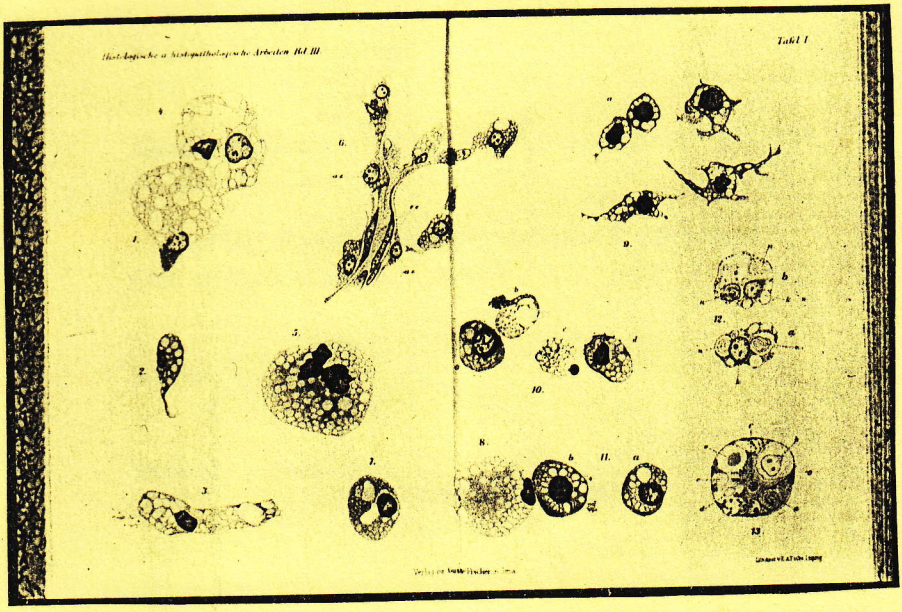
# THE ROLE OF MICROGLIAL CELLS IN HEALTH AND DISEASE

**September 11 - 14, 1996**

Castle Ringberg
Tegernsee
Bavaria
Germany



# PROGRAM

This Symposium is sponsored by

**Boehringer Ingelheim KG**
**Ingelheim/Rhein**
**Germany**

We gratefully acknowledge their generous financial contribution, which enabled us to organize this Symposium.

Furthermore, we want to thank the

**Deutsche Forschungsgemeinschaft (DFG)**
**Bonn**

and

**Max-Planck-Gesellschaft**
**München**

for making travel and accommodations funds available.

**Office Hours:**

| | |
|---|---|
| Wednesday, September 11 | 16.00 - 21.00 |
| Thursday, September 12 | 8.30 - 18.30 |
| Freiday, September 13 | 8.30 - 20.00 |
| Saturday, September 14 | 8.30 - 11.00 |

**Office Phone:** (49) 8022 2792 18
*(during the Symposium)*

**Fax:** (49) 8022 2792 59
*(during the Symposium)*

**Organizing Office:** Meino Gibson
Phone (49) 30 9406 3133
Fax (49) 30 9406 3819

**Duration of the oral presentations:** 30 min. talk + 15 min. discussion

**Posterboards:** Height: 125
Width: 150

## ORGANISATION

Prof. Dr. G. W. Kreutzberg
Neuromorphologie
MPI für Psychiatrie
Am Klopferspitz 18 A
D-82152 Martinsried
Germany
Phone: (49) 89 8578 3650
Fax: (49) 89 8578 3939

PD Dr. Helmut Kettenmann
Max Delbrück Center
for Molecular Medicine (MDC)
Cellular Neurosciences
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany
Phone: (49) 30 9406 3325
Fax: (49) 30 9406 3819

## Wednesday, September 11, 1996

**16.00 Arrival and Registration**

**18.30 Dinner**

**20.00 Welcome Address**
Georg W. Kreutzberg, MPI for Psychiatry, Martinsried, Germany

**Key note lecture**
Henryk M. Wisniewski, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA:
*The role of microglial cells in classical and primitive plaque formation*

**21.00 Informal Get-together**

## Thursday, September 12, 1996

**8.00 Breakfast**

**9.00 Session I**
Chairperson: Henryk M. Wisniewski

Gennadij Raivich, MPI for Psychiatry, Martinsried, Germany
*Signaling molecules and neuroglial activation in the injured central nervous system*

Anne Régnier-Vigouroux, University of Heidelberg, Germany
*Membrane traffic and immune competence of microglia and astrocytes: a comparative analysis*

Lidia Bonfanti, Scuola Normale Superiore, Pisa, Italy
*Reactive microglial cells in neonatal retinae following optic nerve transection*

**10.30 Coffee Break**

**11.00 Session I**

Reinhard Kiefer, Julius-Maximilian University Würzburg, Germany
*Controlling the response of brain microglia and intrinsic macrophages of the peripheral nerve to injury: role of transforming growth factor-ß1*

E. Weihe, University of Marburg, Germany
*Microglial and neuronal reactions to inflammation and ischemia*

Günter Schütz, DKFZ Heidelberg, Germany
*Analysis of glucocorticoid and cAMP signaling by gene targeting*

