S06-17

POU AND PBC HOMEODOMAIN TRANSCRIPTION FACTORS REGULATE DIVERSE CELL FATES IN *C. ELEGANS*.
Bürglin, T.R., Biozentrum der Universität Basel
ceh-6 is a member of the POU-III homeobox family. It is expressed in 10 bilaterally symmetric neurons in the brain, the excretory cell (necessary for osmoregulation), neuroblasts in the ventral nerve cord, and the rectal cells. We generated a knock-out mutation in *ceh-6*. 80% of the animals die during morphogenesis, due to a failure in the rectal cells. These cells however still express the homeobox gene *egl-5* (*Abd-B* orthologue), suggesting that the cells are formed, but do not differentiate properly. The other 20% of the animals die as larvae containing many vacuoles, consistent with a defect in the excretory cell. *ceh-20* is an orthologue of the protooncogene *PBX1* and *Drosophila exd*. During early embryogenesis the gene is expressed only in the posterior half. During larval stages *ceh-20* is expressed in the ventral nerve cord, the retrovesicular ganglion and other neuronal structures in the body. In collaboration with Dr. Stern's laboratory we are examining mutations in this gene that display phenotypes consistent with the expression pattern.

Cytokines and Inflammation in the Nervous System

S07-01

Immunopathology of the neurovascular lesion in cerebral malaria
Jin Ning LOU, Ralf LUCAS, Yves DONATI, Christine MONSO, Pierre JUILLARD, Christine DECRIND, Horst BLUETHMANN[¶] and Georges E. GRAU
Dept. of Anaesthesiology, Pharmacology and Surgical Intensive Care, University of Geneva, CH-1211 Geneva 4, Switzerland and [¶]Hoffmann-La Roche, Basel, Switzerland.

The mechanisms of microvascular injury were studied *in vivo* in experimental cerebral malaria (CM), a model in which TNF has been shown to be a critical mediator. In addition, *in vitro* studies were performed on isolated microvascular endothelial cells (MVEC), purified from brain or lung. First, since *in vivo* evidence had been obtained for a possible role of platelets as effector cells in CM, we evaluated this on purified brain MVEC. TNF was found to increase adherence or fusion of platelets to brain MVEC, as assessed by radiolabelled platelet binding, electron microscopy and flow cytometry. This phenomenon resulted in the expression of platelet antigens on the surface of endothelial cells and led to increased adhesiveness for leukocytes. Second, *in vitro*, brain MVEC from CM-susceptible mice appeared more sensitive to TNF than their counterparts isolated from CM-resistant animals. TNF-induced cytolysis was abrogated in TNF-R1 mutant-derived MVEC, but unchanged in TNF-R2 mutant derived cells. Surprisingly however, when assessing the susceptibility of these mutant mice to CM-induced lethality, TNF-R2 -, but not TNF-R1 -, mutant mice were fully protected. Moreover, in mice susceptible to CM, an increased expression of TNF-R2, but not TNF-R1, was observed on brain microvessels. Although most *in vitro* data point out a critical role of TNF-R1 in cytolysis, these results suggest a significant role of TNF-R2 in pathological events occurring *in vivo*. Alternative mechanisms, such as TNF-induced platelet-endothelium interactions, could be important modulators in pathological conditions where TNF is central.

S07-03

THEILER'S VIRUS INDUCED DEMYELINATION IN IMMUNE KNOCKOUT MICE: A MODEL FOR MULTIPLE SCLEROSIS
Moses Rodriguez, Mayo Clinic, Rochester,MN USA

We utilized mice with genetic deletion of Class I MHC, Class II MHC, CD8 and CD4 to understand the role of immune system in controlling Theiler's virus infection and contributing to demyelinating disease. Deletion of Class I or Class II MHC in animals of resistant H-2b haplotype resulted in virus persistence and chronic demyelination. Class II deficient mice but not Class I deficient mice developed neurological deficits. Genetic deletion of CD4+ or CD8+ T-cells in susceptible SJL/J or PLJ mice enhanced demyelination and persistent virus infection. The most extensive demyelinating and clinical disease was observed in CD4 knockout mice. In conclusion, both Class I-restricted CD8+ T-cells and Class II-restricted CD4+ T-cells are important in resistence to demyelinating disease. Class I MHC and CD8+ T-cells appear to be necessary for the development of the neurological deficits associated with demyelination.

