drives Schwann cell expression. However, these reporter constructs also exposed higher levels of regulatory organization; e.g., when M3 is dissociated from neighboring flanking sequences it acquires the ability to drive transient expression in Schwann cells. Thus, to complement the reporter gene studies, and to explore the role of M3 and M4 in the integrated mbp regulatory system, we derived mice bearing deletions of M3 or M4 within the endogenous mbp gene. Oligodendrocytes in the M3 knockout mice accumulate only 60% of wildtype mbp mRNA levels and form proportionately thinner myelin sheaths. Despite this disruption, the accumulated mRNA levels realized from other myelin genes, such as plp, are unaltered. No change in mbp mRNA levels or myelin was observed in Schwann cells. Notably, only M3 bearing reporter constructs are expressed in remyelinating oligodendrocytes suggesting that M3 may play an essential role in regulating mbp during myelin elaboration in the mature CNS. To determine if this conclusion extends to the integrated regulatory mechanism operating at the endogenous mbp locus, we have initiated remyelination experiments with the M3 knockout mice.

B41

The proximal promoter of myelin basic protein (MBP) gene restricts expression to myelinating oligodendrocytes

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The goal of this investigation is to identify and characterize the minimal combination of regulatory elements sufficient to drive expression of a myelin gene, MBP. Transgenes containing various deletions and mutations of the MBP proximal promoter were inserted in a single copy at the hypoxanthine phosphoribosyl transferase (Hprt) locus. The MBP proximal promoter, composed of 377 bps upstream of the ATG start site, drives expression of b-galactosidase reporter gene specifically in oligodendrocytes. The 252-bp subsequence located between nucleotides −377 and −125, is capable of driving expression when cloned in reverse orientation with respect to a 300-bp heat shock protein (HSP68) minimal promoter. Although a further truncated sequence (−377 to − 228) also drives expression in oligodendrocytes, it does so at detectable but low levels. Within this 149-bp subsequence, the 40 bps between −340 and −300 are highly conserved amongst different species and reporter gene experiments demonstrate that it contains element(s) that are required, but not sufficient, for expression. The proximal promoter also plays a role in restricting expression in other cell types since mutation of a putative nuclear receptor binding site between nucleotides −91 and −80 results in ectopic reporter gene expression. In summary, this investigation so far reveals that the MBP proximal promoter achieves oligodendrocyte-specific expression using a combination of negative and positive regulatory elements.

B42

Myelinogenic cell behaviour after ethidium bromide injection in the brainstem of Wistar rats submitted to the streptozotocin diabetogenic model

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Schwann cell disturbance followed by segmental demyelination in the peripheral nervous system occurs in diabetic patients. As Schwann cell and oligodendrocyte remyelination in the central nervous system (CNS) is a well known event in the ethidium bromide (EB) demyelinating model, the aim of our investigation was to observe the behaviour of both cells after local EB injection in the brainstem of streptozotocin diabetic rats. Adult male Wistar rats were used and some received a single intravenous injection of streptozotocin (50 mg/kg), being submitted 10 days later to a single injection of 10 μl of 0.1% EB or 0.9% saline solution into the cisterna pontis. Ten microlitres of 0.1% EB were also injected in non-diabetic rats. The rats were anaesthetized, perfused through the heart from 7 to 31 days after EB or saline injection and brainstem sections were collected and processed for light and transmission electron microscopy studies. Comparison between the final balance of myelin repair in diabetic and non-diabetic rats at 31 days was assessed using a semi-quantitative method for documenting the extent and nature of remyelination in semithin sections from the EB-induced lesions. Diabetic rats presented delayed macrophagic activity and lesser remyelination in comparison to non-diabetic rats. Although oligodendrocytes were the major remyelinating cells in the brainstem, Schwann cells invaded EB-induced lesions, first appearing at 11 days in non-diabetic rats and by 15 days in diabetic rats. Results indicate that short-term streptozotocin-induced diabetes hindered both oligodendrocyte and Schwann cell remyelination (mean remyelination scores of 2.57 ± 0.77 for oligodendrocytes and 0.67 ± 0.5 for Schwann cells) in comparison to non-diabetic rats (3.27 ± 0.85 and 1.38 ± 0.81, respectively).

B43

Spinal cord remyelination following local injection of ethidium bromide under cyclosporine treatment

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The model of toxically-induced demyelination by ethidium bromide (EB) was used to study the cellular events involved in spinal cord remyelination under an attempt of pharmacological intervention in the process with the immunomodulating agent cyclosporine (CsA). Male Wistar rats were used, some of them submitted to lumbar spinal cord injection of 10 microlitres of 0.1% EB or 0.9% saline solution, and others taken as histologic controls (group I). The animals injected with EB were divided into 2 groups - those that did not receive any immunosuppressive treatment (group II) and those that, from the day of the gliotoxic agent injection, were treated by intraperitonial route with CsA (group III), using

10mg/kg/day during 7 days with a maintenance schedule of the same dose 3 times a week. As for the animals of group IV, they were injected with saline solution and treated with CsA. Spinal cord sections were collected from the 15th to the 31st day after EB or saline injection for light and transmission electron microscopy studies. In the different evaluated times, the presence of phagocytic cells and non-degraded myelin debris in the extracellular space was noted, as well as demyelinated and remyelinated axons. The remyelination was carried out by Schwann cells and surviving oligodendrocytes, the first appearing specially around blood vessels and in subpial areas. Groups of infiltrating pial cells, hypertrophic astrocytes and some lymphocytes were noted. In rats from group III, the repair process of the EB-induced lesions was similar to that of group II. However the most important finding was the presence of a higher density of oligodendrocytes near the remyelinating axons at the periphery compared to group II.

B44

FGF Receptor-2 in oligodendrocytes and myelin

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FGF Receptor-2 (FGFR2) is expressed coincidentally with major myelin proteins as OLs enter terminal differentiation; it is expressed by OLs in myelinated fiber tracts of adult rodent brain, spinal cord and optic nerve.

We have shown (1) that activation (or inhibition) of FGFR2 expression leads to stimulation (or inhibition, resp.) of OL process outgrowth and myelin-like membrane formation in culture. Here we show that FGFR2 is present in OLs and myelin in three isoforms: glycosylated 120 and 105kD isoforms correspond to the inclusion of three and two Ig-like extracellular domains, resp.; and a novel 50kD isoform lacking the extracellular ligand binding region. The 120kD form is the most abundant tyrosine phosphorylated protein in OLs even in the absence of FGF stimulation. All three isoforms are associated with the detergent insoluble, "lipid raft" fraction, the proportions being more in myelin (50%) than in OLs (25%). The expression of FGFR2 by neurons and astrocytes is low or absent; FGFR2 expressed by astrocytes in culture has a higher molecular weight than in OLs, is not associated with lipid rafts, and it does not include a 50kD isoform.

Recently (2) we demonstrated that mice lacking both FGFR2 and CNP display a schizophrenia-like syndrome associated with dopamine-related hyperactivity; correspondingly, we find that FGFR2 mRNA is significantly down-regulated in human schizophrenia brains. Molecular characterization of FGFR2 is expected to provide a better understanding of FGF-mediated signaling in both normal function and neurological disorders.

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NEUROIMMUNOLOGY AND NEUROINFLAMMATION

B45

Dual role of CD38 in microglial activation and activation induced cell death

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Microglia, the resident immune cells of the central nervous system, are normally quiescent but become activated after infection or injury. When activated they change their properties and promote both repair and damage process. The numbers of activated microglia is strictly regulated, in part by 'activation-induced cell death' (AICD) which is mediated via the effect of nitric oxide (NO) on the mitochondrial pathway. Although many aspect of microglia AICD's mechanism have been unraveled very little is known about the connection between the activation and the cell death processes. We show here using primary microglial cultures that the ectoenzyme CD38, via its calcium mobilizing metabolite cyclic ADP ribose (cADPR), plays an important role in promoting the lipopolysaccharide (LPS) and interferon (IFN)γ (LPS/IFNγ)-induced microglial activation and AICD, therefore linking between these two processes. Accordingly, CD38 expression and activity as well as intracellular calcium concentration $[Ca^{2+}]_i$ are increased by the LPS/IFNγ treatment in primary microglia. Moreover, CD38 deficiency and treatment with cADPR antagonists confer partial resistance to LPS/IFNγ-AICD and reduced $[Ca^{2+}]_i$. Importantly, microglial activation as indicated by induction of NOS2 (iNOS), TNFα ,Interleukin (IL)-12 p40 and IL-6 expression, was attenuated by CD38 deficiency and treatment with cADPR antagonists. The effect of CD38 on microglial activation is most likely mediated via a cADPR-dependent increase in $[Ca^{2+}]_i$ whereas the effect on AICD by regulation of NO production. Our results thus suggest CD38 plays an important role in the regulation of the number and function of activated microglia and that this effect can be important in brain injury and repair processes.

B46

A functional role for EGFR signaling in myelination and remyelination

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Cellular strategies for oligodendrocyte regeneration and remyelination involve characterizing endogenous neural progenitors capable of generating oligodendrocytes during development and after demyelination, and identifying the molecular signals that enhance oligodendrogenesis from these progenitors. Migratory NG2+ progenitor cells of the postnatal SVZ express higher EGFR levels than non-migratory, cortical NG2+ cells. The higher endogenous EGFR expression in SVZ NG2+ cells is causally related with their migratory potential in vitro and in vivo after cell engraftment. EGFR overexpression in cortical NG2+ cells converted these cells to a migratory phenotype in vitro and in vivo. Finally, cortical NG2+ cells purified from a transgenic mouse in which the human EGFR (hEGFR) is overexpressed under the CNP promoter exhibited enhanced migratory capability. CNP promoter-driven hEGFR overexpression accelerates remyelination following focal demyelination of corpus callosum (CC). Repopulation of the lesion by NG2+Mash1+Olig2+ progenitors and remyelination occur more rapidly in CNP-hEGFR mice than in wild-type. hEGFR overexpression in SVZ and CC during early postnatal development expands this NG2+Mash1+Olig2+ progenitor population, enhancing oligodendrocyte generation and axonal myelination. Retroviral injection into the SVZ after focal demyelination demonstrated: i) direct migration of NG2+Mash1+Olig2+ progenitors into the lesion, and ii) a large increase in the number of migrating progenitors in the CNP-hEGFR mouse, as compared to wild-type. Analysis of EGFR-null mutant mice confirmed that EGFR signaling regulates oligodendrogenesis and remyelination by NG2+Mash1+Olig2+ progenitors. Our study identifies EGFR targeting as a new strategy to enhance oligodendrocyte regeneration and myelin repair. Supported by NINDS.

B47

The astroglial culture and the uninvited microglia: a cautionary tale

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Primary rodent astroglial-enriched cultures are the most popular model to study astroglial biology in vitro. Many variations of the original protocols exist that result in cultures in which the astrocyte is predominant, but astrocytes are not 100% of cells in these preparations. The aim of this poster is to bring attention to the presence of microglia in astroglial cultures since in my opinion the proportion and the role that microglial cells play in astroglial cultures are often underestimated. In this presentation specific markers that allow the estimation of the microglial proportion in an astroglial-enriched culture, as well as factors on the culture protocol that affect the proportion of microglia will be reviewed. Too often the presence of microglia in astroglial-enriched cultures is ignored and small amounts of microglia can be responsible for effects observed on astroglial-enriched cultures. If the relative contributions of astrocytes and microglia are not properly assessed an observed effect can be erroneously attributed to the astrocyte. The case of NO production is in my opinion a clear example of such an error. In a great number of publications NO production and NOS-2 expression in activated astroglial-enriched cultures are attributed to astrocytes. However, a careful review of the literature suggests that such effects are produced basically by ''contaminating'' microglial cells. In summary: 1) when claiming astroglial-enriched cultures (>95% astrocytes) authors should use adequate protocols to estimate and to minimize the presence of microglial cells, and 2) when working with astroglial cultures authors should clearly demonstrate which is the cell type (astrocytes, microglia, other contaminating cells ...) that is responsible for an observed effect and not assume that it is caused by the astrocyte just because this is the predominant cell type.

B48

Distinct signalling pathways and modes of microglia activation associated with different brain pathologies

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Microglia are highly plastic and multifunctional immune cells of the brain that execute various functions and rapidly respond to pathological insults. In neurodegenerative diseases and acute brain injuries, activated microglial cells express pro-inflammatory cytokines and release toxic factors. Inflammatory microglial signalling involves activation of NFκB and MAP kinases as critical signal transducers. Inhibition of acute microglia activation attenuates brain injury. The most dangerous brain tumours gliomas recruit microglia to the tumour site, and transform into tumour-supportive cells with no typical features of inflammation. We hypothesized that microglia activated under various conditions may be functionally different. We demonstrate that in contrast to lipopolysaccharide (LPS)-induced activation involving all MAP kinases and production of inflammatory mediators, glioma-derived factors induce: p38 MAPK and JNK signalling, release of factors promoting glioma invasiveness without pro-inflammatory responses. Instead, glioma-exposed microglial cells produce anti-inflammatory mediators: TGFβ1 and IL-10, which stimulate motility and invasiveness of glioma cells, microglial migration and phagocytosis. Gene expression profiling revealed further differences in profile and extent of gene expression in microglia exposed to LPS- or glioma-derived factors. We demonstrate that while morphologically similar, glioma-associated microglia differs from ''inflammatory'' microglia and differences in signal transduction may be responsible for its distinctive functions. We found that immunosuppressants FK506 and Cyclosporin A (CsA) affect LPS- or glioma-induced activation of primary microglial cultures. Both drugs efficiently inhibit morphological transformation, migration and phagocytosis of microglial cells, but only FK506 strongly inhibits LPS-stimulated generation of mature IL-1β forms and cytokine secretion. An inhibitory effect of immunosuppressants can be mediated by an inhibition of MAPK signalling pathways, which govern microglial responses. Identification of specific features of microglia in brain pathology will allow fine-tuning of its functions.

B49

Proteinase-activated receptors and extracellular signal-regulated kinases mediate thrombin-induced regulation of surface antigens in microglia

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Brain injury triggers numerous phenotypic changes in microglia including regulation of immunomodulatory

surface antigen expression. The serine proteinase -thrombin is generated at sites of vascular injury in the brain and induces profound changes in neural cell physiology. We recently demonstrated that pharmaceutical-grade recombinant human -thrombin (rh-thr) also induces a restricted set of proteolysis-dependent changes in microglial physiology. Here we used flow cytometry to characterize the ability of rh-thr to regulate expression of a panel of surface antigens in the N9 mouse microglial cell line. Stimulation with 100 U/ml rh-thr increased expression of CD95/Fas (86%) and CD40 (73%), decreased expression of CD80/B7-1 (23%) and CD11b (7%), and had no effect on expression of MHC II, CD86/B7-2 or CD93/C1qRp. Dose response studies demonstrated that 20 U/ml rh-thr was sufficient to induce smaller, but still significant, changes in surface antigen expression. Time course studies revealed maximal effects at twenty-four hours. Using proteinase-activated receptor (PAR) activating peptides; we demonstrated that rh-thr-induced effects on CD95/Fas and CD40 could be reproduced by PAR1, but not PAR4, selective agonists. We next screened a panel of mitogen activated protein kinase (MAPK) inhibitors for their ability to block the rh-thr-induced responses. We found that extracellular signal-regulated kinase (ERK1/2, also known as p44/42 MAPK) pathway inhibitor U0126 completely blocked rh-thr-induced responses whereas neither c-Jun N-terminal kinase (JNK) or p38 MAPK pathway inhibitors (SP600125 or SB203580, respectively) did. Using a bead-based multiplex assay with anti-phospho-MAPK-specific antibodies we then directly demonstrated rh-thr-induced phosphorylation of ERK1/2, but not JNK or p38 MAPK. Our results suggest that PARs and ERK1/2 mediate rh-thr-induced regulation of cell surface antigens in microglia. These results are consistent with some, but not all, aspects of previously reported results using non-pharmaceutical-grade thrombin preparations.

B50

FK506 modulates pro-inflammatory and cytotoxic responses of cytokine-stimulated rat astrocytes.

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Reactive astrogliosis is implicated in many acute and chronic neuropathological conditions and involves astrocyte proliferation, activation and hypertrophy accompanied by production of cytokines, growth factors and metabolic alterations. Astrocyte activation may exert both beneficial and detrimental effects on nervous system cells, therefore its modulation is an attractive target for neuroprotective therapies. We have demonstrated that a widely used immunosuppressant FK506 was a potent inhibitor of gliosis in vivo and improved recovery in a rat stroke model (Zawadzka and Kaminska Glia, 49:36–51, 2005). To dissect the mechanism of FK506 action on activated astrocytes, we employed a model of ''reactive astrogliosis in vitro'' based on primary rat astrocyte cultures stimulated with the mix of pro-inflammatory cytokines (IL1-beta, IFN-gamma and TNF-alpha). Cytokine cocktail activated p38 and JNK MAPK signalling pathways followed by cellular hypertrophy, increase of GFAP staining, nitric oxide production and expression of mRNA for pro-inflammatory or cytotoxic molecules (il-6, cox-2 and trail). FK506 treatment reduced the astrocyte hypertrophy, decreased the level of activated p38 MAPK, as well as down-regulated cox-2 and trail mRNA. Our data suggest that FK506 may exert neuroprotective effect partially via an inhibition of the pro-inflammatory astroglia activation and implicate a calcineurin as a new candidate for triggering of astrogliosis.

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B51

Challenge with innate and protein antigens induces CCR7 expression by microglia in vitro and in vivo

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Since activated microglia are able to phagocytose damaged cells and subsequently express major histocompatibility complex class II (MHC-II) and co-stimulatory proteins, they are considered to function as antigen presenting cells (APCs) in the central nervous system. The maturation and migratory potential of professional APCs is associated with the expression of chemokine receptor CCR7. We therefore investigated whether immunological activation of microglia induces CCR7 expression. We here present that activation of cultured microglia by both the innate antigen lipopolysaccharide and protein antigen ovalbumin rapidly induces CCR7 expression, accompanied by increased MHC-II expression. Moreover, it is shown that CCR7 expression in IBA-1 positive cells is induced during the symptom onset and progression of experimental autoimmune encephalomyelitis, a rodent model for multiple sclerosis. These results suggest that microglia express CCR7 under specific inflammatory conditions, corroborating the idea that microglia develop into APCs with migratory potential towards lymphoid chemokines.

B52

Normal appearing white matter in MS is in a subtle balance between inflammation and neuroprotection

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Multiple Sclerosis is a chronic inflammatory disease of the central nervous system (CNS). Although progressive axonal injury and diffuse inflammatory damage has been shown in the chronic phase of the disease, little is known about the molecular mechanisms responsible. In order to identify these mechanisms, we have studied the gene expression profile in non-lesion containing tissue, the so called normal appearing white matter (NAWM). We performed differential gene expression analysis and quantitative RT-PCR on subcortical white matter from 11 MS and 8 control cases. Differentially expressed genes were further analyzed in detail by in-situ hybridization and immunofluorescence studies. Here, we demonstrate that particular immunomodulatory genes are upregulated in MS NAWM. Immunofluorescence colocalization analysis for STAT4 and STAT6 revealed increased expression of STAT4 in microglia and astrocytes in the MS NAWM, whereas STAT6 expression was mainly detected in oligodendrocytes. In-situ hybridization analysis for

HLA-DRalpha and HIF-1alpha showed an increased expression in MS NAWM compared to control white matter. In this study, we show several genes involved in pro- as well as anti-inflammatory signalling pathways being upregulated in MS NAWM. The upregulation of genes involved in anti-inflammatory mechanisms may protect the CNS environment and thus limit lesion formation, whereas the activation of pro-inflammatory mechanisms may support disease progression. Altogether, this suggests an endogenous inflammatory reaction throughout the whole white matter of MS brain preceding lesion formation.

B53

Tissue inhibitor of matrix metalloproteinase-1: mediator of neuroinflammation?

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Tissue inhibitors of metalloproteinases (TIMPs) are a family of closely related proteins known as specific inhibitors of matrix metalloproteinases (MMPs). However, TIMPs have also been shown to exert growth factor activities and have recently been described to play a role in inflammatory processes too. This study focused on the role of TIMP1 in the CNS by using a transgenic mouse model in which TIMP1 is specifically expressed in astrocytes. Several lines of transgenic mice that express TIMP1 at different levels in various areas of the CNS were established. Low transgenic TIMP1 expression induced no constitutive phenotype at all whereas moderate transgenic TIMP1 expression caused early microglia activation preceding inflammatory infiltrates and calcifications leading to neurodegeneration in areas of maximum transgene expression. When mice of this line were crossed on a Rag2−/− background, the TIMP1 expressing mice revealed calcification and neurodegeneration in the absence of lymphocytic infiltration indicating that TIMP1 may induce the pathology observed. Transgenic mice that expressed very high levels of TIMP1 revealed cerebral morphological pathology such as hydrocephalus. In order to further elucidate the role of TIMP1 in inflammatory CNS-disease, EAE was induced in mice of two mouse lines that showed maximal TIMP1 expression in the spinal cord. These studies showed that, compared to wild type mice, animals expressing low levels of TIMP1 had a more severe mean clinical disease score (MCDC) and did not recover. In contrast to this, mice that expressed high levels of TIMP1 developed a lower MCDC and did recuperate better. These results suggest that TIMP1 may have different dose-dependent modes of action in the context of inflammatory disorders. While low levels of TIMP1 might aggravate inflammation by actions on lymphocytes and/or macrophages, high levels of TIMP1 might act counter-inflammatory by MMP-inhibition. The mechanisms involved in both pathways remain to be elucidated.

