S05-20

VARIATIONS OF CALRETININ EXPRESSION IN THE HUMAN ADENOCARCINOMA CELL LINE HT-29. R. Cargnello, V. Gotzos and M.R. Celio. Institute of Histology and General Embryology, University of Fribourg, Pérolles, CH-1705 Fribourg.

Calretinin (CR) is a Ca^{2+}-binding protein which is not expressed by normal human enterocytes. On the contrary it is expressed by some colon adenocarcinomas. Its function is not yet elucidated, but some evidences lead to hypothesize its involvement in cell proliferation. In order to see whether this protein is related to a particular cellular state, its expression was studied in the adenocarcinoma clonal cell line HT29-18, which can differentiate into enterocytes-like cells by replacing glucose with galactose in the culture medium. After treating the cells with galactose we observed: 1) the appearance of microvilli at the apical surface of the cells, 2) a greater CR expression in undifferentiated HT29-18 cells, grown in glucose-containing medium than in differentiated cells, grown in galactose-containing medium.

Therefore we suggest that CR could be involved in carcinogenesis.

Drosophila and Nematode Embryogenesis

S06-01

Controlling the functional specificity of homeotic proteins

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Much of the *in vivo* functional specificity of the Hox proteins seems to be controlled by poorly understood protein-protein interactions on composite regulatory elements. We have studied such a composite element regulated by the DEFORMED (DFD) protein of *Drosophila*. This *Dfd* epidermal autoregulatory element (*Dfd*-EAE) is also activated in the posterior head of mouse embyros, perhaps in response to mouse DFD-like homologs. Important non-homeodomain binding sites that are required for this element to be regulated by DFD and not other homeotic proteins reside in a 50 bp region that binds a presumptive DFD co-factor protein called DEAF-1.

Other putative homeotic co-factors have been identified in a genetic screen for second-site mutations that enhance weak *Deformed* mutant phenotypes. Using this strategy, we have screened approximately 12,000 third, 9,000 second, and 4,000 X chromosomes for mutations that interact with *Dfd*. Some of the known genes for which mutations were isolated in the *Dfd*-modifier screen are *extradenticle*, *cap n' collar*, and *hedgehog*. How these and other modifier genes contribute to the posterior-head determination pathway along with *Dfd* will be discussed. For example, our results indicate that *extradenticle* is a crucial co-factor for DFD, as it is for many other homeotic proteins. However, its role in controlling the selectivity of homeotic response elements may be mediated through its function as a repressor, not as a selectively binding co-activator with different homeotic proteins.

S06-02

Mechanisms of *wg* signal transduction

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The *Drosophila* segment polarity gene *wingless* and its vertebrate homologue, the mouse *Wnt-1*oncogene, encode secreted growth factor-like molecules that play key roles in generating positional information during development.

In the *Drosophila* embryo, *wg* signaling elicits two distinct cellular responses to pattern the wild-type epidermis. *wg* activity is required for the generation of diverse cell fates in the anterior of each embryonic segment and for the secretion of naked cuticle in the posterior of each segment. These two signaling activities are separately mutable within the Wg protein: EMS-induced mutations have been isolated that disrupt each function independently.

To assess the cellular basis of these altered Wg signaling activities, mutant *wg* transgenes have been constructed and expressed ubiquitously under control of the heat shock promoter. The phenotypic consequences of this manipulated expression will be presented and the implications for *wg* / *Wnt* signal transduction mechanisms will be discussed.

S06-03

Cloning and identification of the *smoothened* gene

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We have identified the transcription unit that rescues the *smoothened* (*smo*) phenotype. The gene encodes a seven-pass membrane protein that has homology to the *Drosophila* Frizzled protein, a putative G-protein-coupled receptor involved in mirror-symmetry pattern formation of adult cuticular structures. The *smo* gene is most abundantly expressed during the blastoderm stage in the trunk region and much less at both poles of the embryo. The gene is also expressed in the head region, similar to the head patch expression of *paired*. At later blastoderm stages, *smo* expression becomes segmentally modulated and remains striped after germ band extension. Since Hh-dependent *wg*-expression is absent whereas Wg-dependent *en*-expression is present in *smo* mutant embryos, the function of *smo* is required in the transduction of the Hedgehog (Hh) and not of the Wingless (Wg) signal. Based on the predicted physical characteristics of the Smo protein and on its position in the Hh signaling pathway, we propose that *smo* may encode the Hh receptor. Its homology to a putative G-protein-coupled receptor may indicate the presence of a G-protein-mediated signal transduction pathway in maintaining positional information within the embryo.