S07-04

CHEMOTACTIC FACTORS IN BACTERIAL MENINGITIS
Karl Frei, Department of Neurosurgery, University Hospital Zürich, CH-8006 Zürich

In bacterial meningitis, the recruitment of leukocytes across the blood-brain barrier into the central nervous system (CNS) is essential for effective elimination of pathogens. However, part of the tissue damage occuring during bacterial meningitis is mediated by cytotoxic factors released by inflammatory cells. In regard to the accumulation of phagocytes within the CNS we asked whether chemokines - a new family of cytokines - might be involved. As shown by Northern analysis, brains of mice infected intracerebrally with *Listeria monocytogenes* (LM) express mRNA for three chemokines, the macrophage inflammatory protein (MIP)-1α, MIP-1β and MIP-2. *In situ* hybridization revealed blood-derived PMNs and monocytes, infiltrating the meninges and the ventricular system, as the major source of the chemokines. Cytological differential of the cerebrospinal fluid (CSF) exudate and histological examination of the infiltrate revealed a shift of the predominant cell type from PMN's to monocytes during the course of infection. In the CSF a time-dependent increase of antigenic MIP-1α and MIP-2 was found. CSF taken from mice 24 h postinfection (CSF-LM24) induced migration of both human monocytes and PMN's when treated in chemotactic chambers *in vitro*. Neutralizing antibodies to chemokines identified MIP-1α and MIP-2 to be responsible for CSF-LM24 mediated chemotaxis of monocytes and PMN's, respectively. CSF obtained from mock-infected animals contained no MIP-1α or MIP-2 and did not lead to migration of leukocytes.

When testing CSF-LM24 on mouse spleen cells, the chemotactic activity detected for mononuclear cells was only partially inhibited by antibodies to MIP-1α and -1β. Thus, in addition to MIP-1 and -2 other not yet defined chemotactic factors are of importance for recruitment of leukocytes in bacterial meningitis.

S07-05

PROTEINS BINDING TO RNA STABILIZATION ELEMENTS IN GM-CSF mRNA 3'UTR.
Verena Bichsel, Alfred Walz and Matthias Bickel. Laboratory of Oral Cell Biology and Theodor Kocher Institute, University of Bern
GM-CSF mRNA stabilization is achieved by sequences located in its 3'UTR. A functional, 60 ribonucleotide long TPA-response element has previously been characterized in the murine GM-CSF mRNA. By using this specific response element as a probe, we identified specific binding to proteins from either murine (EL-4) or human T-cells (Jurkat). The human GM-CSF 3'UTR counterpart that lacks sequence homology in this region also bound to proteins from either cell line. The apparent molecular mass of the murine and human binding proteins was 93 and 94 kDa, respectively. The size of the protein-bound RNA was determined by digesting the complex with ribonuclease A. Electrophoretical separation revealed a 44- and 38-ribonucleotide long sequence in mouse and man. The binding site is thus smaller than the functionally mapped site. Whether the protein binding relates directly to the stabilization that is mediated by the functional element is currently under investigation. Supported by grant: SNF 31 365 06.92

S07-06

COMPARISON OF ENDOTHELIN FUNCTION IN RAT ENDOTHELIAL CELLS AND MACROPHAGES.

Peduto Eberl L;Aubert JD and Juillerat-Jeanneret L. Institute of Pathology and Division of Pneumology, Lausanne, Switzerland.