B54

Proteinase-activated receptor-1 and proteinase-activated receptor-2 induce chemokine GRO/CINC-1 release from rat astrocytes evoking neuroprotective protective pathways

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Thrombin at low doses is an endogenous mediator of protection in ischemic and hemorrhagic models of stroke. However, the mechanism of thrombin-induced protection remains unclear. Astrocytes play an important role in the brain after injury. We report that thrombin upregulated secretion of the chemokine growth-regulated oncogene/cytokine-induced neutrophil chemoattractant-1 (GRO/CINC-1) in primary rat astrocytes. PAR-1-induced GRO/CINC-1 release was mainly mediated by c-Jun N-terminal kinase (JNK) activation. Activation of both proteinase-activated receptor (PAR)-1 and PAR-2 resulted in release of GRO/CINC-1. JNK was identified in both signaling pathways to play a pivotal role. By isoform-specific loss-of-function studies using JNK (1–3) small interfering RNA, we demonstrate that different JNK isoforms mediated GRO/CINC-1 secretion, when it was induced by either PAR-1 or PAR-2 activation. JNK2 and JNK3 isoforms were both activated by PAR-1 and essential for chemokine GRO/CINC-1 secretion, whereas PAR-1-mediated JNK1 activation was mainly responsible for c-Jun phosphorylation, which was not involved in GRO/CINC-1 release. Further studies demonstrated that PAR-1 activation as well as application of recombinant GRO/CINC-1 protected astrocytes from C2-ceramide-induced cell death. Protection occurred with suppression of cytochrome c release from mitochondria. The inhibition of cytochrome c release was largely reduced by the antagonist of chemokine receptor CXCR2, SB-332235. Importantly, a specific JNK inhibitor significantly abolished the protective action of PAR-1. These results for the first time demonstrate that PARs play an important role in anti-apoptosis in the brain by regulating the release chemokines, acting through the receptor CXCR2 to preserve cells from toxic insults. JNK-mediated chemokine GRO/CINC-1 release occurred in a JNK isoform-dependent fashion and invoked PAR subtype-specific mechanisms. Furthermore, activation of PAR-2, as well as PAR-1, rescued astrocytes from ceramide-induced apoptosis via regulating chemokine GRO/CINC-1 release. PAR-1 and PAR-2 can activate separate pathways under certain pathological conditions, to rescue neural cells from cell death.

B55

JNK and AIF are indispensable for TNF and TRAIL -induced death of human adult oligodendrocytes.

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TNF and TRAIL induce apoptotic-like cell death of oligodendrocytes. The ligation of TNF and TRAIL receptors induces several signal transduction pathways including caspase cascade, MAP kinase cascade and mitochondrial derived factors like apoptosis inducing factor (AIF). The intracellular signaling pathways in TNF and TRAIL-induced death of mature human oligodendrocytes (OLs) have not been well characterized. OLs were prepared from human brain specimen. TNF and TRAIL-induced OLs death, was detected by flow cytometry with annexinV-FITC and PI staining, and was non-caspase dependent, as evidenced by: lack of generation of caspase-8, -1 and -3 active subunits; lack of cleavage of caspase-1 and -3fluorogenic substrates assessed; and lack of OLs death inhibition by the general caspase inhibitor, ZVAD.FMK. The intracellular signaling involved in

TNF and TRAIL-induced death of OLs was associated with strong activation of JNK measured by induction of phosphorylated form of JNK or by kinase assay. Accordingly, a dominant negative mutant of MKK4/SEK1, MAP kinase upstream of JNK, inhibited TNF and TRAIL-induced death of OLs. JNK activation occurred prior to mitochondrial membrane dysfunction. Electrophoresis of TNF-exposed OLs DNA revealed large scale DNA fragmentation characteristic for AIF-mediated cell death, and co-localization experiments showed that AIF translocation to the nucleus occurred upon exposure to TNF. AIF depletion by an antisense strategy prevented TNF-induced OLs death but not mitochondrial membrane dysfunction. These data suggests that TNF and TRAIL stimulation induced JNK activation leading to mitochondrial membrane dysfunction, which results in AIF translocation into the nucleus.

B56

Macrophages and brain inflammation

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One of the earliest responses to brain injury is the activation of microglia and astrocytes. Later events include immune infiltration. Interplay between glial and immune cells can modulate the glial response. Glial response to entorhinal axonal lesion includes production of chemokines. These are implicated in recruitment of macrophages and T cells to the hippocampus. We have addressed the role of macrophages in glial response and T cell entry by using intravenous injection of clodronate-loaded mannosylated liposomes, in C57Bl/6 mice. As expected, clodronate-liposome treatment resulted in depletion of peripheral F4/80+ and MOMA-1+ macrophages from spleen. Sequential clodronate-liposome treatment 4, 2 and 0 days before axotomy resulted in significant reduction of infiltrating CD45high CD11b+ macrophages in the hippocampus at 1, 2 and 3d post-lesion, measured by flow cytometry. There was also a slight delay in the expansion of CD45dim CD11b+ microglia in clodronate-liposome-treated mice, but macrophage depletion had no effect on the infiltration of T cells. Lesion-induced TNFalpha mRNA expression was not affected by macrophage depletion, suggesting that activated glial cells are the primary source of this cytokine in the injured brain. It remains to be determined whether, and the extent to which, this differs from inflammatory autoimmune infiltration in EAE, where macrophages are a prominent source of TNFalpha and their depletion preventa parenchymal T cell infiltration and disease.

B57

Microvesicle shedding in the brain: a role for aSMase

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Shedding of membrane particles represents an important mean of intercellular communication in hematopoietic cells. We have recently demonstrated that also microglia are able to form and release membrane vesicles upon P2X7 receptoractivation; shed vesicles are used to release the pro-inflammatory cytokine IL-1beta. Aim of the present study was to get further insights into the mechanism of P2X7-induced vesicle shedding from microglia. WB analysis revealed that acid sphingomyelinase (aSMase) is significantly enriched in vesicles relative to homogenates. aSMase catalyzes the hydrolysis of sphyngomyelin into ceramide thus changing plasma membrane fluidity. We measured aSMase activity in microglial cells exposed to the P2X7 agonist BzATP and found a peak of aSMase activity 2 min after agonist exposure. An involvement of aSMase in P2X7-induced vesicle shedding was also observed by spectrophotometric evaluation; in particular, a significant lower amount of vesicles was detected in the medium of microglia exposed to aSMase blockers. Furthermore, experiments carried out on aSMase KO mice confirmed the crucial role of the enzyme in the P2X7-mediated vesicle shedding. To evaluate whether P2X7 ability to trigger large pore formation is relevant for aSMase activation, microglia were pretreated with a specific MAPK p38 inhibitor, SB203580. We found that SB203580 strongly inhibited aSMase activation and completely blocked vesicle shedding induced by P2X7. All together our results indicate that both p38 MAPK and aSMase act downstream P2X7 and that their activation is necessary for P2X7-induced vesicle shedding. In line with this hypotesis, a 85–90% reduction of P2X7-dependent IL 1-beta release was assayed in microglia pre-treated with either aSMase or p38 MAPK inhibitors. Overall our results indicate that inhibition of vesicle shedding is an efficient way to reduce IL-1 beta release from microglia, thus opening new strategies for the pharmacological treatment of neurodegenerative diseases characterized by inflammatory components.

B58

Mechanical lesion activates newly-identified NFATc1 in primary astrocytes: implication of purinergic receptors

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Despite the large range of functions assigned to astrocytes, the intracellular pathways leading to astrocyte activation remain elusive. The nuclear factor of activated T-cells (NFAT) is a family of transcription factors. In response to increased intracellular Ca++ levels they are dephosphorylated by calcineurin and translocated to the nucleus. Interestingly, calcineurin is activated in astrocytes in response to proinflammatory stimuli and its expression is increased in the reactive astrocytes that surround the brain lesions associated to the Alzheimer pathology. In addition, the calcineurin inhibitor cyclosporine A has been shown to reduce the release of pro-inflammatory cytokines from astrocytes and to increase astrocyte survival after ischemic insults. Since NFAT is a key target of calcineurin, a number of astrocyte functions could be mediated by NFAT. However, whereas NFAT proteins are essential for immune cell activation, the expression and function of NFAT members in astrocytes remain unclear, NFATc3 being the only NFAT isoform described to date. Our work further characterizes the NFAT family in astrocytes. Expression of NFATc1 in primary astrocyte cultures was clearly observed using a combination

of quantitative RT-PCR, Western blotting and immunofluorescence microscopy techniques. We also found that NFATc1 is located in the perinuclear cytosol of resting astrocytes, rapidly translocating to the nucleus in response to a wound lesion, ATP and intracellular calcium-raising ionomycin. Finally, NFATc1 activation by lesions is mediated by ATP release and stimulation of purinergic receptors. In summary, activation of NFAT in astrocytes may be a critical signalling event associated to brain trauma.

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B59

Ex vivo analysis of microglia from various CNS regions

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Microglia become markedly activated in the inflammation process that accompanies central nervous system (CNS) injury. Recent in vivo studies on CNS injury indicate that activated microglia can adopt both a phagocytic, macrophage-like phenotype (''innate defense'') and an antigen presenting, dendritic cell-like phenotype (''adaptive response''). It seems conceivable that these activated microglia influence the course of the inflammation process. While general knowledge on microglia phenotypes is expanding, up to now it is largely unknown whether microglia phenotypes and function depend on the region where they reside. To determine microglia phenotype and function in various CNS regions, a method was developed to isolate microglia for ex vivo analysis from adult mouse optic nerve, striatum, hippocampus, spinal cord, cerebellum and cerebral cortex. Within two to three hours, mechanical dissociation and subsequent density-based cell separation yielded per mouse between 3.000 microglia for optic nerve and 75.000 microglia for cerebral cortex. Up to 100% of the microglia were viable, according to trypan blue staining. Flow cytometry with antibodies to microglial surface markers CD11b and CD45 and selection on size, granularity and viability, showed a 90–95% microglia purity. With both additional perfusion and immunomagnetic separation with CD11b binding magnetic beads the microglia purity could be enhanced to $>$95%. Currently, microglia from various CNS regions are ex vivo phenotyped with markers specific for phagocytosis and antigen presentation, using flow cytometry and real-time polymerase chain reaction analysis. Knowledge on microglia phenotypes and function is likely to offer more insight into the role of microglia in the inflammation process that accompanies CNS injury.

B60

In vitro analysis of astrocyte activation: differential effects of pro-inflammatory molecules on rat cortical astrocytes.

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In vitro analysis of astrocyte activation: differential effects of pro-inflammatory molecules on rat cortical astrocytes. Romina Macco, Alessandra Di Cesare, Alessandra Consonni, Barbara Bettegazzi, Ilaria Pelizzoni, Franca Codazzi, Daniele Zacchetti, Fabio Grohovaz San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Italian Institute of Technology - Research Unit of Molecular Neuroscience, Milano, Italy Astrocytes are recognized as key players in many physiological and pathological processes of the central nervous system. They contribute to the formation of the blood-brain barrier, direct neuronal migration, provide energetic support to neurons, and regulate the extracellular concentration of various ions, metabolites and neurotransmitters. Astrocytes can dynamically and rapidly change their phenotype in a process known as 'activation', contributing to the maintenance of the central nervous system homeostasis. The interplay between astrocytes and microglial cells is thought to control this process via secretion of biologically active molecules, such as pro-inflammatory cytokines. Chronic neuro-inflammation can also produce a status of activation of astrocytes, which can play either a neuroprotective or a neurotoxic role, in particular after brain injury or during neurodegenerative diseases. We used cultures from rat brain cortex to set up an in vitro model of astrocyte activation in which we studied the different phenotypes that are induced upon administration of various stimuli (e.g. inteleukin-1 beta, tumor necrosis factor alpha, interferon gamma). With this model we first investigated the effect of lypopolysaccharide on the expression of markers of activation in pure astrocytic or mixed glial cultures. We also analyzed the intracellular pathways leading to the changes in the astrocytic phenotype, with particular attention to the link between protein kinase C alpha activation and the ensuing phosphorylation of the MAP kinases ERK1/2.

B61

Profile of early microglial activation by unconjugated bilirubin

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Severe jaundice in the neonatal period can lead to deposition of unconjugated bilirubin (UCB) in the central nervous system. The immunostimulant effect of UCB in astrocytes, which has been previously demonstrated, may directly injure neurons or contribute to the development of long-term neuropathological effects. Since microglia play an important role as resident immunocompetent and phagocytic cells in the CNS and are capable of releasing potentially cytotoxic substances such as pro-inflammatory cytokines, it can be hypothesized that UCB-induced microglia reactivity may increase the extracellular levels of IL-1beta and IL-6, further contributing to neuronal injury during hyperbilirubinemia. This study aimed to explore the reactivity of microglia to UCB, characterizing alterations in morphology and the profile of cytokine release, as well as the activation of MAPKs, known as signaling pathways of inflammation. Primary cultures of rat microglia were incubated for 5 min to 2 h with 50 microM UCB in the presence of 100 microM HSA, at 37°C. Alterations of microglial morphology were assessed by immunocytochemistry. Activation of MAPKs (p38, JNK1/2, ERK1/2) was determined by Western blot and cytokine secretion was evaluated by ELISA. Stimulation of microglia by UCB leads to cell activation with characteristic morphological changes, as well as to fast and transient

activation of all the MAPKs tested, with the peak levels of each phosphorylated-MAPK occurring at 15–30 min after UCB addition ($p<0.05$). UCB also enhances the release of pro-inflammatory cytokines IL-1beta and IL-6 ($p<0.05$), with peak levels at 30 min to 2 h. These results reinforce the role of microglia in the pathogenesis of encephalopathy during hyperbilirubinemia and point to these cells as the first brain cells to respond to UCB neurotoxic stimulus.

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B62

Cellular mechanisms underlying dysfunction of immature neurons by hyperbilirubinemia and inflammation

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Deposition of unconjugated bilirubin (UCB) in the central nervous system can be neurotoxic and premature babies display a higher susceptibility to UCB-induced brain damage. Increased levels of UCB trigger the release of pro-inflammatory cytokines by glial cells, which may have toxic effects in neurons. This work was designed to investigate the events resulting from exposure of immature neurons to UCB and pro-inflammatory cytokines, alone or in combination, in order to clarify the mechanisms underlying neuronal dysfunction by hyperbilirubinemia and inflammation.

Rat neurons at 3 days *in vitro* were incubated with 50 μM UCB, and/or 10 ng/mL of both recombinant IL-1beta and TNF-alpha, in the presence of 100 μM human serum albumin, for 24 h at 37°C. NO production, caspase-3, -8 and -9 activities, glutamate release and reduction of MTT [3-(4,5-dimethylthiazol,2-yl)-2,5-diphenyltetrazolium bromide] were determined by spectrophotometry. Protein oxidation was evaluated by slot blot analysis.

Exposure to UCB led to an increase of NO production and protein oxidation (~1.7-fold and ~1.1-fold respectively, $p<0.05$), together with capase-3, -8 and -9 activation (~55%, $p<0.05$), reflecting oxidative disruption and apoptosis. UCB also promoted glutamate release (~5.4-fold, $p<0.01$) and a decrease in MTT reduction (18%, $p<0.01$), biomarkers of excitotoxicity and cell activity impairment, respectively. Incubations with TNF-alpha and IL-1beta increased protein oxidation (~1.2-fold, $p<0.05$), as well as caspases 3 and 9 activities (~1.4-fold, $p<0.05$), and induced a decline in MTT metabolism (~8.9%, $p<0.01$). Co-incubation with UCB slightly elevated caspases activation, protein oxidation and glutamate release (N.S.), while cell functionality was further impaired ($p<0.05$).

These results provide evidence that immature neurons are susceptible to oxidative damage, cell dysfunction and death by apoptosis induced by either UCB or pro-inflammatory cytokines. These data also demonstrate that the impairment of cell functionality by hyperbilirubinemia is further aggravated by inflammation.

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B63

Axonal lesion-induced microglial proliferation and microglial cluster formation in the mouse

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Microglia are innate immune cells and form the first line of defence of the central nervous system. Proliferation is a key event in the activation of microglia in acute pathology, and has been extensively characterised in rats, but not in mice. In this study we investigated axonal-lesion induced microglial proliferation and surface antigen expression in C57BL/6 mice. Transection of the entorhino-dentate perforant path projection results in an anterograde axonal and a dense terminal degeneration that induces a region-specific activation of microglia in the dentate gyrus. Time-course analysis showed activation of microglial cells within the first week post lesion and cell counting demonstrated a significant 1.6 fold increase in microglial numbers 24 hours post lesion reaching a maximal 3.8 fold increase 3 days post lesion compared to controls. Double staining for the microglial Mac-1 antigen and the proliferation marker bromodeoxyuridine, injected 1 hour prior to perfusion, showed that lesion-reactive microglia accounted for the vast majority of proliferating cells. Microglia proliferated as soon as 24 hours after lesion and 25% of all microglial cells were proliferating 3 days post lesion. Immunofluorescence double staining showed that most activated, proliferating microglia occurred in multicellular clusters and co-expressed the intercellular adhesion molecule-1 and the hematopoietic stem cell marker CD34. In conclusion, this study extends observations of axonal lesion-induced microglial proliferation in rats to mice and provides new information on early microglial proliferation and microglial cluster formation and surface antigen expression in the mouse.

B64

Alpha-SMA and nestin expression in reactive astrocytes in multiple sclerosis lesions: potential regulatory role of TGF-beta 1

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Rapid and extensive activation of astrocytes occurs subsequent to many forms of central nervous system (CNS) injury. Recent studies revealed that the expression profile of reactive astrocytes comprises antigens present during astrocyte development. Elevated levels of the injury-related cytokine transforming growth factor-beta 1 (TGF-beta 1) secreted by microglial cells and invading macrophages have been correlated with the reactive astrocyte phenotype and glial scar formation. In the present study, we investigated the expression profile of alpha smooth muscle actin (alpha-SMA) and nestin, two cytoskeletal proteins expressed during astrocyte development, in multiple sclerosis (MS) lesions. In active lesions and in the hypercellular margin of chronic active MS lesions, immunostaining for alpha-SMA revealed a subpopulation of reactive astrocytes, whereas the majority of reactive astrocytes expressed nestin. Alpha-SMA and nestin

expressing reactive astrocytes were always in close relationship with macrophages or microglia. In addition, alpha-SMA and nestin expression were analyzed in rat primary astrocyte cultures in response to TGF-beta1. Our in vitro experiments showed that TGF-beta1 regulated the organization and expression of alpha-SMA and nestin in astrocytes. We suggest that the in vivo re-expression might be under regulation of TGF-beta1. These results further clarify the regulation of astrocyte activity after CNS injury which is important for the astroglial adaptation to pathological situations.

B65

Altered pattern of glial activation in mixed glial cultures from ICAM-1 KO mice

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In a previous in vitro study we observed that astrocytes potentiate nitric oxide (NO) production in microglia following an inflammatory stimulus, being mediated by non-soluble factors present in the astrocyte cell membrane. This observation suggests that the interaction between different types of activated glial cells enhances glial reactivity. This effect could be critical when glial activation has a negative influence in the outcome of injury in pathological conditions. The identification of non-soluble factors involved would be interesting as possible targets to control glial activation. Among different molecules expressed in the astrocyte cell membrane, intercellular adhesion molecule-1 (ICAM-1) is a possible candidate. Inflammatory stimuli increase ICAM-1 expression in cultured astrocytes, and ICAM-1 is a ligand for the integrin receptors LFA-1 and Mac-1, which are present in microglia. In the present work, we determined whether ICAM-1 plays a role in the induction of the production of inflammatory factors in mixed populations of reactive glial cells. We used primary mixed glial cultures, mainly constituted by microglia and astrocytes, from brains of newborn C57BL/6 WT and ICAM-1 KO mice. Confluent cultures were treated with LPS +/− IFN-gamma, and we compared the pattern of glial activation induced in the two types of culture. We observed a decreased NO production in cultures from ICAM KO versus WT mice. NO production occurred in microglia, as stated by immunocytochemical detection of inducible NO synthase expression in these cells. No differences in the percentage of microglia between WT and ICAM-1 KO cultures were observed. These results suggest that the interaction between reactive microglia and astrocytes through ICAM-1 may contribute to the production of inflammatory mediators by reactive glial cells. Other inflammatory mediators are currently being evaluated.