S06-04

***schnurri* is Required for Drosophila dpp Signaling and Encodes a Zinc Finger Protein Similar to the Mammalian Transcription Factor PRDII-BF1**

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Cytokines of the TGFβ superfamiliy were shown to activate receptor complexes consisting of two distantly related serine/threonine kinases. Previous studies indicated that Drosophila dpp (decapentaplegic) which is a homologue of BMP-2 and BMP-4, uses similar complexes and strictly requires the thick veins (type I) and punt (type II) receptor to transduce the signal across the membrane. We present the characterization of the *schnurri* (*shn*) gene and show that it is required for many aspects of dpp signaling. Genetic epistasis experiments indicate that *shn* functions downstream of the dpp signal and its receptors. The *shn* gene encodes a large protein containing zinc finger motifs and is similar to a fimily of vertebrate transcription factors. The shn protein might therefore act as a nuclear target in the dpp signaling pathway directly regulating the expression of dpp-responsive genes.

S06-05

Isolation and Characterization of Downstream Signal Transduction Components of the TGF-b Family of Cytokines in *Drosophila*

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Extensive genetic analysis has made the *Drosophila dpp* gene an attractive model for studying TGF-ß signaling. The products of the *punt, thick veins (tkv)* and *saxophone (sax)* loci function as DPP receptors. Recently Massagué and colleagues have produced a constitutively activated type I TGF-ß receptor by introducing negatively charged residues within the GS box. We have made similarly activated TKV and SAX receptors. The activated receptors can be used to investigate the different effects of DPP signaling through TKV and SAX. On the other hand, inappropriate activation of DPP signaling was expected to lead to dominant phenotypes similar to DPP. Heterozygosity for downstream components that reduce the flow through the pathway might then suppress the dominant phenotype allowing for the development of a genetic screen for additional genes that mediate DPP signaling. Using the Gal-4 system and enhancer piracy to express activated TKV and SAX in different imaginal discs, we produced adult phenotypes. These lines were tested for interaction with the potential downstream genes *mad, medea* and *shn*. The dominant phenotypes of TKV and SAX were both significantly suppressed in flies heterozygous for *mad* and *shn* mutant alleles, but only in some alleles mutant for *medea*, suggesting that the systems are sensitive to haplo levels of potential downstream genes of the DPP signaling pathway. Performing a small F1 screen, we have been able to identify more than 20 different mutations which significantly suppress the mutant phenotypes. Two of these mutations are allelic to *medea* whereas one is allelic to *mad* which shows that this approach identifies downstream components of the DPP signaling pathway. Analyzing the phenotype of germline mutant embryos indicates, however, that not all mutations are downstream of *dpp* since they show phenotypes different from *dpp*. Novel genes in the pathway are likely to have homologs that function in mediating TGF-ß signaling in mammals as well.

S06-06

The Mechanism of Sequence Recognition by Homeotic Selector Proteins is Conserved in Animals: A Response Element from the Mouse Genome Identified by Activity in *Drosophila*

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The homeotic selector proteins are expressed in a series from head to tail along the bodies of all Metazoans. They specify anteroposterior position so that organs and structures form at the correct sites. The *Drosophila* homeotic selector complexes encode 8 selector proteins and vertebrate *Hox* complexes produce proteins of 8+5 classes, defined by homeodomain sequences. Expression of Hox proteins in *Drosophila* has shown that a functional hierarchy, encoded in the class-specific homeodomain sequences, is conserved between *Drosophila* and vertebrates.

We have found that the intron of the mouse *Hoxa-4* gene acts as a strong homeotic response element in *Drosophila* embryos and in leg imaginal discs. Using ectopic expression of *Drosophila* homeotic selector proteins from *hsp70* promoter constructs and ectopic activation of the response element in antennal discs as an assay, we find that this element responds to *Antp* and more posteriorly-acting proteins, but not to more anterior ones. A 27 bp cluster of homeodomain binding-sites in the target DNA, and four specificity-determining residues in the amino-terminal arm of *Antp* and more posterior proteins mediate this axial switch in homeotic selector response. Mutating the binding sites and testing constructs in transgenic mice indicates that this response element functions equivalently in the neural tube of vertebrates.