**12.30 Lunch**

**14.00 Excursion**

**18.30 Dinner**

## Friday, September 13, 1996

**8.00 Breakfast**

**9.00 Session II**
Chairperson: Hans Lassmann

Alexandre Dobbertin, INSERM U114, Paris, France
*Recruitment of macrophages in the central nervous system (CNS): influences of neurons and glial cells*

Manuel Graeber, University of Munich, Germany
*Microglial MHC class II expression in human astrocytomas*

Christiane Nolte, MDC Berlin, Germany
*Effects of growth factors and inflammatory mediators on microglial cell activation in vitro*

**10.30 Coffee Break**

**11.00 Session II**

M. K. Matyszak, Oxford University, UK
*Leucocyte responses to bacillus Calmette-Guérin sequestered in the CNS*

Richard Banati, Hammersmith Hospital, London, UK
*Imaging neuropathology in vivo with PET*

Wolfgang J. Streit, University of Florida, Gainsville, Florida, USA
*Functional significance of microgliosis*

**12.30 Lunch**

**14.00 Poster Session**

**15.30 Coffee Break**

**16.00 Session III**
Chairperson: Helmut Kettenmann

Hans Lassmann, University of Vienna, Austria
*Macrophages and microglia as effector cells in active multiple sclerosis lesions*

Jean Merrill, BERLEX Biosciences, Richmond, California, USA
*Microglial and astrocyte production of TNF and nitric oxide: the implication in MS and CNS-Aids*

Solon Thanos, University of Tübingen, Germany
*The role of microglial cells in the de- and regenerating retina*

Hans A. Kretzschmar, University of Göttingen, Germany
*The role of microglia in prion disease*

**18.30 Dinner/Bavarian Buffet**

**20.00 Session IV**
Chairperson: G.W. Kreutzberg & H. Kettenmann

**Round Table Discussion**

## Saturday, September 14, 1996

**8.00 Breakfast and Departure**

1. Lidia Faff, MDC, Berlin, Germany
   *The effect of pH changes on ATP-induced calcium elevation in cultured migroglia*

2. Gerhard Hager, MPI for Psychiatry, Martinsried, Germany
   *Extracellular matrix molecules in the facial nucleus after axotomy*

3. Uwe Hanisch, MDC, Berlin, Germany
   *Microglial cells express interleukin-15 and ists cognate receptor complex*

4. Elly Hol, MPI for Psychiatry, Martinsried, Germany
   *Molecular changes in neurons and glia during neuronal regeneration - a search for novel genes*

5. Leonard Jones, MPI for Psychiatry, Martinsried, Germany
   *Regulation of thrombospondin in the regenerating facial motor nucleus*

6. Alexander Werner, MPI for Psychiatry, Martinsried, Germany
   *Localization and regulation of receptors for macrophage colony-stimulating factor (MCSF) in normal and injured mouse cetral nervous system*

7. Joachim von Zahn, MDC, Berlin, Germany
   *Regulation of microglia phagocytosis in vitro*

## *Imaging neuropathology in vivo with PET*

R.B.Banati, J. Newcombe, T. Smith, A. Hewson, L. Cuzner, T. Jones, G.W. Kreutzberg, R. Myers
MRC Cyclotron Unit, CSC, RPMS,Hammersmith Hospital, London

PK11195(1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide is a highly specific ligand for the peripheral benzodiazepine binding site (PBBS). In normal brain, there is minimal binding of PK 11195, but a dramatic increase of PBBS expression is seen after neuronal injury. The expression of PBBS occurs exclusively in non-neuronal cells. Using photoemulsion autoradiography combined with immunocytochemical staining for glial markers, we found that the increase in PK 11195 binding is mostly due to the activation of microglia or, in areas of blood-brain barrier destruction, the invasion of macrophages. This observation was confirmed in a number of animal models, such as peripheral nerve axotomy and other brain lesions and on autopsy material from patients with multiple sclerosis and neurodegenerative diseases.

Labelled with carbon-11, PK 11195 can be used as a ligand in positron emission tomography (PET). Directly visualizing microglial activation in vivo by PET-imaging with [$^{11}$C] PK 11195 should therefore allow to detect and monitor the progression of brain diseases where activated microglia are an important neuropathological hallmark. In one of our current studies of autoimmune disease (multiple sclerosis), we have shown that [$^{11}$C] PK 11195-PET indeed images areas and structures affected by disease that could not be identified by magnetic resonance imaging (MRI). First results from patients with progressive dementia (currently diagnosed as Alzheimer's disease) indicate that also in these conditions microglial activation can be an important feature of the ongoing disease process which might not be obvious in the structural MRI image.

Since the level of microglial activation is closely reflective of the rate by which many CNS diseases progress, imaging microglial activation might also help to assess the efficacy of treatment (a drug efficacy study is currently undertaken scanning MS patients treated with beta-interferon). In such cases, PET-imaging of microglial activation should assist establishing a diagnosis based on cellular pathology and the evaluation of therapy.