B66

Interleukin-10 and its receptor are upregulated in glial cells after an acute injury to the postnatal rat brain

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As compared to the adult, immature brain showed a different pattern of susceptibility and inflammatory response during an acute excitotoxic brain insult. Interleukin-10 (IL-10) is an anti-inflammatory cytokine which is upregulated after acute injuries to the adult CNS. However, regulation of injury-induced IL-10 expression in the immature brain is unknown. The aim of this study is to evaluate the spatiotemporal pattern of IL-10 expression and its receptor (IL-10R) after postnatal excitotoxic brain damage. Excitotoxic lesions were induced by stereotaxic N-methyl-D-aspartate injection into P9 rat sensorimotor cortex. At 10 hours post lesion (hpl), 24hpl, 48hpl, 72hpl and 7 days post-lesion animals were sacrificed and brains were processed for real time mRNA quantification and for immunohistochemical analysis. As compared to non-lesioned animals, excitotoxic damage induced an increase in IL-10R mRNA at 48hpl and in IL-10 mRNA at 72hpl. In front of control brains, where IL-10 immunoreactivity was found in astroglial endfeet surrounding blood vessels, in white matter astrocytes and in processes related to the meninges and ventricle wall, injured brains showed an increase in immunoreactivity mostly in cells morphologically indentified as reactive microglia/macrophages and in unidentified small rounded cells at 24−48hpl. On the other hand, at 72hpl most immunoreactivity was observed in cells morphologically identified as reactive astrocytes. In conclusion, IL-10 and its receptor are upregulated in glial cells after an acute excitotoxic brain injury to the immature brain, suggesting an important role in the final lesion outcome.

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B67

Proinflammatory cytokine expression following striatal excitotoxic injury in the aged rat brain

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Enhanced expression of proinflammatory cytokines has long been linked to neuron-glia and glia-glia cross-talk, modulating the glial response to brain injury. Although aging is one of the major risk factors for suffering brain injuries, little is known about the effect of aging on cytokine expression after damage. To address this question, intrastriatal injection of N-methyl-D-aspartate was performed in adult (3–4 months old) and aged (22–24 months old) male Wistar rats. Animals were sacrificed at different survival times between 6 hours post-lesion (hpl) and 7 days post-lesion (dpl) and the pro-inflammatory cytokines interleukin-1 beta (IL-1β) and tumour necrosis factor alpha (TNFα) were analysed by ELISA. Our results show that cytokine expression is found both in the directly damaged striatum and in the adjacent cortex, but following different expression patterns. In the lesioned striatum, similar IL-1β expression is found in aged and adult animals at all survival times analyzed, whereas TNFα expression is reduced in the aged, mainly at 6–12hpl. However, in the adjacent cortex, and in comparison to the adult, aged animals show diminished expression of IL-1β at all survival times, but enhanced TNFα expression at several timepoints after injury. Our data demonstrate that, after striatal excitotoxic damage, proinflammatory cytokine expression differs in the injured striatum versus the adjacent

cortex, and in the aged rats versus the adults, emphasizing the importance of using aged animals for the study of inflammation in acute age-related brain insults.

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B68

Characterisation of glial cell modulators in isolated and mixed spinal cord glial cultures

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Acute and chronic pain is experienced by millions of individuals. In acute pain, activation of nociceptive transmission to the dorsal horn relays pain information to the brain by adaptive mechanisms. These pain signals can be suppressed, relayed unaltered or amplified. However in chronic pain these adaptive mechanisms seem to fail and pain becomes exaggerated and unending leading to pain states such as hyperalgesia, where a painful stimulus is grossly amplified, and allodynia, perceiving a normally non-painful stimulus as painful. Treatment of chronic pain is rarely completely successful. The focus of many current drug therapies has been neuronal mechanisms but recent discoveries have meant that other cell types are becoming potential targets for drug discovery[1]. Spinal cord glia have been shown to be activated in several animal models of exaggerated pain and glial cell modulators effective in blocking these pain states. Evidence now suggests that there are distinct roles for microglia and astrocytes in the induction and maintenance of chronic pain[2] Here we will describe the use of isolated spinal cord microglia or mixed spinal cord glia cultures for assessing glial cell modulators. Data so far suggests minocycline is effective in reducing IL-6 release in LPS stimulated isolated spinal cord microglial cultures, but less so in mixed spinal cord glial cultures. Effects on cytokine profiles in these culture systems using other tool glial cell modulators will be shown.

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B69

Cell density regulates nuclear localisation of interleukin-1α and β in murine microglia

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Interleukin-1 (IL-1) is the prototypical pro-inflammatory cytokine, and is implicated in the pathogenesis of a variety of acute and chronic neurodegenerative diseases. However classical IL-1 signalling can not fully explain the observed effects of IL-1 in the brain. The IL-1 family agonists IL-1α and β contain a confirmed and a putative nuclear localisation sequence respectively, and intranuclear IL-1α actions have been reported in IL-1 expressing cells. Since microglia are an early source of IL-1 in the injured CNS, we tested the hypothesis that IL-1 isoforms localise to microglial nuclei following inflammatory stimuli. Murine primary microglia and a murine microglial cell line (BV-2) were treated with bacterial lipopolysaccharide to induce IL-1 expression, and IL-1 subcellular localisation was characterised both by cell fractionation and immunoblot analysis and by immunocytochemistry. LPS induced pro-IL-1α and β localised to the cytosol and nucleus of both primary microglia and BV-2 cells. IL-1 was predominantly cytosolic in BV-2 cells at high cell density, and both nuclear and cytosolic at low density. Cytosolic localisation of IL-1 in BV-2 cells cultured at low density was induced by co-culture with a confluent HEK cell monolayer. Nuclear localisation of primary microglial IL-1 was similarly regulated by contact with HEK cells or astrocytes. Thus IL-1α and β localise to microglial nuclei in a cell density regulated fashion, and may therefore have intranuclear actions in microglia regulating CNS inflammatory responses.

B70

The ANP-cGMP-protein kinase G pathway induces a phagocytic phenotype but inhibits inflammatory gene expression in microglial cells.

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Reactive gliosis is a prominent feature of CNS injury that involves dramatic changes in glial cell morphology together with increased motility, phagocytic activity and release of inflammatory mediators. We have recently demonstrated that stimulation of the cGMP-protein kinase G (PKG) pathway by NO or atrial natriuretic peptide (ANP) regulates cytoskeleton dynamics and motility in rat astrocytes in culture. In this work we show that the cGMP-PKG pathway stimulated by ANP, but not by NO, regulates microglial cell morphology by inducing a dramatic reorganization in the actin cytoskeleton. Both ANP (0.01–1.0 μM) and the permeable cGMP analogue, dibutyryl-cGMP (1–100 μM), promote a rapid (maximal at 30 min) and concentration-dependent increase in size, rounding and lamellipodia and fillopodia formation in rat brain cultured microglia. These morphological changes involve an increase and redistribution of F-actin, are prevented by the PKG inhibitor, Rp-8-Br-PET-cGMPS (0.5 μM) and appear to involve inhibition of RhoA GTPase and activation of Rac1 and Cdc42 GTPases. The cGMP-induced amoeboid phenotype shows increased phagocytic activity. However, ANP (1 μM) does not induce NO synthase type 2 (NOS-2) or TNF-α expression and furthermore is able to decrease LPS-elicited induction of these inflammatory genes. The cytoskeleton changes and the inhibition of LPS-induced NOS-2 expression produced by ANP in cultured microglia are also observed by immunostaining in organotypic cultures from rat hippocampus. These results indicate that the ANP-cGMP-PKG pathway plays and anti-inflammatory role in microglial cells.

B71

Leukemia inhibitory factor is immunomodulatory and is secreted by activated macrophages during myelin uptake

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In the chronic disabling disease multiple sclerosis (MS), infiltration of the central nervous system (CNS) by

autoreactive T-cells and macrophages results in demyelination and oligodendrocyte loss. Recent findings indicate that inflammatory cells are not merely detrimental but may also have protective effects. We previously showed that leukemia inhibitory factor (LIF), a member of the neurokine family of neurotrophic factors, is secreted by both T cells and macrophages both in vitro and in inflammatory MS lesions. LIF further protects oligodendrocytes from TNF-alpha induced apoptosis. Among immune cells mainly macrophages express neurokine receptors. Here we investigated the influence of LIF on macrophage functions relevant for MS. We demonstrate that LIF suppresses the secretion of the pro-inflammatory mediators TNF-alpha and oxygen radicals by macrophages in vitro but increases myelin phagocytosis. We further show that macrophages secrete enhanced levels of LIF during myelin uptake when stimulated with LPS. These data suggest that LIF has a role in myelin clearance and has immunomodulatory properties.

B72

Effects of endocannabinoid system on the interaction of CD200/CD200r on neuroinflammation

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Brain immune privilege is due not only to the blood-brain barrier but also to the existence of negative signals that maintain microglia cells in a quiescent state when brain tissue is in basal conditions.The local interaction between CD200 receptor (CD200R) and its ligand CD200 is one of the factors that helps to mantain these resting conditions. CD200 is a membrane glycoprotein that is expressed on neurons and provides an inhibitory signal to cells of the myeloid lineage like microglia. When an inflammatory insult occurs within the brain, microglia is activated and a reduction in the expression of the CD200R takes place, in order to face the damage situation. In the present work, we have analyzed the effects of endocannabinoids on the expression of CD200R in microglial cells. We first studied the time-course of the expression of CD200R in response to a proinflammatory stimulus at mRNA and protein levels by means of RT-PCR and western blotting, respectively. Second, we studied whether anadamide could modify CD200R expression in microglia. We found that anandamide treatment was able to increase the expression of CD200R. In an attempt to investigate the importance of CD200/CD200R interaction in a viral model of multiple sclerosis (Theileŕs virus model), we carried out time-course studies of CD200, CD200R, CD11b and CD200R/CD11b expression by real time PCR (21, 35, 60, 90 and 180 days p.i). Currently, we are investigating the actions of endocannabinoids on microglia-neuron interactions and oligodendrocytes in models of multiple sclerosis.

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B73

The in vivo expression of diseased forms of tau protein induced chronic immune response with apparent influx of leukocytes from the blood

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The neuroinflammation plays the prominent role in the progression of Alzheimer's disease (AD). The inflammatory response in AD may be triggered by the accumulation of misfolded proteins including beta amyloid or tau protein. In recent years, much work has been devoted to the study of the role of amyloid beta as an inducer of inflammatory response in neurodegenerative diseases. Comparatively little is known about the mechanisms that initiate inflammation during tau neurodegenerative process. Therefore we developed first transgenic rat model recapitulating AD characteristic neurodegenerative cascade consisting of tau hyperphosphorylation, formation of argyrophilic tangles and sarcosyl-insoluble tau complexes. Strikingly, the transgenic rats showed that misfolded protein tau triggered massive inflammatory response characterized by activation of microglia and macrophages. In transgenic rat brain several morphologic types of activated microglia were distinguished: hypertrophic, bushy, rod shaped microglia and clustering microglia. The activated microglia in the brain showed increase in immunomarkers such as complement receptor 3, lysosomal glycoprotein CD68, T cell antigen CD4 and leukocyte common antigen CD45. Moreover, the innate immune brain response induced by human truncated tau promotes activation of CD45/RT1B monocytes and CD45/OX62 dendritic cells and their influx into the brain parenchyma. These findings underline the value of transgenic rats as a tool to study the direct interactions between CNS neurons suffering from neurodegeneration of Alzheimer's type and resident and blood-borne inflammatory cells.

B74

Development of a mouse model to study the effect of inflammatory demyelinating brain pathology on the subventricular zone

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Subventricular zone (SVZ) contains multipotential cells capable of generating neurons, astrocytes and oligodendrocytes. In normal rodent brain, the SVZ neural progenitors continuously migrate within the rostral migratory stream (RMS) towards the olfactory bulb where they give rise to new neurons. While it has been shown that experimental autoimmune encephalomyelitis (EAE) induces mobilization of SVZ neural progenitors towards the areas of inflammatory demyelination (Picard Riera *et al.*, 2002), the exact effect of chronic inflammation on this stem cell niche is not clear. Such lack of information is in part due to the fact that currently available protocols for induction of inflammatory demyelinating disease in animal models preferentially target the spinal cord and therefore do not reproducibly generate lesions in the SVZ vicinity. The goal of our project was to develop an experimental protocol that would focalise the MOG peptide (35–55)-induced EAE to the SVZ- proximal white matter tracts in C57/Bl 6 mice. After testing 3 variations of the protocol used by Kerschensteiner and colleagues (2004) to target EAE lesions to the cervical spinal cord of Lewis rats, we determined that CFA/MOG immunization combined with a stereotaxic injection of tumour necrosis factor (TNF) a and interferon (IFN) γ reproducibly induces extensive forebrain inflammation and demyelination. We

have therefore used this model to examine the changes in proliferation/differentiation of the SVZ cells, as well as apoptosis in response to chronic inflammatory brain insult. This project is supported by INSERM-AFM-MRNT and ATC Vieillissement. V.T. holds an FRM post-doctoral fellowship.

B75

Systemic inflammation induces age-dependent differential activation of glial cells through the leptomeninges

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There is increasing evidence that the leptomeninges, which covering the surface of the brain parenchyma, modulate neuronal and glial functions by releasing diffusible factors in addition to their physiological role as the cerebrospinal fluid-blood barrier. During systemic inflammation, leptomeninges produced interleukin-1b (IL-1b), tumor necrosis factor-a (TNF-a), interleukin-6 (IL-6), transforming growth factor-b1 (TGF-b1), as well as prostaglandin E2 (PGE2). At the same time, both microglia and astrocytes in the proximity of the leptomeninges were activated. In the case of the young adult rats, the activated microglia and astrocytes produced anti-inflammatory cytokines including interleukin-10 (IL-10) and TGF-b1 but not any proinflammatory cytokines. Furthermore, TGF-b receptor II was mainly expressed by cortical neurons and the leptomeninges during systemic inflammation. The activated microglia and astrocytes of young animals are considered to be beneficial during systemic inflammation for IL-10 and TGF-b1 are known to exert protective roles on neurons. In the case of the middle-aged animals, however, both the activated microglia and astrocytes mainly produced IL-1b and TNF-a during systemic inflammation. Therefore, the activated microglia and astrocytes may become harmful phenotype during systemic inflammation, because these proinflammatory cytokines are known to induce neuronal death and impair the memory and learning. Based on these observations, we deduce that leptomeninges transduce the systemic inflammatory signals to glial cells leading to be beneficial anti-inflammatory cell phenotype at the young-age, while harmful proinflammatory cell phenotype at the middle-age.

B76

MS-like grey matter lesions in beta-synuclein93–111 immunized Lewis rats

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS widely considered to be autoimmune in nature. In the last few years demyelinated lesions of cortex and spinal cord grey matter have come into focus. In the present study we established an animal model with MS-like grey matter lesions. Immunisation of Lewis rats with beta-synuclein93–111, a neuronal antigen, induces monophasic autoimmune CNS inflammation with complete recovery (Mor *et al.*, 2003). We observed inflammatory infiltrates predominantly in the spinal cord grey matter, paralleling the distribution of beta-synuclein protein. No demyelination was observed. To mimick MS inflammatory-demyelinating pathology, anti-MOG antibodies were transferred into beta-synuclein93–111-immunized rats. Thus, extensive demyelinated grey matter lesions were generated. Microglia/macrophage activation in the grey matter was studied using ED1, ED2, anti-iNOS, anti-HO-1 and anti-MRP14 antibodies. Numbers of APP-positive axons were increased in antibody transferred rats compared with animals receiving beta-synuclein93–111-immunization alone. In addition, transient neuronal pathology was observed. In summary, our studies identify beta-synuclein93–111-induced EAE with transfer of demyelinating antibodies as a valuable tool to study mechanisms of grey matter damage in multiple sclerosis.

B77

Microglial responses to Staphylococcus aureus strains that express capsular polysaccharide 5 or 8 are exaggerated compared to non-capsular isolates.

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Previous studies from our group have demonstrated that microglia respond profoundly to an uncapsulated strain of S. aureus, RN6390, by producing robust amounts of proinflammatory mediators. However, serotyping studies have revealed that most S. aureus isolates recovered from human diseases are encapsulated, producing predominantly the capsular polysaccharides (CP) CP5 and CP8. In this study we compared the inflammatory mediator profiles of primary microglia elicited in response to encapsulated strains of S. aureus Reynolds (wild type; R-wt) and its CP5 or CP8 expressing mutants (R-CP5 and R-CP8 respectively), capsule-negative Reynolds [R-CP(-)], and the non-capsular S. aureus strain RN6390. Interestingly, all Reynolds mutant strains significantly induced microglial proliferation at doses of 107 and 106 colony forming units (cfu)/well as demonstrated by MTT cell viability assays. Examination of microglial morphology revealed that strain R-CP5 but not R-wt induced the formation of microglial aggregates. Quantitative real-time PCR (qRT-PCR) analysis of matrix metalloproteinase 2 (MMP2) and MMP9 mRNA expression revealed that both MMPs were induced in response to either R-wt or R-CP5 strains, whereas MMP2 mRNA expression was induced much more prominently in R-CP5-stimulated microglia. On the other hand, the effects of mutants on the expression of numerous inflammatory mediators including TNF-alpha, MIP-2, IL-12p40 and IL-10 were dose-independent until very low concentrations were reached, whereupon they were still able to induce significantly higher inflammatory mediator levels compared to RN6390 and R-wt. In conclusion, these studies suggest that capsular polysaccharides influence microglial responses and may result in the tailoring of CNS responses to facilitate immune evasion, which may account, in part, for the enhanced virulence of these strains in humans. This study is supported by UAMS Medical Research Endowment award to N.E.

B78

Chronic demyelination is not sufficient to induce acute axonal damage

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The hallmarks of multiple sclerosis are destruction of myelin, loss of myelin producing cells and axonal damage. Axonal damage is supposed to be the main cause for disability in MS patients whereby the exact mechanisms leading to axonal damage are not completely understood. We used the cuprizone model, a toxic demyelination model, to investigate whether demyelination alone without inflammation is sufficient to induce axonal damage. Chronic demyelination was induced by feeding 8 week old male C57Bl/6 mice a 0.2% cuprizone diet for 12 weeks. After withdrawl of the toxin animals stayed on normal chow for another 12 weeks. Paraffin fixed tissue was stained for the myelin proteins PLP and MOG to quantify the extent of de- and remyelination. Immunohistochemical stainings for amyloid precursor protein (APP) and non-phosphorylated neurofilament (SMI32) were used to study axonal damage. Scoring of myelin proteins revealed substantial demyelination of the corpus callosum starting at week 4 of treatment and reaching a plateau after week 6. Withdrawl of cuprizone induced a slow remyelination process. After 12 weeks on normal chow extensive reexpression of myelin proteins could be observed. Despite severe demyelination in the corpus callosum analysis of APP and SMI32 stained sections showed only minor axonal damage. In our model of chronic demyelination the remyelination potential was slow but still present and only little axonal damage could be found. We conclude that the isolated absence of myelin sheaths for several weeks is not sufficient to induce massive axonal damage and further i.e. inflammatory signals are required to explain the early axonal damage observed in MS lesions and some models of EAE.

B79

Neuronal control of microglial phenotype in vitro

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Microglia are resident immune cells in the brain that become activated in response to diverse central nervous system injuries. Microglial phenotypes are dynamic, dependent on their activation state, and may be beneficial or detrimental. The activation of microglia in the brain is complex and involves diverse activators. The aim of the present study was to examine microglial activation states in rat, glial-neuronal cocultures, by analysing microglial morphology. Neurone-derived factors are known to affect microglial activity, and thus particular attention was paid to the microglia associated with neuronal cell bodies. Ameboid microglia were classed as activated, and microglia harbouring processes were classed as resting. Microglia in association with neuronal cell bodies displayed a higher degree of activation as compared to microglia distant to neuronal somas. To determine the role of neuronal activity in this phenomenon, tetrodotoxin (TTX) was applied to glial-neuronal cocultures. Based on morphology, TTX treatment did not affect the morphological states of the neurone-associated microglia. However, the number of neurones associated with microglia increased in response to treatment with TTX. Thus, neurones in culture can affect microglial activation states, and our data indicates that suppression of neuronal activity can increase the association of microglia with neurones. Similar mechanisms of microglial activation could exist in response to CNS injuries with loss of neurone function, and contribute to immune and inflammatory processes in the brain.

B80

Effects of early cannabinoids treatment on TMEV-IDD outcome

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Alterations of the central nervous system (CNS) microvascular endothelium are closely linked to the pathogenesis of several neuroinflammatory diseases. Adhesion molecules are involved in the leukocyte recruitment at the blood brain barrier. Theiler's virus infection of the CNS induces an immune-mediated demyelinating disease (TMEV-IDD) in susceptible mouse strains and serves as a relevant infection model for human multiple sclerosis (MS). Previous work of our group showed that administration of cannabinoids or the pharmacological activation of endocannabinoid tone ameliorate motor deficits, diminish leukocyte infiltration and reduce microglial reactivity (Arévalo-Martín *et al.*, 2003; Mestre *et al.*, 2005). In the present study we investigated whether early cannabinoids treatment could modify TMEV-IDD onset. In particular, we studied the effects of WIN 55,212–2 treatment, for 3 or 7 days after TMEV infection in both, the immune response and the TMEV-IDD motor disturbances. Our data show that early treatment with the synthetic cannabinoid agonist WIN 55,212–2 diminish microglial activation, CD4+ lymphocytes infiltration, intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) expression in the brain of TMEV-infected mice. In addition, immunohistochemical spinal cord analysis 60 days post-infection show that early cannabinoid treatment limits microglial activation and adhesion molecules expression. Moreover, early cannabinoid treatment during 3 or 7 days after TMEV-infection reduce motor deficits. Overall our results suggest that immune response modulation by early cannabinoids treatment may affect the development of CNS pathologies related to neuroinflammation like multiple sclerosis. Acknowledgements: We are grateful to Dr. Moses Rodriguez (Mayo Clinic/Foundation, Rochester, Minnesota, USA) for gentile delivery of Theiler's virus DA strain.