Further investigations on the molecular mechanisms of this homeotic response switch are now in progress.

S06-07

SEGREGATION DISTORTION IN DROSOPHILA MELANOGASTER

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Males of D. melanogaster, which are heterozygous for SD factors (SD = segregation distortion), produce 95% or more progeny containing the SD chromosome, that is more than the expected 50%. In the present study it is shown, that histone - protein transition is in half of the spermatids defect.

S06-08

***sparkling*, a paired box gene required for the positional identity of cone and pigment cells during the ommatidial assembly of the *Drosophila* eye**

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We have isolated the *sparkling* (*spa*) gene which encodes an 844-amino acid protein with a paired-domain near its N-terminus. It is expressed in the central and peripheral nervous system of the embryo from stage 11 till the end of embryogenesis. In eye discs, Spa protein appears in cone cells, which are found five rows behind the morphogenetic furrow. The protein is also expressed in a subset of cells in the antennal and leg discs. Molecular analysis reveals that spa^{1} is a 5.8-kb insertion which reduces the expression of *spa* in the eye disc dramatically, whereas spa^{pol} is a 1.5-kb deficiency which deletes two small exons and flanking intron sequences, resulting in a complete loss of *spa* expression in the eye. On the other hand, the dominant spa^{A} allele is produced by a complicated translocation of the third and fourth chromosomes, which reduces and changes the expression pattern of *spa* in the eye disc. Analysis of eye phenotypes of *spa* mutants indicates that Spa is required for the normal development of cone cells. Without the function of Spa, cone cells lose their positional identity, leading to incorrect assembly of cone and pigment cells during the late pattern formation of the *Drosophila* retina. To understand the role of *spa* during embryogenesis, we are isolating EMS-induced lethal *spa* alleles in the hope that their phenotypes will provide insight into Spa's function in the development of the central and peripheral nervous system.

S06-09

The functional conservation of proteins in evolutionary alleles and the dominant role of enhancers in evolution

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The *Drosophila* genes *prd, gsb*, and *gsbn* all encode transcription factors with a paired-domain and a *prd*-type homeodomain in their N-terminal halves. Li and Noll have shown previously that ubiquitous expression of these genes generates the same phenotypes and that the transgene expressing the *prd* coding region under the control of the *gsb* cis-regulatory region is able to rescue the cuticular phenotype of *gsb* mutant embryos. These results point to the *cis*-regulatory rather than coding regions as being responsible for the essential difference between the distinct functions of the three genes. Here we report that (i) *Drosophila prd* mutants are able to survive to viable adults if supplied with two copies of a *prd*-Gsb transgene expressing the Gsb protein under the control of the entire *cis*-regulatory region of *prd* and (ii) the Pax3 protein, a mouse homolog of Prd, Gsb and Gsbn, when placed under the control of the *prd cis*-regulatory region, is able to rescue the cuticular phenotype of *prd* mutant embryos. These results show that Gsb and Pax3 proteins have conserved all or some functions of the Prd protein that are required for survival of embryos to viable adults although their C-terminal halves appear unrelated. In addition, Pax3 is an even better substitute than Gsb for some functions of Prd. Since the coding regions of *prd*-Gsb, *prd*-Pax3, and *prd* have been derived from a common ancestral gene during evolution, *prd*-Gsb and *prd*-Pax3 may be considered as "evolutionary" alleles of *prd*. We conclude that the acquisition of new *cis*-regulatory regions has been the major device for the functional diversification of the *Drosophila* genes *prd* and *gsb* and the mouse *Pax3* gene.