Endothelin peptides have been initially described as potent endothelial-derived vasoconstrictors. It is now accepted that endothelins can be secreted by various cells, including cells of the mononuclear lineage. However, the effects of endothelin(s) on these different cell types has not been extensively compared. The effet of added endothelin-1 to rat bone marrow-derived macrophages or to endothelial cells expressing endothelin receptor, but which do not secrete the peptide, was evaluated after short (up to 8 hours) or long (up to 5 days) exposure. Endothelin secretion by macrophages was determined. Nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) secretion, and the expresion of enzymatic markers of these cells was measured. Macrophages secreted endothelin-1, but did not respond to endothelin for any of the functions tested. Endothelial cells responded to endothelin-1 by a rapid increase in IL-6 secretion. Long term addition of the peptide induced endothelial contraction, but did not modify the expression of enzymatic markers. Thus, endothelin-secreting macrophages may be involved in regulating the function of endothelial cells expressing endothelin receptor.

S07-07

REGULATION OF THE KYNURENINE PATHWAY BY IFN-γ IN MURINE MACROPHAGES AND MICROGLIAL CELLS.

D. Alberati-Giani and Andrea M. Cesura.
Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, CH-4070 Basel

The regulation of the kynurenine pathway enzymes by interferon-γ (IFN-γ) in murine macrophages (MT2) and microglial (N11) cells was studied. In both cell lines, IFN-γ induced the expression of indoleamine 2,3-dioxygenase activity. Whereas other enzymes of this pathway were constitutively expressed in both cell lines to a similar extent, kynureninase activity was much higher in MT2 than in N11 cells. In addition, this enzyme was markedly stimulated by IFN-γ only in MT2 macrophages. These results support the notion that activated invading macrophages may constitute the major source of neuroactive kynurenines in brain inflammation.

S07-08

ERYTHROPOIETIN AND ITS RECEPTOR IN THE MOUSE BRAIN

Digicaylioglu M., Bichet S., Marti HH., Bauer C. and Gassmann M., Dept Physiol., University of Zurich-Irchel, CH-8057 Zurich.

The main physiological regulator of erythropoiesis is the hematopoietic growth factor erythropoietin (EPO), which is primarily produced in the kidney of adult mammals and is induced in response to hypoxia. Binding of EPO to the EPO-receptor (EPO-R) controls the terminal maturation of red blood cells. So far, EPO has been reported to act mainly on erythroid precursor cells. However, we have detected mRNA encoding both EPO and EPO-R in mouse brain by reverse transcriptase-mediated PCR (RT-PCR). Homologous competition experiments on mouse brain sections, visualized with autoradiography revealed defined binding sites at distinct brain areas. The specificity of EPO binding was assesed by using two monoclonal antibodies against human EPO, which are known to be inhibitory and non-inhibitory, respectively, for binding of EPO to EPO-R. Major EPO binding sites were observed *in the* capsula interna, cortex, ependym, fibre tracts, hippocampal formation and midbrain areas (*PNAS, 92:3717,1995*). A monoclonal antibody raised against human EPO-R was used to identify the cells expressing the EPO-R. In preliminary immunohistochemical studies we have detected the EPO-R on neurons in hippocampus, cortex, midbrain, fimbria and amygdala of the mouse brain. Functional expression of the EPO-R and hypoxic upregulation of EPO suggest a novel role of EPO in the brain.

S07-09

THE HUMAN MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG) GENE: SEQUENCE AND STRUCTURE

Arnold B., and Matthieu J.-M.
Laboratoire de Neurochimie, CHUV, Lausanne

MOG is the primary target autoantigen in auto-immune demyelinating diseases. It contains 218 amino acids, one single N-glycosylation site which is part of the Ig like variable domain, and 2 putative transmembrane domains. We isolated and sequenced human genomic clones. This gene contains 8 exons and spans over 15 kb. The size of the introns vary from 242 to 6484 bp. Exon 1(Ex1) is formed by a short (9 bp) 5' non-coding sequence followed by the 88 bp coding for the signal peptide. Ex2 (348 bp) codes for the Ig-like domain. Ex3 (114 bp) codes for the first transmembrane domain and Ex4 & 5 (21 bp each) for the intracellular loop. Ex6 (127 bp) codes for the 2nd transmembrane domain while Ex7 (21 bp) and 14 bp of Ex8 (1.2kb) code for the extracellular C-terminal region. Currently, we are studying the regulation of the MOG gene.