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B81

Prenatal inflammation impacts glutamatergic synaptic function

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Epidemiological studies have linked maternal infection during pregnancy and the later development of neuropsychiatric disorders such as schizophrenia and autism. Experimental data also showed that inflammation induced by chemicals, bacteria or virus during fetal development impairs offspring's behavioral and cognitive performance. It is now widely accepted that cognitive impairments are due to

synaptic dysfunction but the consequence of prenatal inflammation on synaptic function has poorly been investigated. We have now demonstrated that prenatal inflammation suffices to induce deferred glutamatergic synaptic dysfunction in adult. Indeed, we found a transient perinatal brain inflammation in mice mutated on the microglial gene DAP12 which display altered glutamatergic transmission in adulthood. In addition, mice experimentally exposed to brain inflammation during embryonic development display comparable synaptic defects. Finally, neurons cultured more than two weeks without microglia, from neonates born to KARAP/DAP12−/− mutant or inflamed mice also display altered glutamatergic synaptic function. This alteration of glutamatergic neurotransmission provides a cellular basis to the neuropsychiatric alterations due to prenatal inflammation.

B82

Transcriptional regulation of CCR5 in microglia and astrocytes

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Several chemokines and chemokine receptors, including CCR5, are implicated in the pathogenesis of multiple sclerosis (MS). We show that expression of CCR5 is enhanced in MS lesions of various disease stages. Earlier, we have shown that MS lesions are hallmarked by enhanced expression of 'stress-response' transcription factors such as IRF-1, NF-κB and CREB-1, leading to enhanced expression of both classes of MHC molecules. The expression of these molecules overlaps with the expression of CCR5 in MS lesions. Therefore, we investigated whether these factors are also involved in the transcriptional regulation of CCR5. Using promoter evaluation assays we determined that neither IRF-1 nor NF-κB is involved in the activation of the CCR5 promoter. Additionally, we show that these factors are not involved in the induction of transcription of endogenous CCR5 in human primary microglia and astrocytes, as determined by RT-PCR. In contrast, we found that CCR5 expression is regulated by the cAMP/CREB pathway. However, the restricted expression pattern of CCR5 does not correspond with the ubiquitous nature of CREB-1 expression, which suggests the involvement of additional regulatory mechanisms. Therefore, we are currently investigating the contribution of epigenetic mechanisms such as DNA methylation and post-translational histone modifications in the transcriptional control of CCR5. Taken together, we conclude that expression of CCR5 is regulated by several genetic and epigenetic regulatory mechanisms and that alterations in these mechanisms could account for the aberrant expression of CCR5 in MS lesions.

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B83

Effect of the PPAR-β agonist GW501516 in an in vitro model of antibody-induced demyelination

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Active demyelinating lesions in multiple sclerosis (MS) are surrounded by inflammatory foci. Physiological pathways have been discovered which control the inflammatory process. The peroxisome proliferator-activated receptor (PPAR) transcription factors participate in these endogenous anti-inflammatory pathways, and PPARs may be involved also in the maturation of oligodendrocytes. Therefore, PPAR-specific agonists appear as promising candidates for the therapy of demyelinating diseases such as MS. Among the three PPAR isoforms, PPAR-β is the most abundant in the brain. Aggregating brain cell cultures were used to study the effects of GW501516, a PPAR-β-specific agonist, on demyelination, and remyelination, as well as on the demyelination-induced inflammatory response. Changes in the expression of the inflammatory markers glial acidic fibrillary protein (GFAP), tumor necrosis factor-α; (TNF-α;), and interleukin-6 (IL-6), and of the myelin-related markers myelin oligodendrocyte glycoprotein (MOG), and myelin basic protein (MBP) were measured by quantitative RT-PCR. Demyelination was induced by the treatment with anti-MOG antibodies and complement, causing decreased expression of the specific markers MBP and MOG, while PPAR-β expression remained unchanged. The demyelinating agents also induced a strong inflammatory response as indicated by the reactivity of microglial cells and astrocytes, and the increased TNF-α; expression. GW501516 did not prevent demyelination nor did it promote remyelination. In resting cultures, GW501516 strongly increased the expression of IL-6 and decreased TNF-α; expression, whereas in reactive cultures, it decreased the expression of TNF-α;. GW501516 also up-regulated PPAR-β expression in resting but not in reactive cultures. Thus, despite its ability to modulate the inflammatory response, the PPAR-β specific agonist had no protective effect on myelin. In addition, it induced distinct changes of the inflammatory marker expression pattern in the resting compared to the reactive situation. This suggests that the effects of PPAR-β depend on the inflammatory and pathological conditions.

B84

Apoptotic microglia translocate condensed chromatin from the nucleus into the cytoplasm

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In most organisms apoptosis is morphologically characterized by chromatin condensation, nuclear fragmentation, and formation of apoptotic bodies. Recently, we reported on a new apoptotic phenotype lacking nuclear fragmentation in the microglial cell line BV-2 after UV-irradiation (Brain Research, 1121: 12–21, 2006). In the present study, we extended our data about the fate of condensed chromatin during apoptosis in UV-irradiated BV-2 cells by performing transmission electron microscopy using high pressure frozen cells or chemically fixed cells, as well as immunogold-labelling of the histone H3. Regardless of the method of cell preparation we confirmed our previous morphological observation, that apoptotic BV-2 cells contain condensed chromatin not only in the nucleus but also in the cytoplasm. Cytoplasmic chromatin is always located at the nuclear envelope opposite to nucleoplasmic chromatin. We identified

condensed cytoplasmic chromatin in 66% of the cells three and five hours (but not one hour) after induction of apoptosis by UV-irradiation. Additionally, dilation of the nuclear membrane was accompanied by pinching-off of electron-lucent vesicles, implying that the reduction of the nuclear volume by chromatin extradition is complemented by a shrinking nuclear envelope. Furthermore, we confirmed our morphological data about cytoplasmic chromatin in apoptotic cells by using a polyclonal antibody to histone H3. This antibody labelled nuclear as well as cytoplasmic chromatin. Therefore, our data indicate that chromatin is translocated from the nucleus to the cytoplasm during apoptosis in BV-2 cells.

B85

Anterograde axonal and terminal degeneration promotes infiltration of inflammatory lymphocytes in T-cell recipient mice

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Background: Axonal transection takes place in early lesions of multiple sclerosis (MS). Here, we investigated if axonal transection promotes recruitment of inflammatory cells into areas of anterograde axonal (Wallerian) and terminal degeneration distal to the site of transection. If so, this might contribute to disease progression in patients with MS. Methods: To mimic axonal transection in early lesions in MS, T-cells reactive to myelin basic protein (MBP) were transferred to female SJL/N mice, that subsequently were subjected to perforant pathway transection resulting in a dense anterograde axonal and terminal degeneration in the dentate gyrus. Brains from mice with: 1) T-cell transfer and PP-lesion with 7 day survival post lesion, 2) T-cell transfer alone, 3) PP-lesion alone, or 4) unmanipulated mice, were processed immunohistochemically for T-cells, microglia-macrophages and for MBP to evaluate clearance of myelin debris. Results: Axonal lesion resulted in a massive infiltration of CD3+, CD4+ and to a smaller extent CD8+ cells in the deafferented dentate gyrus of the PP-lesioned T-cell recipient mice compared to PP-lesioned mice, T-cell recipient mice, and control mice. PP-lesioned T-cell recipient mice also showed enhanced activation of CD11b+ microglia, and signs of enhanced macrophage recruitment. Observation of reduced amounts of MBP+ particles in the PP-lesioned T-cell recipient mice compared to PP-lesioned mice suggested that the presence of T-cells in deafferented dentate gyrus enhanced microglial-macrophage clearing of myelin debris. Conclusion: Anterograde axonal and terminal degeneration distal to the sites of axonal transection may precipitate the development of new inflammatory lesions along the anterogradely degenerating axons.

B86

The role of Toll-like receptors in CNS glial responses

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Glial cells (astrocytes and microglia) are early responders to injury and infection and become activated in brain diseases such as Multiple Sclerosis and Alzheimer's Disease. Glial activation is accompanied by increased expression of toll-like receptors (TLRs), innate pattern recognition receptors that are implicated in pathogen recognition. We wish to understand the role of TLRs in glial responses to sterile injury. Immunohistochemistry showed TLR2 protein to be upregulated specifically on activated microglia and not activated astrocytes in murine models of anterograde (perforant path lesion, Babcock *et al.*, J Neurosci. 2006 Dec 6;26(49):12826–37) and retrograde neuronal degeneration (transection of the facial nerve, Holm, data not shown). Message for other TLRs was also upregulated though cellular specificity has not yet been established. However, both microglia and astrocytes express TLR receptors in vitro, both cell types responding by production of TNFalpha to ligands for TLR2 (PAM3-CSK4, a synthetic peptide, Zymosan, a yeast cell wall component, and lipoteichoic acid, LTA a gram-negative cell wall component) and for TLR4 (LPS, a gram-positive cell wall component). Flow cytometric analysis of mixed glial cultures showed that the basal level of TLR2 expression by microglia was approximately 25 fold higher than that by co-cultured astrocytes. This difference was abolished following stimulation with LPS (10ng/ml) for 24 hours after which both cell types expressed TLR2 at equivalent levels. Current experiments are aimed at comparing basal and injury-responsive levels of TLR expression by astrocytes and microglia in vivo.

B87

Semaphorin 3A and 3F: key players in myelin repair in Multiple Sclerosis?

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In MS, plaques of demyelination occur in the CNS. Some can remyelinate, with migration of oligodendrocyte precursors (OPCs) into the plaque and their subsequent maturation into myelinating oligodendrocytes. Chronically demyelinated plaques either contain plentiful OPCs which do not remyelinate, perhaps secondary to differentiation arrest, or are devoid of OPCs, perhaps lacking an attractive migratory signal or producing a repulsive one. In development, Sema3A is repulsive and Sema3F attractive to OPCs and we hypothesise that they are similarly involved in MS. Using post-mortem brain, we assessed active and chronic MS plaques, and controls, using in situ hybridisation to detect Sema3A and 3F, followed by immunohistochemistry to identify cell type. We found that neither semaphorin is detectable in control white matter or MS white matter containing chronic plaques. However, around and within active MS plaques, they were re-expressed mainly in astrocytes and activated microglia, with more Sema3F around very active plaques and more Sema3A around less active plaques. There was also twice the number of neuronal cell bodies expressing Sema3A or 3F in MS cerebral cortex ($63 \pm 6.3\%$ or $70 \pm 13.3\%$) compared to control cortex ($33 \pm 6.6\%$ or $30 \pm 2.9\%$ respectively). We reproduced these results in rats with intraspinal injections of lysolecithin to demyelinate the corticospinal tract. After three days, many

astrocytes and microglia expressed Sema3A or 3F around and within demyelinating plaques, and there was an increase in the number of neuronal cell bodies in the contralateral motor cortex expressing Sema3A or Sema3F. These results suggest that demyelination provokes expression of Sema3A and 3F in astrocytes and activated microglia locally, and distantly, in the cell bodies of axons traversing demyelinating plaques. We propose that both mechanisms may influence remyelination and we are testing this by lentiviral over-expression of Sema3A or 3F in vitro in myelinating co-cultures and in vivo, in lysolecithin-induced spinal cord demyelination.

B88

Regulation of inflammatory reaction by glucocorticoid receptors in microglia in MPTP- model of Parkinson's disease

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Experimental evidence indicates that microglia activation is associated with enhanced neurodegeneration in animal models of PD and in PD patients. It is not known whether inflammatory changes are responsible for active nerve cell death or whether they have protective functions during degeneration of dopamine neurons. In CNS, the precise actions of glucocorticoid receptors (GRs) are not well characterized. We are analyzing the role of GR in MPTP model of PD using mouse lines in which GR is specifically inactivated in either macrophages/microglia (GRlysCre) or in dopaminergic neurons (GRDATCre). 3–4 month-old GRlysCre and GRDATCre control and mutant mice were injected with MPTP (20mg/kg i.p) and sacrificed 24 hrs, 7 days and 21 days later. The postmortem analysis revealed: i) a significant increase in dopaminergic neuronal death in mutant GRlysCre compared to control littermates whereas there was no difference between GRDATCre mutants and their controls; ii) decrease in striatal dopamine transporter activity in mutant GRlysCre mice with respect to controls after 21 days; iii) nearly x8 fold higher number of GFAP+ cells in SN of mutant GRlysCre compared to control littermates. We are analysing cytokine expression levels in SN and striatum by quantitative PCR and the results so far indicate that the expression of TNFα is significantly elevated in SN of mutant GRlysCre after 24 hours. Overall, the results suggest that GRs in microglia, activated by endogenous glucocorticoids, participate in protecting DA neurons in SN against MPTP intoxication.

B89

Nerve damage or inflammation alter purinergic signaling in satellite glial cells in mouse sensory ganglia

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Satellite glial cells (SGCs) are the main glial cells in sensory ganglia. The morphology of these cells has been described in detail, of but little is known about their physiological and pharmacological properties. We have shown previously that SGCs in trigeminal ganglia (TG) are endowed with functional P2Y purinergic receptors, and the objective of the present work was to find out whether these receptors are altered under pathological states -- axotomy of the infraorbital nerve or complete Freund's adjuvant (CFA) injection in the lower lip of mice. Ganglia were removed 4–7 days after treatments and were tested in vitro. We examined changes in intracellular Ca^{2+} in SGCs in response to ATP using Fluo-3 or Fura-2 as indicators. The effect of lower lip inflammation was tested in short-term (20 h) cultures and that of axotomy in intact TG. In controls the threshold of SGCs response to ATP was 5 μM (20% of the cells responded), and response reached plateau at 100 μM; at least 25 cells were examined at each concentration. In SGCs from treated mice the threshold for ATP was 0.01 μM (i.e., 500-fold lower than control), and reached plateau at 10 μM. These results apply for both treatments.

In this work we showed for the first time that the sensitivity of SGCs in TG to the inflammatory neurotransmitter ATP greatly increased after axotomy and inflammation. We conclude that SGCs are likely to be involved in pathological changes in TG and might contribute to the altered signaling that takes place in chronic pain states.

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B90 Late Substitution

Glial cell calcium signals measured using a genetically encoded FRET based Cameleon Calcium Indicator in Transgenic Mice

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Recent discoveries have highlighted the central role glial cells play in signaling in the brain. Oligodendrocytes and Schwann cells receive signals during action potential traffic and astrocytes monitor neural activity and modulate network activity and plasticity. Glial cell signals rely on voltage and metabotropic Ca^{2+} signaling, and mutual signals between glia and neurons may play a vital role nervous system function including regeneration. To investigate the Ca^{2+}-based neuron-glia signaling in intact brain, we have expressed a Ca^{2+} indicator protein, the YC 3.60 cameleon, under the control of the human S100β promoter, and directed its expression predominantly in astrocytes and Schwann cells. Expression of YC 3.60 within glial cells extended into the entire cytoplasmic compartments including in the fine terminal processes of protoplasmic astrocytes and Schwann cell Cajal bands. In the brain, all known S100β expressing cells expressed significant concentrations of YC 3.60 such that activity could be recorded. While expression was almost exclusive in astrocytes, a number of other cell types which express S100β, such as large motor neurons in the brain stem and some of the NG2 and CNP positive oligodendrocyte progenitor cells (OP cells), also were fluorescent with YC 3.60. Using a variety of known *in vivo* assays, we found that stimuli known to elicit Ca^{2+} signals in astrocytes (glutamate application, electrical stimulation of neural pathways) caused substantial and rapid Ca^{2+} signals in the YC 3.60 expressing astrocytes. Action potential propagation was associated with Schwann cell Ca^{2+} signals in isolated peripheral nerves. These results, for the first time, show that genetically encoded reporter is capable of recording activity dependent Ca^{2+} signals even in the very fine terminal processes of astrocytes that wrap individual synaptic boutons and in Schwann cells.

B91

Pharmacological inhibition of the NO-pathway blocks microglia migration following a laser lesion in the mouse spinal cord in vivo

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The advances in transgenic mouse technology expressing celltype-specifically fluorescent proteins as well as in laser-scanning microscopy allow identifying individual cells in vivo. We were using two-photon microscopy to study the response of microglial cells to a laser lesion in the spinal cord. Adult transgenic mice with microglial-specific EGFP expression were anaesthetized using pentobarbital i. p. and methohexital-Na i. v.. Vital functions were closely monitored and were maintained for a period of at least 12 h. During anaesthesia, a laminectomy was performed at the main input region of the hind leg, at the spinal cord segment L4. After surgery, the mouse was placed underneath the objective of the laser-scanning microscope. To monitor in vivo responses of microglial cells to an acute injury, we applied a laser lesion. In the intact animal, resting microglial cells displayed highly motile branches that continuously surveyed the microenvironment and axonal structures. Following a laser lesion, we observed extension of microglial branches towards the lesion within minutes. In the time course of several hours, microglial cells moved towards the lesion site followed by microglia accumulation indicating strong microgliosis. Local application of non-specific and specific nitric oxide synthase (NOS) and guanylate cyclase inhibitors led to an almost complete blockade of the microglia response around the lesion site. These results indicate that the early response of microglia to a spinal cord lesion involves activation of the NO-system. Pharmacological blockade of the NO-pathway may represent an efficious approach to modulate microglia reaction in acute or chronic spinal cord diseases.

B92

Expression and function of Nox genes in murine microglia

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Reactive oxygen species (ROS) such as superoxide ion and hydrogen peroxide are involved in cell death mechanisms and regulate the activity of redox sensitive enzymes or transcription factors. Microglial cells are known to generate high amounts of ROS during development or in pathological contexts. Part of this ROS production is due to the activity of the leucocyte NADPH Oxidase in which the Nox2 protein acts as the catalytic subunit. A gene family of NADPH Oxidases (Nox), sharing a high homology to Nox2 has been described, but their function in the CNS remains an open question. We have investigated the microglial expression of the Nox gene family. We observed that in addition to Nox2 expression, microglia purified from rodent cerebral cortex express Nox1 gene products. RT-PCR analyses did not reveal Nox3, Nox4 or Duox transcripts in purified microglia, however these cells expressed Noxo1 and Noxa1 gene encoding for Nox1 regulator and activator subunits. Nox1 activity was revealed by detection of superoxide and hydrogen peroxide in microglia derived from Nox2-KO mice. Using Nox pharmacological inhibitors, lentivirus transducing shRNA specific for Nox1 gene as well as microglia derived from wild-type or Nox2-KO mice, we have shown that Nox1 regulates microglial proliferation and production of pro-inflammatory compounds. Our results suggest that Nox1 plays an important role in microglial growth and activation during development or in pathologies.

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B93

Glial cell changes in a rat MOG-EAE model of MS pathology

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Experimental allergic encephalomyelitis (EAE) induced in female Dark Agouti rats by subcutaneous injection of myelin oligodendrocyte protein (MOG) generates inflammation and demyelination primarily in the spinal cord and optic nerve, which are the primary sites affected in neuromyelitis optica (NMO), a variant of multiple sclerosis (MS). Additionally, we found extensive remyelination by Schwann cells in the spinal cord of MOG-EAE rats, which has also been reported to occur in NMO patients. This suggests that astrocytes comprising the glia limitans are unable to maintain effective exclusion of peripheral Schwann cells from spinal cord CNS tissues during the disease process. Furthermore, a key marker protein found in NMO patients has been shown to be autoantibody to aquaporin-4, a water channel protein found primarily on the endfeet of astrocytes which make up part of blood-brain barrier. From both these lines of evidence, it appears that the integrity of astrocyte functions may be particularly critical to the disease process in NMO, and possibly also in MOG-EAE. We have investigated the disposition of astrocytes, microglia and oligodendrocyte precursor cells at different stages in the disease course of MOG-EAE using fluorescent immunohistochemistry. Early stages of the disease are dominated by the appearance of large Virchow-Robin (VR) spaces invaginating from the pial surface: these invaginations are enclosed by extensive scaffolds of microglial processes, which may play a role in their development. Later on, macrophages and other invading peripheral cells penetrate into spinal parenchyma through failing astrocytic barriers at pial and VR surfaces. Following a demyelination phase, Schwann cells are able to penetrate demyelinated tissue and compete favourably against extant oligodendrocytes for axonal substrates. The observed presence of astrocytes in these regions does not effectively inhibit the process.