S06-10

Role of *pox meso* in larval muscle development

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The *Drosophila* gene *pox meso* (*poxm*) encodes a transcription factor containing a paired-domain. It is expressed with a segmental periodicity in the embryonic mesoderm, suggesting a possible role of *poxm* in somatic muscle development. To understand the function of *poxm*, we generated a small deficiency by imprecise excision of a P-element near the *poxm* locus. This deletion removed the entire *poxm* transcript and at least one adjacent locus at the site of the original P-element insertion. Although this deletion uncovers two loci, it is still useful for examining the function of *poxm* in muscle development. Analysis of the phenotype of this small deficiency showed that one of the lateral muscles 18, is missing in the first four abdominal segments A1 to A4 and at least three of the ventral muscles, 15 to 17, are missing in A1 to A7. Additional ventral muscles are missing and disorganized in some segments. The missing muscles correspond precisely to the positions of *poxm* expression in the somatic mesoderm. To exclude the possibility that this defect in muscle development of embryos homozygous or transheterozygous for the small deficiency is caused by the lack of the neighbouring gene's function, we confirmed that all muscles are present in embryos that are homozygous or hemizygous for the lethal P-element insertion in the neighboring locus. These results suggest that *poxm* is indeed important in muscle development and might determine a subset of somatic muscles. Our current work concentrates (i) on rescuing the muscle defect phenotype and (ii) on the elucidation of the role of *poxm* in somatic muscle development, i.e., on the search for its targets and upstream regulators.

S06-11

The *Drosophila net* gene, required for intervein fate in wings, encodes a putative bHLH transcription factor

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The *net* mutant phenotype suggests that *net* plays an important role in wing morphogenesis by regulating the adhesion of the two epithelial cell layers in the wing primordia. In *net* mutants, additional wing veins appear, thus suggesting an apparent role for *net* as a vein suppressor gene. By chromosomal walking, 230 kb of genomic DNA were cloned which span the *net* locus in the cytological interval between 21B1 and 21C1. The region containing *net* was further narrowed down by mapping breakpoints of several insertions and one inversion. Screening cDNA libraries from 4-8-h old embryos and third instar larval discs identified three classes of cDNA clones, two of which overlap but are transcribed in opposite directions. To determine which transcript represents the *net* gene, we used single strand-specific RNA probes from each cDNA class in DIG *in situ* labeling experiments on whole mount embryos and imaginal discs. Surprisingly, only one of the overlapping transcripts exhibited an expression pattern, which, in wing discs, complements the pattern of *rhomboid* (Sturtevant and Bier, Development 121, 785, 1995), a gene required for wing vein formation. The putative Net protein sequence shows homology to the *myc*-type bHLH DNA-binding domain and hence may be a transcription factor. Since the *rho* enhancer contains binding sites for HLH proteins (Ip et al., Genes & Dev. 6, 1728, 1992), we predict a model in which Net binds to the *rho* enhancer and represses *rho* expression. Such a repression of *rho* would result in preventing vein development in intervein areas.

S06-12

CONTROL OF EMBRYONIC BRAIN DEVELOPMENT BY THE HOMEOBOX GENES *otd* AND *ems* IN *DROSOPHILA*.

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We have studied the roles of the homeobox genes *orthodenticle (otd)* and *empty spiracles (ems)* in embryonic brain development of *Drosophila*. The embryonic brain is composed of three neuromeres. *otd* is expressed predominantly in the anterior neuromere. *ems* is expressed in the two posterior neuromeres. Mutation of *otd* eliminates the first brain neuromere. Mutation of *ems* eliminates the second and third neuromeres. *otd* is also necessary for the development of the dorsal protocerebrum of the adult brain. We conclude that the *otd* and *ems* homeobox genes are required for the development of specific segmental brain neuromeres in *Drosophila*. Considering similarities in expression and mutant phenotypes of gene homologs in mammalian brains, we postulate that an evolutionarily conserved program underlies brain development in all higher animals.

S06-13

FUNCTIONAL CONSERVATION OF *PAX6* IN EYE DEVELOPMENT AND EVOLUTION

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The *eyeless* gene of *Drosophila melanogaster* is homologous to the vertebrate genes *Small eye (Pax6)* in mice and Aniridia in humans. These genes encode transcription factors containing a highly conserved paired domain and homeodomain and they show a similar genomic organization suggesting to be true orthologs. In addition, mutations in all of these genes cause severe defects in eye morphogenesis. Gal4-UAS-mediated misexpression of *eyeless* and of mouse *Pax6* cDNAs leads to the induction of light-responsive ectopic eyes in *Drosophila*.