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B94

Disruption of the axo-glial junction within normal appearing white matter of the MS brain

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Alongside focal areas of demyelination, secondary progressive Multiple Sclerosis (SPMS) is also characterised by the presence of global microglial activation and diffuse axonal injury within the 'normal appearing' white matter (NAWM). Early disruption of saltatory conduction in MS may be due to breakdown of the junction between the leading edge of the myelin sheath and the axon that demarcates the node of Ranvier. Oligodendrocyte specific neurofascin (Nfasc155) binds proteins on the axonal membrane to form paranodes, which stabilise Nav+ channels to the node, and Kv+ channels to the juxtaparanode. Here we describe how disruption of the nodal architecture coincides with inflammation in the NAWM. NAWM was scored for perivascular infiltrates, microglia, macrophages and axonal pathology from 17 SPMS cases in comparison to controls. The discreet Nfasc155+ paranodes were often disrupted when in proximity to activated microglia and associated with damaged axons and this correlated with the degree of NAWM inflammation. An encroachment of juxtaparanodal Kv1.2 channels into the paranodal domains, nearer the undisrupted node (Nav1+ structures) accompanied the changes in Nfasc155 expression. Similar nodal disruptions were noted in pre-symptomatic experimental autoimmune encephalogenic animals; indicating Nfasc155 changes in the NAWM to be independent of invading immune cells. Within the periplaque and active demyelinating lesions Kv1.2 channels abutted normally clustered nodes. Nodal structures were only disrupted in the complete absence of Nfasc155, indicating that complete disruption of paranodes and movement of Kv1.2 channels precede nodal alterations. The disruption in oligodendrocyte Nfasc155+ expression in the absence of immune cell invasion suggests evolving damage to the axon-oligodendrocyte complex that may contribute to conduction deficits outside of the demyelinated lesions.

B95

Expression of membrane-type matrix metalloproteinases (MT-MMPs) in CNS inflammation versus CNS injury

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Matrix metalloproteinases (MMPs) are a family of 22 proteases, including six membrane-bound MMPs (MT-MMPs). MMPs are thought to mediate cellular infiltration in CNS inflammation, which is an integral part of the pathogenesis of multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). MMPs may also mediate infiltration after CNS injury. We have investigated the expression of MT-MMPs after brain stab injury, and in spinal cord from mice with EAE. Using real-time RT-PCR, we found in three different models of EAE that expression of four MT-MMPs (MMP-15, 16, 17 and 24) were downregulated in spinal cord of mice with severe EAE, whereas the two remaining MT-MMPs (MMP-14 and 25) were upregulated. After entorhinal cortex stab lesion, which results in activation of microglia and prominent influx of leukocytes to the injured area, MMP-15, 17 and 25 were significantly downregulated. With flow cytometric cell sorting followed by real-time RT-PCR, we found that expression of all but one (MMP-24) of the MT-MMPs could be detected in control microglia. Microglia isolated from mice with severe EAE showed statistically significant down-regulation of MMP-15, 17 and 25. We investigated possible sources of upregulated MMP-25 in EAE spinal cord using immunofluorescent double staining for MMP-25 and the microglia/macrophage marker CD11b. This showed no colocalization of MMP-25 with CD11b+ cells, whereas cells with neuronal morphology in spinal cord grey matter stained positive for MMP-25. These results suggests that activation of microglia in the course of neuroinflammation correlates with reduced expression of certain MT-MMPs. Activated glia cells in the injured CNS may also downregulate MT-MMP expression in the absence of autoimmune inflammation.

B96

Interleukin-11 potentiates oligodendrocyte survival and maturation, and myelin formation.

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Mechanisms regulating oligodendrocyte survival and myelin formation are an intense focus of research into myelin repair in multiple sclerosis (MS). Although demyelination and oligodendrocyte loss are pathological hallmarks of the disease, increased oligodendrocyte numbers and remyelination are frequently observed in early lesions, but diminish as the disease course progresses. In the current study, we used a microarray-based approach to investigate genes regulating repair in MS lesions, and identified interleukin-11 (IL-11) as an astrocyte-derived factor that potentiates oligodendrocyte survival and maturation, and myelin formation. IL-11 was induced in human astrocyte cultures by the cytokines IL-1β and TGFβ1, which are both expressed in MS plaques. In MS tissue samples, IL-11 was expressed by reactive astrocytes, with expression localizing to the myelinated border of both active and silent lesions. Its receptor, IL-11Rα, was expressed by oligodendrocytes. In experiments in human cultures, IL-11Rα localized to immature oligodendrocytes, and its expression decreased during maturation. In cultures treated with IL-11, we observed a significant increase in oligodendrocyte number, and this was associated with enhanced oligodendrocyte survival and maturation. Importantly, we also found that IL-11 treatment was associated with significantly increased myelin formation in rodent CNS cocultures. These data are the first to implicate IL-11 in oligodendrocyte viability, maturation, and myelination. We suggest that this pathway may represent a potential therapeutic target for oligodendrocyte protection and remyelination in MS.

B97

Cytokine-induced reactivation of Jagged-Notch signaling restricts oligodendrocyte maturation and myelin formation

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In the developing CNS, activation of Notch1 receptors on oligodendrocyte progenitors (OPCs) by the ligand, Jagged1, inhibits their maturation, maintaining the progenitor pool. Reactivation of Notch _ignalling has recently been demonstrated in the demyelinating disease multiple sclerosis (MS) and its animal models. In active MS plaques, Jagged1 localizes to reactive astrocytes, while Notch1 and its effector, Hes5, are expressed by oligodendrocytes. In the current study, we examined the mechanisms underlying reactivation of Jagged-Notch _ignalling in CNS glia, and the potential consequences. Expression of Jagged1 was induced in human astrocytes by TGFβ1, a cytokine expressed in MS lesions, and this effect was mediated via the receptor ALK5 and the transcription factor Smad3, with Smad2 contributing a potentiatory nonessential role. Activation of Notch _ignalling in human OPCs restricted their differentiation. Importantly, coculture of human OPCs with TGFβ1-treated human astrocytes also restricted OPC differentiation, and inhibition of astrocytic Jagged1 expression downregulated this effect. In myelinating rodent CNS cocultures, specific abrogation of Notch1 in OPCs significantly potentiated the formation of compact myelin. In parallel *in vivo* studies, we found that OPC differentiation was accelerated in Olig1-Cre x loxP-Notch1 mice. Taken together, these results suggest that astrocytes exposed to cytokines present in the MS lesion signal to oligodendrocytes via Jagged-Notch interactions, and that the consequences may be restrictive in terms of oligodendrocyte maturation and myelin formation.

B98

Interleukin-1β regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program

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Loss of blood-brain barrier (BBB) integrity is an early and significant event in lesion pathogenesis in the inflammatory demyelinating disease multiple sclerosis (MS), and understanding mechanisms involved may lead to novel therapeutic avenues for this disorder. Well-differentiated endothelium forms the basis of the BBB, while astrocytes control the balance between barrier stability and permeability via production of factors that restrict or promote vessel plasticity. Here, we report that the proinflammatory cytokine interleukin-1β (IL-1β), which is expressed in active MS lesions, causes a shift in the expression of these factors to favor plasticity and permeability. The transcription factor HIF-1 plays a significant role in this switch. Using a microarray-based approach, we found that in human astrocytes, IL-1β induced the expression of genes favoring vessel plasticity, including HIF-1α and its target, VEGF-A. Demonstrating relevance to MS, we showed that HIF-1α and VEGF-A were expressed by reactive astrocytes in active MS lesions, while the VEGF receptor VEGFR2/flk-1 localized to endothelium and IL-1 to microglia/macrophages. Suggesting functional significance, we found that expression of IL-1β in the brain induced astrocytic expression of HIF-1α and VEGF-A, and BBB permeability. In addition, we confirmed VEGF-A to be a potent inducer of BBB permeability and angiogenesis, and demonstrated the importance of IL-1β-induced HIF-1α in its regulation. These results suggest that IL-1β contributes to BBB permeability in MS via reactivation of the HIF-VEGF axis. This pathway may represent a potential therapeutic target to restrict lesion formation.

B99

The pro-apoptotic transcription factor p53 impacts the pattern of microglia activation

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Microglia, like macrophages, can adopt classical (M1) or alternative (M2) patterns of activation that lead to toxic or trophic actions respectively. The HIV/gp120 coat protein causes M1 activation in microglia leading to TNF-alpha dependent neurotoxicity in mixed cerebrocortical cell culture. Microglia from p53 deficient mice fail to transmit the toxicity of gp120 to neurons. To determine why p53 is required for gp120 induced neuronal injury, whole mouse genome expression arrays were performed using mRNA extracted from cultured microglia generated from strain matched p53 containing and p53 deficient neonatal mice. We observed that p53 has a dramatic effect on the regulation of gene expression in microglia. We also observed that p53 deficient microglia have increased expression of many genes associated with the M2 pattern of activation and a blunted M1 cytokine response following exposure to interferon-gamma. Taken together, these findings suggest that p53 is involved in promoting M1 activation and in the absence of p53, microglia default to an M2 activation pattern.

B100

Activated microglial production of IL6, IL16 and MIP-1-beta after incubation with IFN-gamma-secreting CD8+ T cells from C. neoformans-infected mouse brain

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Activated microglial production of IL6, IL16 and MIP-1beta*f*nafter incubation with IFN-gamma-secreting CD8+ T cells from Cryptococcus neoformans-infected mouse brain Karen Aguirre1,2, Sherry Pittman1, and Christine Ward1 1Coastal Carolina University, Conway, SC 29526 and 2Trudeau Institute, Saranac Lake, NY 12983 Abstract Cryptococcus neoformans variety grubii is a facultative intracellular fungus that causes meningoencephalitis in individuals with CD4+ T cell deficiency. Interactions between

CD8+ T cells and microglia in non-viral intracellular infections have not been well-characterized. CD8+ T cells are often presumed to act via a cytotoxic mechanism in the CNS, but to date detailed studies have been carried out exclusively in viral systems, where host cell metabolism is significantly compromised. Microglia bearing non-viral intracellular pathogens like C. neoformans variety grubii conceivably deploy defensive mechanisms not available to virally-infected cells. In this study, when T cells harvested from the brains of C. neoformans variety grubii-immunized and re-challenged CD4+ T cell-deficent mice were co-cultured with murine microglia pre-stimulated by opsonized cryptococci, microglial production of IL6 was enhanced and there was a trend toward MIP-1beta induction. Levels of CD4+ T cell chemoattractant cytokine IL16 were also elevated in co-culture supernatants. Cytoxicity was detected in co-culture with T cells from a minority of mice. Thus, T cells and microglia collaborated to mount an immune response that featured IFNgamma-induced potentiation of effector microglia (reminiscent of CD4+ T cell-mediated macrophage activation), chemoattraction of CD4+ cells, and in some cases, T cell mediated cytotoxicity. To our knowledge, this is the first exploration of CD8+ T cell/microglial interactions with an intracellular eukaryotic pathogen. These studies may encourage and inform attempts to construct a CD8+ T cell-based vaccine of value to CD4+ T cell-deficient individuals.

B101

Molecular and cellular aterations in cortical grey matter of multiple sclerosis patients

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system leading to demyelinated lesions. Although MS is regarded as a white matter disease several grey matter changes have been described recently. Insights into axonal pathology as well as grey matter demyelination raised the attention to the significance of grey matter pathology. Little is known about the molecular changes in the cerebral cortex of MS patients. Thus, the aim of our work is to identify molecular and cellular changes in the normal appearing grey matter (NAGM) of cortical brain tissue. Postmortem tissue blocks from primary motor cortex as well as frontal cortex were selected and screened for cortical demyelination using mmunohistochemistry. In this search for NAGM, we identified extensive demyelinated areas in the cerebral cortex in MS patients. The most common type of lesions was characterized by demyelination extending inward from the subpial surface. To identify molecular changes which may be involved in the development of cortical lesions we analyzed the gene expression of MS and control patients using microarray technology. Preliminary analysis of a subset of MS and control cases suggest a deregulation of functionally related genes in NAGM of MS cases.

B102

Inflammatory activation of microglia and macrophages by HIV-1 envelope protein gp120 via chemokine receptors CCR5 and CXCR4 stimulates p38MAP kinase and toxicity in neurons

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In the brain human immunodeficiency virus-1 (HIV-1) infects productively only macrophages and microglia, yet the infection can lead to dementia. Interaction of the viral envelope glycoprotein gp120 with CD4 and chemokine receptors CXCR4 (CD184) or CCR5 (CD195) activates microglia and macrophages and initiates a p38 mitogen-activated protein kinase-dependent signaling cascade that results in neuronal injury and death. Using as a model mixed cerebrocortical cultures, containing neurons, astrocytes and microglia from rodents genetically deficient in CXCR4, or CCR5, or both HIV coreceptors with wild types as controls, we found that either chemokine receptor can critically contribute to HIV/gp120 neurotoxicity. However, we also observed that CCR5–mediated signaling triggered by its physiological ligands MIP-1beta (CCL4) or RANTES (CCL5) can be neuroprotective, in this case via an Akt (PKB)-dependent pathway. Immunofluorescence microscopy revealed coexpression of CXCR4 and CCR5 not only in microglia but also astrocytes and neurons, and Ca2+ imaging experiments suggested that CCR5 ligands modulate CXCR4-mediated responses through heterologous desensitization. Surprisingly, both the microglia/macrophage-activating peptide Tuftsin and its inactivating fragment TKP were as efficient in abrogating HIV/gp120 neurotoxicity as was the depletion of microglia. Taken together, our findings indicated microglia/macrophages play a pivotal role in induction and prevention of HIV-1 neurotoxicity. Supported in part by amfAR and by NIH grants R01 NS050621 (to M.K).

B103

Microglial/macrophage dynamics in normal aging and in Alzheimer's-like disease

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In Alzheimer's disease, innate microglial responses are thought to occur without significant involvement of peripheral immune cells, such as macrophages. We have evaluated microglial/macrophage dynamics in the neocortex of young (4 month) and aged (18 month) wildtype (WT) and amyloid precursor protein (APP)/ presenilin (PS) 1 transgenic (Tg) mice using flow cytometry. Estimates generated by point-counting techniques showed that ~0.10% of the neocortex was occupied by amyloid plaques in 4 month APP/PS1 Tg mice, and that plaque load increased significantly to ~3.5% by 18 months. Even though small amyloid deposits were present, macrophage proportions were not significantly different in young mice. Macrophage proportions increased 2- and 20-fold in aged WT and APP/PS1 Tg mice, respectively. Similarly, microglial numbers were unchanged in young mice, but were increased 3-fold in 18 month APP/PS1 Tg mice relative to age-matched WT control mice. Interestingly, however, rates of microglial and macrophage proliferation were unaffected by age or expression of the APP/PS1 transgene, suggesting alternate mechanisms of population expansion occur. Instead, rates of microglial and macrophage apoptosis were increased in 18 month APP/

PS1 Tg mice, indicating that microglial/macrophage cell loss prevails over capacity for self-renewal in chronic neuroinflammatory disease. This coincided with emergence of microglial subpopulations expressing high levels of CD11c, CD86 and MHC Class I, increased infiltration by CD4+ and CD8+ T cells, and elevated levels of IL-1beta mRNA. Importantly, stereological estimates found no significant changes in numbers of neocortical neurons between aged WT and APP/PS1 Tg mice. Supported by The Lundbeck Foundation, The Novo Nordisk Foundation, and The Augustinus Foundation.

B104

CCL2 binding to primary adult human astrocytes is CCR2-independent: a new role for astrocytes in the regulation of inflammation.

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Astrocytes are major sources of chemokines within the central nervous system (CNS) and are critical components of the inflammatory response in multiple sclerosis (MS). Within MS lesions, an elevated level of CCL2 has been shown to be involved in the recruitment of monocytes, which express the corresponding receptor, CCR2. However, contradictory findings have been reported on the expression of CCR2 by adult human astrocytes in vitro. To understand the role of astrocytes in chemokine regulation at the inflammatory site, we have investigated the expression of CCR2 by astrocytes using real time PCR and flow cytometry following treatment with IL-1â, TNF and IFN α at 0–100 ng/ml for upto 48 h. TNF significantly increased CCR2 mRNA expression at 24 and 48 h; however no protein expression was detected. Despite the absence of detectable CCR2, CCL2 binding to astrocytes by flow cytometry using biotinylated CCL2 followed by avidin-FITC was observed under basal conditions and this was unaffected by proinflammatory cytokine treatment. The absence of CCR2 surface expression was confirmed by the absence of activation of signal transduction pathways (Erk and Akt) by CCL2. In vitro, human adult astrocytes constitutively expressed the chemokine decoy receptor D6. Although D6 immunostaining was observed in human brain tissue section, the role of D6 in CCL2 binding to astrocytes remains to be confirmed. Our results suggest a role for astrocytes in the regulation of inflammation by controlling CCL2 levels to establish a chemokine gradient which may then direct the migration of leukocytes into MS lesions.

B105

Consequences of microglial activation on the survival of oligodendrocytes at defined stages of differentiation

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Activated microglia, the brain's principal immune cell, have been implicated in a number of neurodegenerative diseases. Activated microglia have been identified at sites of neural injury and inflammation, and may have a role in the pathogenesis of disease through the release of cytotoxic factors. It has been suggested that these factors are detrimental to the survival of other neural cells including oligodendrocytes. Oligodendrocytes (OLG), as the major myelin producing cells of the central nervous system, play an important physiological role in myelinating axons. Damage or loss of these cells or their precursors can result in demyelination, axonal damage and neuronal degeneration. Defining mechanisms that can affect the survival of OLG or their precursor cells (OPC) are likely to be relevant in devising strategies to halt the progression of neurodegeneration. In the present study we have investigated the consequences of microglial activation on the survival of oligodendrocytes. Microglial cultures were activated with lipopolysaccharide, a bacterial cell wall component, which was specific for toll-like receptor 4 (TLR4) and the conditioned media collected. OLG and OPC were exposed to the conditioned media and cell survival determined. Here we show that activated microglia release soluble factors which influence the survival of OPC by reducing their proliferative capacity as determined by Ki67 immunolocalisation and BrdU incorporation. This effect was dependent on the stage of maturation affecting OPC only and not differentiated OLG. Microglial conditioned media did not cause cell death in either oligodendrocyte population. Future studies will aim to determine the soluble factors released from TLR-4 stimulated microglia which may be responsible for the observed effect on OPC.

B106

Microglia in aged PS1-APP mice exhibit downregulation of Aβ clearing genes and upregulation of pro-inflammatory cytokines

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Microglia accumulate in Alzheimer's Disease (AD) brains, but their role in AD pathogenesis remains to be elucidated. We showed recently that decreased early microglial accumulation leads to increased β-amyloid (A) deposition and early mortality in AD mouse models. Microglia clear Aβ by secreting Aβ -degrading enzymes and express receptors that promote Aβ phagocytosis, suggesting an early neuroprotective role in AD. In contrast, Aβ can activate microglia to produce cytokines and neurotoxins, hence promoting neurodegeneration. To understand the role of microglia in AD, we developed a method to isolate purified microglia from adult mice and analyzed gene expression in these freshly isolated cells from old transgenic PS1-APP mice, an established mouse model of AD, and from their littermate non-transgenic controls. Aged PS1-APP mice have a large number of A deposits in their brains and a robust microglial response. Using quantitative real-time PCR, we found that PS1-APP microglia have a 2–4 fold decrease in expression of the A -binding scavenger receptors (SRA, CD36, LOX-1), and CD11b and a 2–5 fold decrease in expression of the A -degrading enzymes insulysin, neprilysin and MMP9 compared with control mice. In contrast, PS1-APP microglia had a 3-fold increase in the pro-inflammatory cytokines IL-1 and TNFα. Our results suggest that microglia from mice with advanced AD have a diminished ability to clear A via

phagocytosis and/or degradation, but maintain intact pro-inflammatory and neurotoxic activity. Early microglial accumulation promotes A clearance and is protective in AD, but as disease progresses, microglia loose their A -clearing capabilities and become more pro-inflammatory, therefore contributing to neurodegeneration. Anti-inflammatory therapy for AD should take this dual role for microglia into consideration.

REGENERATION AND REPAIR

B107

Cellular prion protein in astrocytes modulates neuronal survival and differentiation

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The functions of cellular prion protein (PrPc) are under intense debate and PrPc loss-of-function has been implicated in the pathology of prion diseases. Neuronal PrPc engagement with Stress Inducible Protein-1 (STI1) and laminin plays a key role in cell survival and differentiation. The present study evaluated whether PrPc expression in astrocytes modulates neuron-glia crosstalk that underlies neuronal networking. Astrocytes from wild-type mice promoted a higher level neuritogenesis than astrocytes obtained from PrPc-null animals. Remarkably, neuritogenesis was greatly diminished in co-cultures combining PrPc-null astrocytes and neurons. Laminin secreted and deposited at the extracellular matrix by wild-type astrocytes presented a fibrilary pattern and was permissive for neuritogenesis. Conversely, laminin coming from PrPc-null astrocytes displayed a punctate organization, and did not support neuronal differentiation. Additionally, secreted soluble factors from PrPc-null astrocytes promoted lower levels of neuronal survival than those secreted by wild-type astrocytes. PrPc and STI1 were characterized as soluble molecules secreted by astrocytes which participate in neuronal survival. Taken together, these data indicate that PrPc expression in astrocytes is critical for sustaining cell-to-cell interactions, the organization of the extracellular matrix, and the secretion of soluble factors, all of which are essential events for neuronal differentiation and survival.

B108 Late Substitution

Müller glial cells from adult human retina exhibit neural stem cell characteristics and potential to regenerate damaged retina

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BACKGROUND

Recent evidence outlines the role of glia as neural precursors in the adult central nervous system. Müller glial cells exhibit progenitor characteristics in the post-natal chick and rat retinae, but their progenitor-like role in developed human retina has not been clearly elucidated. We have investigated the ability of a subpopulation of Müller glia to differentiate into retinal neurons *in vitro* and upon transplantation *in vivo*.

METHODS

Spontaneously immortalized Müller glial cells were cultured on extracellular matrix (ECM) proteins in the presence of FGF2 or retinoic acid. Expression of transcription factors of neural progenitors, markers of photoreceptor and retinal ganglion cells, as well as markers of other mature retinal neurons were examined by confocal microscopy of immunostained cells, western blotting and RT-PCR analyses. Cells were transfected with the pEGFP vector (Clontech, USA) and transplanted into the subretinal space of experimental models of retinal degeneration. Fate of the transplanted cells *in vivo* was investigated by confocal microscopy of retinal sections co-stained with antibodies to GFP and various retinal cell markers.

RESULTS

In the presence of extracellular matrix and growth factors, spontaneously immortalized Müller glial cells acquire neural morphology, form neurospheres and express neural progenitor markers such as βIII tubulin, Sox-2, Pax-6 and Chx10. Upon the influence of growth factors and ECM they express Nrl, Nr2e3 and Crx, which are factors implicated in the developmental formation of photoreceptor cells. Under various culture conditions they also express ATOH7, Brn3b, HuD and Notch-1, which are involved in the development and maturation of ganglion cells. They also exhibit markers of post-mitotic retinal neurons, including PKC, peripherin, recoverin, HuD, calretinin and Brn3. Immortalized Müller cells grafted into the subretinal space of dystrophic RCS rats or migrated into the different retinal cell layers, where they expressed various markers of retinal neurons.

CONCLUSION

We show that adult human neural retina harbours a population of Müller glia with neural stem cell characteristics. The results suggest that these cells constitute a useful source of neural progenitors with the potential to develop into cell based therapies to treat human retinal disease.

B109

Inhibiting cell cycle progression reduces reactive astrogliosis initiated by scratch injury in vitro and by cerebral ischemia in vivo

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Astrogliosis occurs in a variety of neuropathological disorders and injuries, and excessive astrogliosis can be devastating to the recovery of neuronal function. In this study, we asked whether reactive astrogliosis can be suppressed in the lesion area by cell cycle inhibition, and thus have therapeutic benefits. Reactive astrogliosis induced in either cultured astrocytes by hypoxia or scratch injury, or in a middle cerebral artery occlusion (MCAO) ischemia model were combined to address this issue. Significantly, the cell cycle inhibitor, olomoucine, inhibited hypoxia-induced cell cycle activation by arresting the cells at G1/S and G2/M in a dose-dependent manner, and also reversed hypoxia-induced up-regulation of PCNA. Also in the cultured astrocytes, scratch injury induced reactive astrogliosis, such as hypertrophy and an increase in BrdU(+) astrocytes, both of which were ameliorated by olomoucine. In the MCAO ischemia mouse model, we found that intraperitoneal olomoucine administration significantly inhibited these astrogliosis-associated changes. To demonstrate further that cell cycle regulation impacts on astrogliosis, cyclin D1 gene knockout mice (cyclin D1–/–) were subjected to ischemia, and we found that the percentage of Ki67-positive astrocytes in these mice was markedly reduced in the boundary zone. The number of apoptotic neurons and the lesion volume in cyclin D1–/– mice also decreased as compared to cyclin D1+/+ and cyclin D1+/– mice at days 3, 7 and 30 after local cerebral ischemia. Together, these in vitro and in vivo results strongly suggest that astrogliosis can be significantly affected by cell cycle inhibition, which therefore emerges as a promising intervention to attenuate reactive glia-related damage to neuronal function in brain pathology.

B110

Glial Fibrillary Acidic Protein (GFAP) expression is increased by dexamethasone following local gliotoxic injection in the rat brainstem

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Ethidium bromide (EB) is a gliotoxic agent that causes focal astrocytic and oligodendroglial disappearance. As immunosuppressive and antiinflamatory drugs are known to modify glial expression, this study was designed to investigate astrocyte immunoreactivity to Glial Fibrillary Acidic Protein (GFAP) after EB injection in animals submitted to dexamethasone (DX) treatment. Adult Wistar rats were injected into cisterna pontis with 0.1% EB and received DX (3mg/kg/day, intraperitonial route - group I) or not (group II). Some were injected with 0.9% saline solution and were also treated with DX (group III) or not (group IV). Brainstem samples were collected from 24 hours to 31 days post-injection for GFAP immunohistochemical staining using avidin-biotin method. In groups I and II, extensive lesions were seen in the pons and mesencephalon, with astrocyte disappearance from the central area 24 hours post-injection. Macrophagic infiltration and peripheral astrocytic reaction were noted after 3 days. Marginal astrocytes presented increased immunoreactivity to GFAP and DX-treated animals presented a greater number of GFAP-positive cells. In groups III and IV, discrete pontine lesions were observed, showing central astrocyte preservation and a peripheral GFAP staining less intense comparing to groups I and II. No difference was found in GFAP immunostaining between groups III and IV after saline injection. Astrocytes from the edges of the EB-induced lesions presented increased immunoreactivity to GFAP and DX seemed to increase this expression.

B111

Developing strategies to promote endogenous repair in demyelinating pathologies.

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The mobilization of endogenous stem cells recently appeared as a promising strategy to promote brain repair. Recent studies revealed that, in the case of brain injury (including demyelination lesions), few subventricular zone (SVZ)-derived cells migrate to lesioned areas in a spontaneous repair attempt. Besides, it is now acknowledged that oligodendrocyte progenitors are present in the SVZ. Therefore, the pool of endogenous SVZ progenitors stands as an interesting source of cells for myelin repair. Our goal is to promote endogenous cell mobilization/recruitment in demyelinating pathologies in order to favour cell replacement and myelin repair. We adopted several complementary approaches to stimulate SVZ cells to exit the rostral migratory stream (RMS) and migrate toward periventricular structures. Rearing mice in enriched versus standard laboratory cages, we showed that enrichment increased SVZ cell proliferation and favoured recruitment to demyelinated lesions. Among factors acting both on SVZ cell proliferation and migration, epidermal growth factor (EGF) appeared as an interesting candidate. Indeed, we demonstrated that intranasal HB-EGF treatment not only stimulated cell proliferation but also strongly promoted SVZ cell mobilization toward the demyelinated corpus callosum. Taken together, these results suggest that acting on SVZ cell proliferation might be part of the strategy to develop in order to promote endogenous repair. In a second approach, we wanted to target molecules susceptible to act on the mode of migration of SVZ progenitor cells. We chose Reelin, a glycoprotein acting as a detachment signal on neuronal progenitors reaching the olfactory bulb, by changing their mode of migration from a tangential to a radial orientation. Preliminary results using grafts of SVZ progenitors engineered to produce Reelin into the periventricular structures showed encouraging enhanced recruitment.

B112

Regrowth of transected RGC axons occur despite the persistence of the glial scar in the lizard Gallotia galloti

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We have previously described the spontaneous regeneration of retinal ganglion cell (RGC) axons after unilateral transection of the adult lizard optic nerve (ON) (Lang *et al*; '98, '02). Our purpose is to study the glial scar in the lesion site during this process. We performed an immunohisto-

chemical study using astrocyte markers as Vimentin (Vim) and GFAP, the transcription factor Pax2, and the proliferating cells marker PCNA in lesioned lizards (after 0.5, 1, 3, 6, and 12 months postlesion). Reactive astrocytes showing upregulation of Vim and GFAP were observed from 0.5 to 12 months postlesion despite that the reinnervation of the retinorecipient tectal layers already occurs at 6 months postlesion. In addition, GFAP+/PCNA+ and Vim+/PCNA+ reactive astrocytes were firstly stained from 0.5 and 3 months postlesion, respectively. Interestingly, Pax2+/Vim+ and Pax2+/GFAP+ astrocytes were observed in control animals and reactive astrocytes also upregulated the Pax2 after lesion. We conclude that 1/ the persistent glial scar do not interfere in the regrowth of RGC axons, 2/ the Pax2 is an astrocyte marker in the lizard optic nerve and this transcription factor could regulate the upregulation of the GFAP and Vim. 3/ the upregulation of Vim+/PCNA+ and Pax2+ astrocytes at the lesion site could play a key role in the regenerating process of the lizard RGC axons.

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B113

Analysis of cellular environment of the injured rat spinal cord following treatment with cyclosporin A

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The immunosuppressant drug Cyclosporin-A (CsA) is routinely used to prevent rejection following organ or bone marrow transplantation. Considerable evidence has arisen in the last number of years that it may have neuroprotective properties which can be exploited in the treatment of spinal cord injury (Ibarra & Diaz-Ruiz, Curr Med Chem. 2006;13(22):2703–10). Spinal cord injury not only causes direct damage to the tissue but also results in a secondary injury where a glial scar forms around the site of injury forming a lesion. CsA was administered to a group of rats (n = 4) one week after they endured a contusion injury. The rats were sacrificed two weeks later and the spinal cords were sectioned, stained using immunohistochemical methods and analysed. Using stereological methods the volumes of the lesions in the CsA treated and control groups and the volumes of individual cell types contributing to the lesion were calculated. Little difference in lesion volume was observed between the two groups. However, there were some notable differences in the proportion of individual cells contributing to the lesion. Double immunohistochemical staining showed co-expression of various cell markers by the different cell types. CsA administration may be used as a technique to control the cell population of the lesion, making it more permissive to neuronal regeneration.

B114

Axonal regeneration of retinal ganglion neurons using immortalized adult human olfactory ensheathing glia

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Olfactory ensheathing glia possess a remarkable capacity to promote axonal regeneration in the central nervous system (CNS). Efficient establishment and maintenance of human olfactory ensheathing glia (hOEG) primary cultures with neuroregenerative competence is fundamental to obtain a source of cells for transplantation in the injured CNS. However, the availability of human tissue to prepare cultures is restrictive and maintenance of primary cultures is limited to a few passages. To solve these problems we have prepared primary cultures of hOEG from adult olfactory bulbs from necropsies. Thereafter, we have reversibly immortalised these primary hOEG by lentiviral gene transfer of human telomerase and murine Bmi-1 and established clonal lines. For this, we used a system of reversible immortalization based on LoxP and CRE recombinase. While primary hOEG cultures can only be expanded for about 10 passages, our immortalized clonal lines, which express OEG typical markers, divide indefinitely and conserve neuroregenerative properties: both primary and immortalised hOEG are able to promote axonal regeneration of adult rat retinal ganglion neurons (RGN) in coculture. We aim to de-immortalise hOEG clonal lines by lentiviral transfer of CRE recombinase and confirm that such de-immortalised (''primary-like'') cells maintain neuroregenerative capacity.

B115

Leukemia Inhibitory Factor Signaling modulates both central nervous system demyelination and myelin repair

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Leukemia Inhibitory Factor (LIF) receptor signaling limits the severity of inflammatory demyelination in experimental autoimmune encephalomyelitis, a T cell dependent animal model of Multiple Sclerosis (MS) (1.). To identify whether LIF exerts direct effects within the central nervous system (CNS) to limit demyelination, we have studied the influence of LIF upon the phenotype of mice challenged with cuprizone, a copper chelator that produces a toxic oligodendrocytopathy. We find that exogenously administered LIF limits cuprizone-induced demyelination. Knockout mice deficient in LIF exhibit both potentiated demyelination and oligodendrocyte loss (number of GST-Pi positive oligodendrocytes [mean +/− SEM]: wild-types 1095 +/− 142, LIF knockouts 429 ± 142; p = 0.03) after 3 weeks of cuprizone challenge, an effect that is ameliorated by exogenous LIF, arguing for a direct beneficial effect of endogenous LIF receptor signaling. Numbers of oligodendrocyte progenitor cells in cuprizone-challanged mice are not influenced by either exogenous LIF or LIF deficiency, arguing for direct effects upon the differentiated oligodendrocyte. Studies of the influence of LIF upon remyelination after cuprizone-challenge fail to reveal any significant effect of exogenous LIF. The LIF knockout mice do, however, display impaired remyelination, although oligodendrocyte replenishment, previously identified to occur from the progenitor pool, is not significantly compromised (64.9% of baseline levels for wild-types and 59.2% for LIF knockout mice; p = 0.3). Thus

endogenous LIF receptor signaling is not only protective of oligodendrocytes but can enhance remyelination and exogenous LIF has therapeutic potential in limiting the consequences of oligodendrocyte damage.

REFERENCE

Butzkueven. *et al.* (2002). *Nat Med.* 8, 613–9.

B116

Zebrafish myelination: a transparent model for remyelination

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Multiple Sclerosis (MS) is an autoimmune disease, which may lead to chronic demyelination of CNS axons. This prevents saltatory conduction and eventually leads to nerve degeneration. Current therapies aim to reduce damage but there are at present none that promote regeneration of lost myelin sheaths (remyelination). Our aim is to develop and validate a zebrafish model of remyelination and use it to screen compounds for their potential as remyelination therapies. The zebrafish offers the possibility of high-throughput, in vivo modelling that is unavailable in other vertebrate models and there is good homology between zebrafish and mammalian myelin systems. Our approach is to first assess the recruitment and differentiation of oligodendrocyte progenitor cells (OPCs) in response to compound libraries. OPC recruitment is assessed in a primary screen using a transgenic olig2:EGFP line of zebrafish and analysing the number of dorsally migrating OPCs along the spinal cord. OPC differentiation is assessed in a secondary screen using oligodendrocyte differentiation and myelin markers. Positive results will then be assessed in a tertiary screen for remyelination ability in a lesion model; oligodendrocytes, and therefore myelin, will be laser-ablated in vivo and the speed of oligodendrocyte repopulation used as a measure of remyelination efficiency. Results will be validated using rodent remyelination models. Results so far indicate that several compounds cause significant, concentration-dependent increase in OPC recruitment in the primary screen and the secondary screen is currently being developed.

B117

Effect of lentiviral vector expressing neurotrophin-3 on dorsal root ganglia neurite outgrowth

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In injured CNS tissue neurotrophins can act to increase the intrinsic neuronal growth state by downregulating the growth cone response to inhibitory signals and increasing the degradation of extracellular inhibitory factors. Neurotrophin-3 (NT-3) has been shown to promote spinal axonal elongation and enhance myelination by oligodendrocytes in injured CNS. In this study we are investigating the use of lentiviral vectors expressing NT-3 to promote neurite outgrowth in vitro. This lenti NT-3 vector will ultimately be used in vivo in a model of CNS injury to induce astrocytes within the glial scar to produce NT-3. We harvested dorsal root ganglion (DRG) neurons from E15 rat embryos. We tested the transduction efficiency of DRG neurons using lentiviral vector expressing green fluorescent protein. DRG neurons were also transduced with a lentiviral vector expressing NT-3. NT-3 concentration in DRG media was analysed using ELISA. The Neu7 astrocyte cell line and primary rat astrocytes were also used in these experiments to test transduction with lentiviral GFP and NT-3. Lenti NT-3 transduced Neu7 cells and astrocytes were co-cultured with DRG neurons. These co-culture experiments show successful transduction of cells with lenti NT-3 resulting in a comparable increase in neurite outgrowth in DRG neurons co-cultured with primary astrocytes but not with Neu7 astrocytes. This lentiviral vector shows promising potential for growth of neurites in injured CNS tissue. We will introduce this lenti NT-3 vector into a contusion model of spinal cord injury.

B118

An immunohistochemical staining for p-38 as a marker for the satellite cells mediating indirect reactions of the dorsal root ganglia to nerve injury

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Nerve injury induces neuropathic pain and activation of p38 mitogen-activated protein kinase (p38) in different populations of DRG neurons and spinal cord microglia. We have investigated an immunohistochemical location of activated p38 protein in the C7–C8 and L4–L5 dorsal root ganglia (DRG) of naïve rats and those operated for unilateral L4–L5 spinal nerve ligature (SNL), sciatic nerve ligature (ScNL), and sciatic nerve transection (ScNT). In contrast to published results an enhanced p38-IF was found not only in DRG neurons but also in the satellite cells (SC), particularly after ScNL and ScNT for 1, 2, and 4 weeks. An increased p38-IF was observed in the SC of contralateral L4–5 DRG and those of cervical segments in comparison to very low p38-IF in the cells of ipsilateral L4–5 DRG. In contrast, small and medium-sized neurons displayed increased p38-IF only in the ipsilateral L4–5 DRG. A week SNL induced elevated p38-IF in the cervical DRG neurons, but reduced p38-IF in the ipsi- and contralateral L4–5 DRG. The DRG neurons related to SNL displayed an increased p38-IF until 2 weeks. An increased p38-IF in the SC was observed in DRG not associated with damaged nerve. Our results suggested a nerve damage signaling that spreads in the nervous system to induce expression of the critical p38 mitogen-activated protein kinase. In addition, our results indicate that the signaling in the DRG not associated with injured nerve may be mediated by satellite glial cells. Supported by MSM0021622404.

B119

Exploring the requirement of Cdk2 for normal white matter development and myelin repair

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Type 2 cyclin-dependent kinase (Cdk2), which controls G1/S transition in eukaryotic cell cycle, was recently shown to be dispensable during embryonic development since Cdk2-null mice develop normally until adulthood. Previous work showed that Cdk2 controls oligodendrocyte progenitors (OPC) cell cycle progression and is downregulated in adult OPCs in vitro. We assessed here the requirement of Cdk2 for proliferation of CNS precursor cells that generate newborn oligodendrocytes in specific regions of the adult brain. We analyzed subcortical white matter, corpus callosum, striatum and cerebellar white matter areas with a broad spectrum of antigenic markers for distinct stages of oligodendroglial maturation (CNPase, MAG, NG2, and Olig2), and found that oligodendroglial lineage development was normal in adult Cdk2-null mice. We next used a model of focal lysolecithin-induced lesion of the corpus callosum in order to challenge the role of Cdk2 in OPC proliferation and oligodendrogenesis following acquired non-autoimmune demyelination. We observed an increase of Ki67+ (cell proliferation marker) cells in the sub-ventricular zone (SVZ) and in the lesion of demyelinated WT mice compared to demyelinated Cdk2-null mice. Altogether, our data provide evidence that Cdk2 does not appear to be essential for normal developmental myelination, suggesting the function of Cdk2 may be effectively compensated in neonatal OPCs. However, it seems to be involved in cell cycle kinetics in adult OPCs following demyelination, and thus could alter myelin repair.

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B120

Expression of Nogo-A and Nogo-66 receptor during development and optic nerve regeneration in the lizard, Gallotia galloti

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Nogo-A is a potent oligodendrocyte and myelin-associated neurite growth inhibitor and contributes to the failure of axon regeneration in the mammalian CNS. Inhibition of axon growth by Nogo-A is mediated by the Nogo-66 receptor (NgR). We have previously shown that retinal ganglion cell (RGC) axons regenerate successfully after optic nerve transection in the lizard, Gallotia galloti. Against this background, we analysed the expression of Nogo-A and NgR in the developing visual pathway and during RGC axon regeneration in the lizard, using immunohistochemistry and Western blot techniques. Moreover, the effect of recombinant Nogo-A protein on growth of lizard RGC axons was evaluated by in vitro time-lapse analysis. Expression of Nogo-A was found to be associated primarily with myelinated axon tracts in the adult lizard visual pathway and became up-regulated during RGC axon regeneration. During development, Nogo-A expression at a lower level preceeded myelination and was associated with neurons. NgR was expressed at low levels in the intact visual pathway, but became strongly up-regulated in RGCs following optic nerve injury. During development, levels of NgR expression were high around stage E32 when RGC axon growth occurs, but decreased towards adult levels around stage E39. Recombinant Nogo-A had no effect on growth lizard RGC axons, but caused collapse of rat sensory neurites in vitro. These results indicate that expression of Nogo-A and NgR do not appear to block RGC axon growth and regeneration in the lizard. However, regulation of their expression levels suggests a role for both proteins during axon growth in the visual pathway.