To corroborate the hypothesis of a putative monophyletic origin of the eye we have identified and characterized *Pax6* homologs in a nemertean, a mollusc and an urochordate. They all show a high degree of sequence and gene structure conservation and their expression pattern implies that they are involved in the formation of the eyes. In the case of the ascidian *Phallusia mammillata* we found expression of the *Pax6* homolog in the neural fold and later in development in the spinal cord and the brain. Ectopic expression of the ascidian gene in *Drosophila* also leads to the formation of supernumerary eyes.

These data suggest that a *Pax6* gene was present in the last common ancestor of these phyla and that it plays a crucial role in eye morphogenesis and evolution.

S06-14

Antisense RNA inhibition of the *labial*-like gene in *Caenorhabditis elegans*

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ceh-13 is a member of the *C. elegans* homeobox gene cluster that includes *lin-39, mab-5* and *egl-5*. Its structure corresponds to both insect and vertebrate *labial* orthologue genes which mediate anterior positional information during embryonic development. Genetic and molecular analyses have shown that *lin-39, mab-5* and *egl-5* provide positional cell fate information along the anterior-posterior axis in nematode development.

ceh-13 is expressed earlier than the other genes of the cluster, namely at the onset of gastrulation and persists into later stages in many cell types (Wittmann *et al.*, in prep.). This suggests that *ceh-13* may have several important functions in the development of the nematode.

We are interested in elucidating the function(s) of *ceh-13*. We have used the antisense RNA injection technique to inhibit the *ceh-13* activity. A typical Vab (variable) phenotype was reproducibly observed in the progeny of injected hermaphrodites. The different visible developmental defects were limited to body regions where *ceh-13* would normally be expressed. Based on these results, we are now performing a mutagenesis screen to isolate a *ceh-13* null mutant.

S06-15

A SCREEN FOR FACTORS REGULATING *CEH-13* EXPRESSION IN *C.ELEGANS*

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ceh-13 is the *C.elegans* homologue of *labial*, the most anteriorly expressed homeotic gene of the HOM-C/HOX gene cluster in *D. melanogaster* and vertebrates. The function of *ceh-13* is currently being investigated (see abstract of K. Brunschwig *et al.*). *ceh-13* expression begins very early during embryogenesis and shows an interesting asymmetry. At the 28-cell stage, CEH-13 protein appears in the two cell lineages AB and E. The AB lineage at this stage comprises 16 cells, but only the eight posterior daughters of the last division express CEH-13. Similarily, among the two daughters of the E blastomere, only the posterior cell (Ep) expresses CEH-13. We are interested in determining how these asymmetries are established during early embryonic development. We hope that *ceh-13* promoter analysis and a screen for maternal-effect lethal mutations that result in the aberrant expression of CEH-13 will lead to the identification of cis- and trans-regulatory elements.

S06-16

CEH-2, CEH-14, CEH-26*: THREE HOMEOBOX GENES IMPORTANT FOR THE DEVELOPMENT OF *C. ELEGANS

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ceh-14 encodes a LIM domain and a homeodomain.The LIM domain is known to play an important role in protein-protein interactions. Promoter-*lacZ* constructs showed an expression in the nervous system. We have now isolated cDNAs, which will be used to raise antibodies against Ceh-14.

ceh-26 is an orthologue of the *Drosophila* gene *prospero*. It is expressed in the nervous system during development. Knock-outs in *Drosophila* display cell lineage defects affecting certain neuroblasts. We isolated *ceh-26* cDNAs. Once we obtain an antibody against the protein, we will determine the expression pattern of *ceh-26* to explore its role in the development of the nervous system.

From ***ceh-2*** only the 3' end containing part of the homeodomain is known. Antibodies against a peptide 3' to the homeobox stain cells in the anterior of the pharynx. GFP- and *lacZ* reporter plasmids are being constructed to generate transgenic animals to verify these data. Several *ceh-2* specific fragments have been obtained by PCR of cDNA libraries and will be used to find *ceh-2* cDNA clones.

S06-17

POU AND PBC HOMEODOMAIN TRANSCRIPTION FACTORS REGULATE DIVERSE CELL FATES IN *C. ELEGANS*.
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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
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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
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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
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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