B121

Mechanisms of fibroblast growth factor 2 signalling and oligodendrocytes differentiation in vivo

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The differentiation of oligodendrocytes progenitor cells (OPCs) into myelinating oligodendrocytes (OLs) is under the control of multiple factors, including fibroblast growth factor 2 (FGF2). In vitro studies show that FGF2 is a potent mitogen for OPCs and inhibits their differentiation into OLs. We have shown that administration of FGF2 in vivo has strong dose-dependent actions, whereby raising FGF2 levels initially increases the numbers of OPCs and OLs, but raising FGF2 to high levels decreases the number of OLs and inhibits myelination. Here, we have examined the mechanisms of action of FGF2 in vivo in transgenic mice in which expression of the fluorescent proteins DsRed is driven by proteolipid protein (PLP). All procedures were carried out in accordance with the Animals Scientific Procedures Act 1984. Mice were deeply anaesthetised under isofluorane and FGF2 or the glycogen synthase kinase 3α (GSK3α) inhibitor indirubin were injected into the cerebrospinal fluid (CSF) of the lateral ventricles, twice daily for three days commencing at postnatal day (P)6; saline was injected in controls. At P9, animals were killed by sodium pentobarbital overdose and brains were analysed by confocal microscopy and immunohistochemistry for NG2, PDGFαR, Olig02, APC, and MBP. For comparison, developmental changes in the distribution and antigenic phenotype of oligodendrocyte differentiation stages were characterised in the forebrain, hippocampus and cerebellum of untreated PLP-DsRed mice. Administration of FGF2 or indirubin promoted oligodendrocyte differentiation and induced a significant increase in the numbers of OPCs, immature and myelinating OLs, throughout periventricular areas of the brain. We are currently examining these effects using live cell imaging on brain slices maintained in organotypic culture conditions. These analyses indicate FGF2 triggers GSK3α signalling pathways in OLs, which has clinical implications as a therapeutic target for promoting repair in MS.

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B122

Grafts of Schwann cells engineered to express PSA-NCAM promote functional recovery after spinal cord injury

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Schwann cells (SCs) are among the most attractive cellular candidates for the development of remyelination therapies for CNS lesions. Yet, their integration in the CNS is inhibited by astrocytes and therefore the use of genetically modified SCs with improved properties is an alternative promising approach. Our strategy for ameliorating the therapeutic potential of SCs has been to alter their adhesive properties by expressing on their surface the polysialylated (PSA) form of the neural cell adhesion molecule NCAM. In the present study, SCs from transgenic GFP-mice were transduced with a retroviral vector encoding sialyl-transferase X, the enzyme responsible for transferring PSA on NCAM. Engineered STX-GFP-SCs with sustained PSA expression were thus generated and were found to have improved ability to associate with astrocytes in vitro. Importantly, when these cells were transplanted in vivo in a mouse model of spinal cord injury they promoted faster and significantly greater functional recovery as compared to using SCs transduced with a control retroviral vector or no cells at all. Morphological analysis indicated that the improved functional recovery correlated with earlier and enhanced remyelination by grafted STX-GFP-SCs, increased remyelination by host SCs as well as enhanced differentiation/remyelination by resident oligodendrocyte progenitors. Moreover, sprouting of regenerating serotonergic nerve fibers, which are known to be important for locomotion and recovery after injury, was observed into and across the lesion site. These results underline the potential therapeutic benefit of early activation of myelin-forming cells to differentiate and remyelinate severed axons thus restoring functions in CNS trauma and/or demyelinating diseases.

B123

Increased GFAP immunoreactivity by astrocytes in response to contact with dorsal root ganglia cells in a 3D culture model.

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Failure of repair mechanisms in the injured CNS is widely attributed to the inhibitory environment of the lesion site, most notably the formation of the glial scar which forms a physical and physiological barrier to axon regeneration. We developed an in vitro 3D cell culture model to investigate the response of astrocytes to cells found at the inhibitory interfaces formed following damage to the spinal cord. CellTrackerTM labelled dissociated DRGs were seeded onto astrocyte-populated collagen gels and maintained in culture for 5 days. Astrocytes near the DRG interface showed marked GFAP up-regulation and adopted a reactive morphology which was observed up to 1mm away. Intensity of GFAP fluorescence at this interface was 3 fold higher than that seen away from the interface or in controls (astrocyte only gels). Furthermore, the presence of DRG conditioned medium was not capable alone of eliciting this response. In conclusion this model may provide a useful tool for understanding reactive astrogliosis in response to cells found at inhibitory interfaces following spinal cord or dorsal root injury. The contact between astrocytes and satellite cells may be enough to induce astrocyte reactivity and formation of the gliotic scar, or this contact may induce the secretion of a soluble factor which is not released from DRG cultures under physiological conditions.

B124

Evaluation of the inflammatory reaction after transplantation of olfactory ensheathing cells into the rat spinal cord

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Application of olfactory ensheathing cells (OECs) in the treatment of spinal cord injuries in humans requires the evaluation of the safety of their transplantation. Cell grafting into the central nervous system is accompanied by a local inflammatory response, which intensity depends on the degree of invasiveness of the operative approach, the type of the transplanted cells, their maturity, and immunogenicity. Most approaches of OECs transplant-mediated spinal cord repair assume the usage of purified cell cultures. However some authors claim that OECs from nasal mucosa should be transplanted together with cells found in their natural milieu which may enhance their neurotrophic properties e.g. olfactory nerve fibroblasts, neural stem cells. This issue raises the question about the safety of the intraspinal transplantation of such mixed cell populations. Our aim is to analyze the extent of transplantation-associated inflammatory response in the the intact rat spinal cord following microinjection of syngeneic unpurified nasal OECs and the impact of this procedure on the neurological state of the animals. In this work we present and discuss our controversial results. The study was approved by the Local Ethical Committee.

B125

Transplantation of Schwann cells over-expressing L1 enhance functional recovery in a mouse spinal cord compression model

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Functional recovery after spinal cord lesion remains an elusive goal. A combination of inhibitory molecules and the lack of the appropriate permissive factors in the lesioned spinal cord result in failure in fiber tract reconnection and function. Experimental transplantation in rodent and primate models of CNS injuries has led to the idea that Schwann cells (SC), the myelinating glial cells of the PNS, are good candidates for cell therapy of CNS lesions. However, the motility and integration of the transplanted cells is low within the CNS a factor presenting serious limitations for the

development of therapeutic strategies. In this study we used retroviral transduction to genetically modify SC in order to over-express the cell adhesion molecule L1, a protein implicated in mechanisms of neuronal migration, axonal guidance and myelination during development and regeneration, and its secreted form L1-Fc. Subsequently, L1- and L1-Fc- transduced SC derived from â-actin GFP mice were transplanted rostrally to the lesion site of adult of C57/6J mice that had been subjected to spinal cord compression injury. Assessment of the animals 2 and 4 weeks after injury revealed that the group of mice transplanted with L1-expressing SCs exhibited faster and greater functional recovery as compared to the groups where SCs were transduced with a control vector or with no cells at all. Additionally, morphological analysis of the lesioned area indicated enhanced sprouting of regenerating serotonergic nerve fibers, which are known to be important for locomotion and recovery after injury, into and across the lesion site in the L1-transduced group of animals as compared to controls. Further phenotypic analysis of the lesioned spinal cords is in progress in order to investigate the molecular mechanisms triggered by L1 over-expression in SC, resulting to enhanced axonal regeneration and subsequent locomotory recovery.

B126

Schwann cells but not olfactory ensheathing glia (OEC) secrete a factor that induces characteristics typical of hypertrophy in astrocytes

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Schwann cells and OECs are candidates for transplant-mediated repair of spinal cord lesions since they have been shown to provide cellular environments that promote axonal outgrowth in several models of CNS injury. However, when Schwann cells are in contact with astrocytes, the latter develop a stress response characterised by reactive astrocytosis/proliferation, boundary formation and increased GFAP and CSPG expression. OECs do not induce this stress response, freely intermingling with normally appearing astrocytes. The similar was observed in vivo by demonstrating that astrocytes mingle with OECs but not Schwann cells after injection into normal spinal cord. Here we demonstrate that the factor(s) secreted by Schwann cells are able to induce this reactive phenotype in OEC/astrocyte co-cultures. The effect is mediated through the FGFR1 receptor but cannot be induced by FGF2 alone. Furthermore, when heparin is added to OEC/astrocyte cultures the astrocyte stress response is induced whereas boundary formation is reduced when Schwann cells/astrocyte cultures are treated with heparitinase or chlorate. In vivo, FGF2 and FGFR1-immunoreactivity was increased over grafted OECs and Schwann cells compared to the surrounding tissue and HSPG immunoreactivity is increased over reactive astrocytes bordering the Schwann cell graft. These data suggest that components of the astrocyte stress response including boundary formation, astrocyte hypertrophy and GFAP expression are mediated by a FGF family member and HSPG. Identification of other factors secreted by Schwann cells that induce this negative response in astrocytes would further our ability to manipulate the inhibitory environment induced after injury to promote regeneration.

B127

Fibrin(ogen) inhibits neurite outgrowth via beta3 integrin-mediated transactivation of the EGF receptor

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Regeneration failure of adult mammalian central nervous system (CNS) neurons is due to the non-permissive CNS environment. Identification of the inhibitors in the injured CNS is essential for the understanding of nerve regeneration. Although spinal cord injury (SCI) is associated with extensive vascular damage, the effects of blood components in the inhibition of CNS regeneration have not been examined. Here we show that the blood protein fibrinogen is a novel inhibitor of neurite outgrowth that is massively deposited in the spinal cord after injury. Fibrin deposition spatially and temporally correlates with damaged axons in three animal models of SCI, namely dorsal hemisection in the mouse and dorsal column wire knife and contusion SCI models in the rat. Fibrinogen inhibits neurite outgrowth in neurons in vitro in a concentration-dependent manner and its inhibition capacity is similar to myelin. Fibrin-mediated inhibition of neurite outgrowth is reversed by blocking the fibrinogen receptor beta3 integrin or blocking phoshorylation of EGFR. Fibrinogen induces co-immunoprecipitation of its receptor alphavbeta3 with EGFR resulting to phosphorylation of EGFR in neurons. These results identify fibrin as a novel inhibitor of neurite outgrowth and shows that fibrin stimulated integrin-growth factor cross talk in neuronal cells functions as a molecular mechanism that inhibits regeneration processes.

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B128

Axon regeneration in modeling the glial scar in vitro

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Spinal cord and brain injuries cause permanent damage because severed axons are unable to regenerate past the barrier of the glial scar. Mechanical trauma and exposure to non-neural cells rapidly induce astrocyte reactivity and cause glial scar formation. We established a novel in vitro model of the glial scar by combining abrupt mechanical stretch and meningeal cell contact. Astrocytes responded to these stimuli

with stellation and clustering, as well as upregulation and accumulation of GFAP and nestin. Expression of axon growth inhibitors (phosphacan, neurocan and tenascins) was increased. Importantly, stretched astrocyte-fibroblast cocultures profoundly inhibited neurite growth from cortical, spinal and dorsal root ganglion neurons. Unbiased quantification of neurite growth in this scar model was possible using automated and interactive tracing. Neurite lengths were significantly reduced on astrocyte surfaces in the scar-like lawns compared to control astrocytes, and neurites were thin, beaded and possessed retractive end bulbs. Neurite regeneration was poorer in early postnatal neurons compared to their embryonic counterparts, and was further reduced in week-old neurons. Inhibition on the stretched astrocyte-fibroblast cocultures was comparable to that on CNS myelin; however combining myelin with the scar model did not increase the inhibition. We tested whether Schwann cell precursors (SCPs), axon-growth associated glia of peripheral nerves (Wanner *et al.*, Glia 54: 424 and 439, 2006), could improve scar-related neurite growth inhibition. SCPs were represented by the cell line Spl201 (Lobsiger *et al.*, Glia 36: 31, 2001). They migrated readily on the scar-like lawns and promoted neurite growth of CNS neurons found associated with their surfaces. Thus, we have developed a reproducible, controlled and quantifiable model of the glial scar useful to investigate the causes of reactive astrogliosis and axon growth inhibition. The potential of neurite growth promoting cells can be studied using this model in developing future therapeutic strategies for CNS injuries.

B129

Differential gene expression following chick spinal cord injury at permissive and non-permissive stages for regeneration

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Given its accessibility in ovo, the chick provides an ideal system for studying the molecular mechanisms underlying loss of regenerative ability within the central nervous system with development. Chick spinal cord resection results in complete functional recovery until around E13. The ability to regenerate prior to E13 might be due to several and non mutually exclusive factors: i) more limited secondary injury response, ii) plasticity of neural progenitors, iii) reduced glial response, iv) permissive environment. The aim of this study was to identify molecules/signalling pathways that play a key role in spinal cord regeneration by comparing the response to injury at stages of development permissive (E11) and non-permissive (E15) for regeneration. E11 and E15 spinal cords were injured at the thoracic level by crushing them with sharp forceps. Spinal cord segments were collected 24 hours after injury in the region surrounding the lesion and from sham-operated embryos. Five individual spinal cord samples per group were used for Genechip screening (GeneChip® Chicken Genome Array, Affymetrix, containing >28.000 genes). As expected, significant changes in gene expression were observed between E11 and E15 in control animals. This included genes involved in myelination, such as myelin basic protein. A difference in the expression of genes associated with astrocytes and microglia (such as ferritin and S100) was also observed, further validating our screening. Analysis of molecules differentially regulated in response to injury at E11 and E15 identified several molecules associated with cell survival/apoptosis and neural plasticity. Analysis of the nestin-like protein transitin, confirmed up-regulation of this protein 24 hours after injury at E11. Such an increase in this protein expression, however, was not observed in E15 injured spinal cords, suggesting a loss of plasticity in spinal cord neural progenitors with development. Animal experimental procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986.

B130

Expression of the neurosteroid 3alpha,5alpha-tetrahydroprogesterone in neurogenic zones of the adult rat brain

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We have demonstrated that neural progenitors isolated from newborn rat brain and expressing the polysialylated form of NCAM (PSA-NCAM) were able, in vitro, to synthesize the neurosteroid 3alpha,5alpha-tetrahydroprogesterone (3a,5a-THP), a neuroactive metabolite of progesterone (Gago *et al.* [2001] Glia 36:295–308). We also established that 3a,5a-THP stimulated the proliferation of these cells through GABAA receptors (Gago *et al.* [2004] J Neurosci Res 78:770–783). These results suggest the existence of a paracrine/autocrine control of neural progenitor biology involving 3a,5a-THP. The aim of the present work was to determine whether such regulation exists in neurogenic zones of the adult rat brain in vivo. We have investigated by immunofluorescence the distribution of the enzyme 3alpha-hydroxysteroid oxidoreductase (3a-HSOR) responsible for the 3a,5a-THP synthesis, in neurogenic zones expressing PSA-NCAM, in the adult male rat brain. PSA-NCAM+ cells corresponding to neural progenitors or newly formed cells expressed 3a-HSOR in the olfactory bulb (OB), particularly in the internal granular layer, the mitral cell layer, the glomerular layer and in the accessory OB. PSA-NCAM+ progenitors exhibiting a less intense staining for 3a-HSOR were also found in the rostral migratory stream, the subventricular zone and the subgranular zone of the dentate gyrus. In these neurogenic zones, PSA-NCAM+ cells not always expressed 3a-HSOR but were in tight relationship with 3a-HSOR+ cells. Other PSA-NCAM+/3a-HSOR+ cells were observed covering the lateral ventricle, and the dorsal part of the fimbria of the hippocampus. Immunoreactive fibers for both markers were observed tangentially to the wall of the lateral ventricle caudally and in the cerebellum, presumably in the subpial layer. These results bring anatomical evidence suggesting that 3a,5a-THP could contribute in the control of neural progenitor biology in vivo.

B131

Expression of interleukin-6 receptor alpha (IL6-Ra) in normal and crush injured rat sciatic nerves

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Interleukin-6 (IL-6) is a pleiotropic cytokine synthesized by many different cells after appropriate stimulation. IL-6 binds first to the interleukin-6 receptor alpha (IL6-Ra) and then the

complex binds to the signal-transducing gp130 receptor, forming a functional hexameric receptor complex. Using immunohistochemical techniques, we show that IL6-Ra is expressed in the rat sciatic nerve. High levels of IL6-Ra expression are observed in non-myelinating Schwann cells. In myelinating Schwann cells it is present as discrete dots in the perinuclear and in distinct cytoplasmic membrane domains of the Schwann cell sheath. It is also express at the nodes of Ranvier and in the vicinity of the nodes. Upon nerve crush, IL6-Ra is gradually up-regulated in the degenerating myelinated fibres. The highest levels of expression correlates with the maximum state of demyelination of the axons and the maximum peak of Schwann cell proliferation. The expression of IL6-Ra is down-regulated again during axon regrowth and remyelination. Western blot against IL6-Ra of whole sciatic nerve, cerebral cortex, spleen, and pancreas revealed two bands of ~80 kDa and ~110-kDa. The ~80-kDa band corresponds to the glycosilated monomer of the mature form of IL6-Ra, while the 110-kDa might correspond to the non-glycosilated dimer of IL6-Ra. All the tissues we studied express IL6-Ra, but differences in the levels of expression are observed.

B132

Olig2 translocation determines an astrocytic fate in adult NG2 positive progenitors responding to traumatic brain injury

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Introduction: Astrogliogenesis dominates in glial scar formation after brain injury and little is known about its molecular mechanism. We investigated the effect of traumatic cortical injury on the fate of endogenous adult progenitors. Methods: Using a forebrain stab-injury model in rat, we examined the response of endogenous adult glial progenitors to traumatic brain injury. We analyzed mRNA and protein expression of olig2 with RT-PCR, Western blot and immunohistochemistry. Tissue from the vicinity of the cortical injury was collected at 2, 4, 6 and 8 hours, 1, 2, 4, 7 days and 2, 4 weeks after injury. Proliferative cells were labeled using BrdU 2 hours before sacrifice and quiescent BrdU-containing progenitors in subventricular zone (SVZ) were labeled by BrdU daily injection for 5 days and followed by a 10 day chase period. Results: Almost all NG2 positive progenitors expressed olig2 and all olig2 positive cells are NG2 positive. Brain injury induced up-regulation and translocation of olig2 in the injury site in a time dependent manner. The olig2 translocation was observed in a narrow time window and it correlated with NG2 redistribution and regulation. The olig2 translocated cells were proliferative and differentiated into new or mature astrocytes while the olig2 non-translocated cells differentiated towards oligodendrocytes. Furthermore, some quiescent BrdU-contained olig2 progenitors migrated from SVZ into the vicinity of the traumatic brain injury. Conclusion: Traumatic brain injury induced translocation of olig2 identified proliferative astrocyte progenitors. Olig2 non-translocated progenitors retained oligodendrocyte precursor identity. Adult olig2 positive progenitors in SVZ contributed to the cortical astrogliogenesis. This study implicates Olig2 translocation is involved in injury induced astrocytic fate specification.

B133

Proteome changes in developing and injured chick spinal cord

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Spinal cord injury usually results in severe disability. The chick embryo provides an ideal model for understanding the mechanisms underlying changes in CNS regeneration with development. Resection of chick spinal cords results in complete functional recovery until around E13, but thereafter irreversible damage occurs as in adult birds and mammals. Although studies have focused on inhibitory effects of myelination in regeneration, and we have evidence for significant differences in secondary injury response between regenerating and non-regenerating spinal cords, key differences in response to injury and their significance remain to be delineated. In this study, a 2-DE-based approach was used to detect proteome changes as opposed to protein-by-protein analysis of potential targets. This approach does not only allow identification of changes in protein expression, but also of potentially important post-translational modifications. Protein samples from the peri-lesion area of individual spinal cords of E11 chick embryos (regeneration permissive) and E15 chick embryos (non permissive) collected either 2 or 24 hours after injury were compared to corresponding sham-operated spinal cord samples. Proteins were separated using immobilised pH gradients for isoelectric focusing (pI range 4–7) in the first dimension followed by large format second-dimension gels. Several developmentally-regulated and injury-associated proteins were detected. We focused on 34 proteins whose expression level is differentially increased or decreased (at least 1.5 folds) after injury at E11 or E15, and their identification by mass spectrometry is in progress. Among the differentially expressed proteins already identified there are putative molecules involved in early response to injury, which play a role in controlling cell survival/apoptosis and neurogenesis. All animal procedures were performed according to the Animal (Scientific Procedures) Act 1986.

B134

Peripheral nerve regeneration through acellular and genetically modified grafts

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Peripheral nerve (PN) injuries are usually associated with considerable functional morbidity, exacerbated when the extent of the injury requires the insertion of a graft. Autografts are considered the ''gold standard'', although availability of suitable lengths of appropriately sized nerve segments is a problem. Nerve sheaths from donors which have been made acellular may be a suitable alternative, since they are not as prone to immunological rejection (Gulati 1995). Suitability was tested by grafting acellular sheaths into 1cm nerve defects in sciatic nerves.. Immunohistochemical quantification after three months revealed that autografts contained many regenerated axons but allogeneic acellular grafts also contained large numbers of axons, supporting their suitability as an alternative. PN regeneration is primarily driven by activated Schwann cells (SCs), which provide a

range of neurotrophic factors (NTFs) and matrix molecules important for neuronal survival and axonal regrowth. However, many damaged neurons die, perhaps due to depletion of NTFs, and although neurons regenerate, there is often atrophy of denervated muscles. Acellular PN sheaths were seeded with heterologous SCs and grafted onto transected sciatic nerves. Analysis revealed a reduction in dennervated muscular atrophy when SCs were present in grafts and that SCs remained there for extended periods of time, suggesting they could be used to deliver NTFs to injured neurons. Lentiviral vectors used to genetically modify donor SCs to over-express ciliary neurotrophic factor (CNTF) revealed a considerable number of regenerating fibres in CNTF/SC-grafts and calcitonin gene-related peptide (CGRP) immunostaining revealed a higher number of CGRP-positive fibres in CNTF/SC-grafts.

B135

Myelin protein mediated inhibition of oligodendrocyte precursor cell differentiation

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In the central nervous system oligodendrocytes extend processes which form myelin sheaths around axons allowing for rapid propagation of action potentials by saltatory conduction. Following a demyelinating incident a multipotent precursor cell population (oligodendrocyte precursor cells, OPCs) of the adult CNS responds to demyelination by undergoing rapid proliferation, migration and differentiation into new oligodendrocytes. While the adult central nervous system has a remarkable capacity to regenerate myelin sheaths under physiologic conditions, remyelination often fails in disease. One possible explanation for the failure of remyelination is the inhibition of OPCs by myelin breakdown products that accumulate in acute lesions of various etiologies.

We have recently shown that myelin break down products in acutely demyelinating are a major obstacle for differentiation of OPCs into mature oligodendrocytes. By plating purified primary rat OPCs onto substrates prepared from myelin membrane preparations we give evidence that 1) proteins in myelin exist which exert profound inhibitory effects on OPC differentiation and 2) that the inhibitory activity can be distributed to distinct fractions prepared by column chromatography.

TUMOURS

B136

Actin-rich protrusions and non-localised GTPase activation in merlin-deficient schwannomas

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Schwannomas are benign Schwann cell tumours that occur spontaneously or in patients with neurofibromatosis type 2. They lack both alleles for the tumoursuppressor merlin, a membrane-cytoskeleton-linker that has been linked to the Rac-PAK pathway. Rac1, as well as Cdc42, are members of the RhoGTPases that induce formation of actin-rich membrane protrusions like lamellipodia/ruffles and filopodia, when activated. Until now little is known about RhoGTPase activation and the role of protrusions in the dedifferentiation of schwannoma cells. We investigate those aspects in our schwannoma in vitro model of human primary schwannoma cells and for comparison healthy human primary Schwann cells. Using scanning electron microscopy we here show that Schwann cells exhibit few actin-rich protrusive structures; mostly one single lamellipodium (sheet-like) with few filopodia (finger-like), whereas schwannoma cells are characterised by multiple ubiquitously occuring lamellipodia/ruffles and filopdia. Rac1 and Cdc42 are highly activated in schwannoma. Both are found all around the cell periphery in colocalisation with their effector phospho-PAK indicating that both are activated in a non-localised random manner. Using live cell imaging, we further demonstrate fast and continuous remodelling of the many actin-rich protrusions in schwannoma cells. The underlying cytoskeleton of these structures is thin, extensively branched and the Arp2/3 complex, a major regulator of actin-branching, is enriched in the lamellipodia/ruffles of schwannoma cells. In conclusion we claim that Rac1 and Cdc42 are activated in a non-localised manner in schwannoma and that this unusual activation leads, via different effectors, to many lamellipodia-like zones that finally result in many, non-directional occurring, highly dynamic actin-rich protrusions.

B137

Direct infusion of gamma-linolenic acid into C6 rat gliomas implanted in vivo alters the mrna expression of proteins involved in angiogenesis, cell cycle control and invasion

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Gamma-linolenic acid (GLA) is an inhibitor of tumour cell proliferation in both in vitro and in vivo conditions. The aim of the present study was to identify specific targets of GLA action by analyzing the mRNA expression profiles of proteins of importance for glioma proliferation and invasion. Immunohistochemical analysis of angiogenesis-related proteins was also performed by light and electron microscopy (TEM). GLA was infused into the tumour bed with Alzet osmotic pumps over a 14 day period at a concentration of 5mM and 0.5µl/hr. GLA decreased the expression of E2F1, p16, p53, p65, bax, Ku70, Ku80, PPARg, ERK1, nm23b, matrix metalloprotease 2, prostaglandin receptors EP1, EP2 and EP3, vascular endothelial growth factor A (VEGFA), VEGF receptor Flt1 and cyclooxygenase 2 (COX2). No changes were found for cyclin D1, c-myc, pRb, p21, p27, bcl2, nm23a, tenascin C and prostaglandin receptor EP4. GLA decreased the protein expression of VEGF and its receptor Flt1, but not Flk1. In conclusion, the infusion of GLA caused marked changes in mRNA expression of proteins critical to glioma

progression and induced a visible reduction in tumour size, with apoptosis identified by TEM. These findings increase our knowledge of the action of gamma-linolenic acid in gliomas and show its effects on both cell cycle and angiogenesis-related pathways, both of which are clear targets for cancer chemotherapy. These data lend further support to the proposed use of gamma-linolenic acid as an adjuvant therapy in the treatment of patients with malignant gliomas.

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B138

Connexin 43 is involved in the antiproliferative effect of tolbutamide in glioma cells

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Our previous work has shown that tolbutamide increases gap junctional permeability in poorly coupled C6 glioma cells and that this effect is similar and additive to that found with dbcAMP, a well-known activator of gap junctional communication. Furthermore, the increase in gap junctional communication promoted by tolbutamide or dbcAMP is concurrent with the inhibition of proliferation of C6 glioma cells. In the present work, we show that tolbutamide and dbcAMP increase the synthesis of the tumor suppressor protein Cx43 and that they decrease the level of Ki-67, a protein expressed when cells are proliferating. These effects were accompanied by a reduction in the phosphorylation of pRb, mainly on Ser-795, a residue critical for the control of cell proliferation. The decrease in the phosphorylation of pRb is not likely to be mediated by a reduction in the levels of D-type cyclins, since instead of decreasing the expression of cyclins, D1 and D3 increased slightly after treatment with tolbutamide or dbcAMP. However, the Cdk inhibitors p21 and p27 were up-regulated after treatment with tolbutamide and dbcAMP, suggesting that they would be involved in the decrease in pRb phosphorylation. When Cx43 was silenced by siRNA, neither tolbutamide nor dbcAMP were able to up-regulate p21 and consequently to reduce glioma cell proliferation, as judged by Ki-67 expression. In conclusion, tolbutamide and dbcAMP inhibit C6-glioma cell proliferation by increasing Cx43, which correlates with a reduction in pRb phosphorylation due to the up-regulation of the Cdk inhibitors p21 and p27.

B139

Concerted action of Ski and Rb determines cell type-specific TGFbeta downstream effect on the cell cycle

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TGFbeta induces Schwann cell proliferation, whereas it promotes epithelial cell differentiation. We show that the proto-oncogene Ski plays a key role in mediating these effects. Ski is a negative regulator of the TGFbeta pathway. TGFbeta strongly down-regulated Ski in epithelial cells, but not in Schwann cells. Ski can interact with the nuclear Retinoblastoma protein (Rb). In Schwann cells but not in epithelial cells, Rb (and in particular its hyperphosphorylated form) was up-regulated upon TGFbeta treatment, and both Ski and Rb moved to, and interacted in the cytoplasm. In sciatic nerves, Ski and Rb interacted in the cytoplasm of proliferating Schwann cells, whereas Rb was mostly restricted to the nucleus of myelinating Schwann cells. Overexpression of Ski induced Rb up-regulation, and colocalization of both proteins in the cytoplasm of Schwann cells and epithelial cells. Proliferation was also stimulated, and TGFbeta failed to promote epithelial cell differentiation. Conversely, down-regulation of Ski in Schwann cells prevented TGFbeta-induced proliferation and Rb relocalization to the cytoplasm. Our findings reveal a critical function of Ski in the control of TGFbeta effects on the cell cycle, and strongly indicate that Ski and Rb act in concert to mediate TGFbeta-induced Schwann cell proliferation.

B140

Stem cell markers in astrocytomas

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According to new hypotheses astrocytomas / gliomas either arise from or attract neural stem cells (NSCs). Biological markers, particularly antigenic markers, have played a significant role for the characterization of these tumour stem cells (TSCc). Because these studies have been performed with single experimental samples mostly from gliomas, we investigated the expression of the stem cell markers CD133 / Prominin, Nestin, Sox-2, Musashi-1, CXCR4, Flt-4 / VEGFR-3 and CD105 / Endoglin in 72 astrocytomas of different WHO-grades and compared it to normal adult human brain. Expression of their mRNA was quantified by quantitative RT-PCR, of their protein by counting immunopositive cells. In contrast to normal brain, tumour samples showed a high variability for the expression of all markers. However, their mean expression was significantly increased in astrocytomas, but this depended on the WHO grade only for CD133, Nestin, Sox-2 and Musashi-1. Confocal microscopy revealed that these markers mostly could be co-stained with glial fibrillary acidic protein (GFAP), a marker for astroglial cells, but less frequently with the proliferation marker Ki-67 / MIB-1. These markers sometimes, but not necessarily could be co-stained with each other in complex patterns. Our results show that most astrocytomas contain considerable portions of cells expressing stem cell markers. It appears that some of these cells originate from tumour genesis (supporting the stem cell hypothesis) while others are attracted by the tumours. Further functional markers are required to differentiate these TSC-types.

B141

TI-VAMP-mediated secretion in astrocytes and gliomas: implications for cathepsin release.

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Gliomas encompass all primary central nervous system tumours of glial-cell origin. The infiltrating nature of malignant brain tumours mainly derives from the local

destruction of the extracellular matrix formed by the adjacent normal tissue. Tissue invasiveness correlates with the ability of tumour cells to digest the extracellular matrix by secreting proteolytic enzymes, such as aspartic (Cathepsin D) or cysteine (Cathepsin B) peptidases. It has been therefore suggested that effective anti-proteolytic therapy might provide additive or synergistic treatment benefits if used in combination with conventional therapeutics for glioma treatment. Aim of the present proposal is to identify pathways of secretion responsible for cathepsin release,with the intention of identifying new strategies for the treatment of highly invasive tumours. Here we provide the first indication of a vesicular pathway deputed to the storage and release of Cathepsin B in astrocytes and gliomas. Cell fractionation and immunofluorescence experiments revealed that these vesicles differ from either VAMP-2 positive clear vesicles releasing glutamate or SG II-positive large dense core granules releasing ATP, and were characterized by the presence of the v-SNARE TI-VAMP/VAMP-7. TI-VAMP vesicles were enriched at the tips of astrocytic and glioma processes and were able to undergo fusion with the plasmamembrane. Immunoisolation of TI-VAMP positive vesicles followed by ELISA assay revealed that these organelles contain Cathepsin B. Moreover down-regulation of TI-VAMP expression by RNA-interference negatively modulated Cathepsin B release from ADF glioma cell lines, as detected by ELISA assay. These data indicate a role for TI-VAMP in cathepsin secretion and suggest the possibility that down-modulation of protein expression may reduce cathepsin release.

B142

The integrin alpha5 beta1 promotes glioma growth

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Inhibition of cell adhesion to the ECM is a fundamental step for activation, survival, targeting and migration of many cells like activated endothelial cells, tumor cells and inflammatory cells. Many of these interactions are mediated by integrins, a family of multifunctional cell adhesion receptors. We focus on the integrin alpha5 beta1, which is strongly expressed in proliferating endothelium, upregulated on blood vessels in human tumors and in proliferating or migrating cells such as macrophages, myofibroblasts or RPE cells. We now show, that an inhibitor of alpha5 beta 1 integrin attenuated glioma growth in an experimental mouse model. After 21 days, the tumor volume was significantly smaller after treating animals for 14 days with JSM6427 (24.8 ± 5 mm3; n = 6) as compared to controls (45.2 ± 6.2 mm3; n = 12). Moreover, we observed a reduction in microglial density at the tumor border. The density of Iba1-immunoreactive (microglial) cells in JSM6427 treated animals was 51.1 ± 8.1 per mm2 (± SEM), the vehicle treated 188.9 ± 20.8 per mm2. We could demonstrate the expression of integrin alpha5 beta1 on both microglia and glioma cells using flow cytometry. We observed a similar effect of JSM6427 in a slice culture were we injected glioma cells. After 5 days, we quantified the tumor size in the slices by measuring the area covered by the fluorescently labeled glioma cells. Slices treated with the integrin inhibitor JSM6427 showed a significant reduction in tumor size to 65.9 % ± 4.9 (n = 36) as compared to control (100 %, n = 30). Depleting microglial cells from the slice culture by clodronate treatment abrogated the effect of JSM6427 on glioma invasion indicating that the presence of microglia is required. We show further, that microglial migration, stimulated by ATP was attenuated dose-dependently by JSM6427.

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In vitro characterizaiton of sphere-forming glioma 'stem' cells-what is the stemness?

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A concept of cancer stem cells has been recently proposed as a minor, self-renewing subset of cancer cells that continuously produce their descendant tumor cells constituting the major fraction of tumors. Cancer stem cells have been isolated in vitro from primary gliomas and many other cancers, and have been attracting attention because they are anticipated to represent the tumorigenic fraction, and, potentially, important therapeutic targets. It has been reported that cancer stem cells can be isolated even from established cell lines. We herein examined biological features of sphere-forming glioma cells driven from different glioma cell lines (C6, U373, U251, U87). In all the cell lines, most of the tumor cells formed spheres in serum-free medium consisting of Neurobasal medium, B-27 supplement, L-glutamine, EGF and bFGF, and each cell line also formed secondary spheres. When dissociated, the sphere-forming cells expressed astroglial, oligodendrocytic, and neuronal markers some of which were never detected in the parental cell lines cultured in the conventional, serum-containing medium. Sphere forming cells driven from C6 cell line were tumorigenic when transplanted in rat brains as well as their parental cells. These findings support the idea that tumor stem cells exist in established cell lines. Importantly, however, most of the cells in these cell lines were capable to form spheres when cultured in low-binding plates even in the serum-containing medium. Under the differentiating condition, they also exhibited multipotentiality. We thus conclude that sphere-formation per se results from the intrinsic anchorage-independency of the tumor cells and most of the tumor cells could differentiate to all the three lineages if cultured under appropriate conditions. Our results indicate that the current methods of sphere assay, at least in part, merely causes juvenilization of the major fraction of tumor cells, and call for the necessity of carefully seeking the 'true' stem cell population.

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Differential proteome analysis of human gliomas stratified for loss of heterozygosity on chromosome arms 1p and 19q

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Gliomas, the most common primary brain tumors, are histologically classified on the basis of morphological and

immunohistochemical features as defined in the World Health Organization (WHO) classification of tumors of the nervous system. In addition to the histological assessment, certain genetic factors, such as allelic losses on chromosome arms 1p and 19q, are able to provide clinically useful information that may help to stratify gliomas into prognostically distinct subgroups. In particular, recent randomized trials have strongly associated 1p/19q-deletion with response to radio- and chemotherapy as well as longer survival in patients with anaplastic oligodendrogliomas and anaplastic oligoastrocytomas (Cairncross *et al.*, J. Clin. Oncol. 24, 2707–14, 2006; van den Bent *et al.*, J. Clin. Oncol. 24, 2715–22, 2006). To identify proteins that are differentially expressed between gliomas with and without 1p/19q-deletion, we performed a proteomic analysis on oligoastrocytomas using differential gel electrophoresis (DIGE) followed by MALDI-TOF/TOF mass spectrometry. Thereby, we identified 46 differentially expressed proteins ($\Delta \geqslant 2.0$, $p \leqslant 0.05$). From this candidate protein pool, we selected the first promising proteins for further analysis and confirmed their differential expression by using western-blot analysis and immunohistochemistry. Further clinical validation of these differentially expressed proteins on larger patient cohorts is ongoing to assess their utility as potential biomarkers for classification and prognostic assessment.

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Analysing the mechanism causing the OSM cell cycle arrest in human glioblastoma cell lines

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Oncostatin M (OSM) induces a very efficient growth arrest on various tumour cell lines including human glioma cell lines. We have recently shown that OSM treatment of glioma cell lines elicits a cell cycle arrest in the G1 phase of the cell cycle and the differentiation into GFAP positive astrocytes. This process seems to be a consequence of the modified expression of various cell cycle regulators including upregulation of the CDK inhibitors p21WAF1 and p27Kip1 while simultaneously downregulating cell-cycle stimulating proteins like cyclin A and Skp2/Cks1. In order to dissect the contribution of the cell cycle regulator proteins for the OSM-induced cell-cycle arrest we have generated cell lines which overexpress downregulated proteins while we inhibited the expression of the CDKI proteins by siRNA knockdown. Furthermore, we analyzed the contribution of the CDKI proteins in vivo by inducible expression of these proteins. In order to identify the transcriptional mechanism by which OSM downregulated the expression of the Skp2 and Cyclin A genes we performed promoter analysis of these genes. The particular relevance of the transcriptional downregulation of these genes was investigated by quantitative RT-PCR analysis of these genes in glioma cell lines responding to OSM by cell-cycle arrest vs glioma cell lines not responding to OSM.

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PDGF drives the proliferation and migration of neonatal glial progenitors leading to the formation of malignant gliomas

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To test the ability of neonatal SVZ progenitors to expand beyond their developmental limits, we infected neonatal rat pups with a retrovirus that expresses PDGF and green fluorescent protein (GFP). Infiltrative tumors that closely resembled human glioblastomas developed in 100% of the animals by 10 days post injection. The tumors were composed of a mixture of retrovirus infected (GFP+) and uninfected (GFP-) cells, suggesting that PDGF was driving tumor formation via both autocrine and paracrine signaling. Rats co-injected with retroviruses that express PDGF-IRES-dsRED and GFP formed tumors that contained a mixture of DsRed and GFP+ cells. When separated by FACS and transplanted into neonatal rats, the DsRed+ cells formed new tumors whereas the GFP+ cells did not form tumors unless they were co-injected with DsRed cells. Time-lapse microscopy of slices cultures showed that both GFP and DsRed populations were highly migratory and proliferative. Furthermore, adding PDGF to the slice culture media stimulated the migration and proliferation of glial progenitors in the white matter. These results show that constitutive expression of PDGF keeps glial progenitors migrating and proliferating for an extended time and that this leads to the rapid formation of tumors that closely resemble glioblastomas.

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Sphere formation is not essential for the derivation of tumour initiating stem-like cells from human glioblastoma specimens

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Objective: Our aim was to develop a protocol to reliably isolate human glioblastoma-initiating cells with stem cell-like characteristics and confirm their ability to initiate tumour formation in vivo.

Methods: Fresh glioblastoma specimens were collected, dissociated and cultured in serum free media with FGF2 and EGF to produce primary spheroid bodies. These spheroids were mechanically dissociated and re-cultured as free floating secondary spheres to generate further spheroid colonies. Comparison was made with cells that grew as adherent monolayers on coated flasks in identical defined media.

Differentiation was induced by the withdrawal of mitogens and the addition of 1% foetal calf serum. Expression of markers associated with multipotentiality and phenotypic differentiation were examined. Tumour formation *in vivo* was tested by orthotopic implantation into SCID mice.

Results: Under serum free conditions spheroid aggregates containing $CD133^{+}$ cells were readily generated from dissociated tumour tissue. However, we were unable to demonstrate self-renewal of these populations, tumour formation in vivo was not observed but implanted cells migrated extensively in the host brain. Under differentiating conditions markers associated with astrocytes, oligodendrocytes and neurons could be identified within the spheroid colonies.

Cells cultured as monolayers and passaged at confluence were initially slow growing. However, self-renewal was observed and maintained by passage every 8 weeks. Attempted passage at shorter intervals resulted in cell loss and failure of self-renewal. After a limited number of passages

we observed that self renewal could be maintained at shorter passage intervals of 3–4 weeks. Under differentiating conditions these cells also expressed markers associated with astrocytes, oligodendrocytes and neurons. Grafting of this cell population resulted in tumour formation with local invasion of the host brain.

Summary: Human glioma stem-like cells can be isolated by culture with EGF and FGF2 under serum free conditions. However, the formation of spheroid bodies is not essential for this process.

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Time-dependent effects of a novel ruthenium complex containing the non-steroidal anti-inflammatory drug (nsaid) ibuprofen on the mRNA expression of proteins involved in cell cycle control, Angiogenesis and Invasion in C6 rat glioma cells *in vitro*

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Malignant gliomas have poor patient prognosis due to their highly invasive nature and their resistance to conventional radiotherapy and chemotherapy. The NSAIDs are known cyclooxygenase-2 inhibitors and have anti-tumour effects. Ruthenium containing compounds have been shown to exhibit anti-tumour activity, which involves binding to both DNA and proteins. The novel ruthenium-ibuprofen complex (RuIb) significantly inhibits proliferation of C6 rat glioma cells *in vitro* at a concentration of 100μM after 72hrs of treatment. The aim of the present study was to identify specific targets of RuIb action and the time-dependence of these changes by analyzing the mRNA expression profiles of proteins of importance for glioma proliferation and invasion. RT-PCR was performed using specific primers in order to study the mRNA expression pattern of proteins after 100μM RuIb exposure *in vitro* at 3, 6, 12, 24, 48 and 72hrs of treatment. RuIb decreased the expression of cyclin D1, EP1, EP4 (48hrs), PPARγ, pRb and increased the expression of Bax, Bcl-2, p21, p27, p53, p65, c-myc, E2F1, ERK2, nm23α and β, VEGF-A, Flt-1 and EP4 (6hrs). C6 cells did not express p16 and COX2, under control conditions or after RuIb exposure. The alterations in the expression of cell cycle and apoptosis-related proteins indicate that RuIb has multiple targets, which translate into the inhibition of proliferation and induction of cell death.

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