**REACTIVE MICROGLIAL CELLS IN NEONATAL RETINAE FOLLOWING OPTIC NERVE TRANSECTION.**

Bonfanti, L.*, Rabacchi, S. , and Maffei, L..
*Scuola Normale Superiore, Pisa, * Present address Consorzio Mario Negri Sud, Laboratory of Molecular Neurobiology, S. Maria Imbaro, Chieti, Italy.*

Glial cell responses occur following a damage in the central and peripheral nervous system. In a central nerve axotomy, where regeneration is largely inhibited and a massive neuronal death is the most dramatic response to trauma, the reaction of microglial cells are traditionally associated to the recognition and engulfment of degenerating cells. When neuronal degeneration is present, microglial cells develop cytotoxic and phagocytic properties, change morphology (from ramified to round). In the present study, we have analysed the microglial response, in the developing retina, after optic nerve section in order to detect the first sign of microglial response. Neonatal rats are sacrified at various times after the lesion (5, 10, 15, 24 hours). Microglial cells are examined by using a lectin derived from *Grifonia Simplicifolia*, that labels both resting and activated microglia, and by immunocytochemistry using the antibody ED1 that specifically recognizes phagocytic microglia. Our quantitative results show that the number of lectin-labeled microglial cells is similar to normal, unlesioned retina (12,000 ±SD 1,000) until 20 hours post-lesion, while it significantly increases at 24 hours post-lesion (18,000 ±SD 4,000 $p<0.05$). Our morphological analysis with ED1 labeling reveals that the number of round cells, with few or no cytoplasmic processes, increase from 350 (±SD 100) in unlesioned retinas to 1,800 (±SD 900) at 5 hours post-lesion when pyknosis is still very limited.
In conclusion our findings show that retinal microglial cells respond to optic nerve section in two phases. The late microglial response occurs at 24hr after the lesion and it is appears at the time of massive neuronal cell death. A very early phase occurring 5 hours after the lesion at this time microglial cells change their shape from ramified to round. The early response of microglia prior to death of axotomised neurons suggests that retinal microglial cells respond to some early signal triggered in the retina by axotomy. In the light of our observations it is possible that, in the developing retina, the early response of microglial cells may be involved in critical role in the following RGCs degeneration.

**Recruitment of macrophages in the central nervous system (CNS) : influences of neurons and glial cells.**

Dobbertin, A. and Mallat, M.
*INSERM U114, Paris, France.*

Growth and reactions of microglia induce CNS infiltration and proliferation of mononuclear phagocytes stemming from hematopoïetic organs. In vitro cultures were used to investigate influences of rat or mouse CNS cells on purified adult rodent bone-marrow derived macrophages (BMM). Survival and proliferation of isolated BMM required addition of M-CSF to culture medium. A coculture system allowing exposure of BMM to soluble compounds released by CNS-derived cells, indicated that neurons from different developing brain regions produce factors which dramatically enhance mitogenic effect of M-CSF. Using saturating concentration of M-CSF (10 ng/ml of recombinant human M-CSF), the presence of neurons led to a 2-fold increase of the number of BMM within 72h. In contrast, cultured astrocytes or microglia failed to enhance M-CSF mitogenic effect. $^{3}$H-thymidine labeling of synchronized BMM indicated that the neuronal stimulation involved shortening of the G1 phasis cell cycle rather than induction of proliferation of a BMM subpopulation unresponsive to M-CSF alone. Moreover, neuron-derived factors failed to rescue BMM from death in the absence of exogenous M-CSF. Potentialisation of M-CSF mitogenic effect by neurons was also observed using cultured ameboid microglia or peritoneal macrophages instead of BMM. Molecular characterization of these neuron-derived factors is in progress. Considering that astrocytes can produce M-CSF, our results suggest a cooperation between different CNS cell lineages in promoting microglial growth or reactions.

## The effect of pH changes on ATP-induced calcium elevation in cultured microglia

Faff L. and Kettenmann H.
*Max-Delbrück-Center for Molecular Medicine, Cellular Neurosciences, Robert-Rössle-Str. 10, 13122 Berlin*

ATP is released in the CNS as a cotransmitter from neurons and as a modulatory substance from endothelial cells. Furthemore, ischemic, injured and dying cells release ATP in a large amount. As has been previously reported, microglia, the resident macrophages of the CNS respond to extracellularly applied ATP with an increase in the intracellular $Ca^{2+}$ $(Ca^{2+})_i$, due to the purinergic receptor activation [Walz et al., (1993) *J. Neurosci.*, **13,** 4403]. Because most traumatic or pathologic events in the CNS are accompanied by changes in extracellular pH ($pH_0$) it is thus likely that $pH_0$ could affect $pH_i$ in microglial cells and modulate ATP-induced elevation of the $(Ca^{2+})_i$. In this study we have investigated the effect of $pH_0$ on $pH_i$ and the effect of $pH_i$ on ATP-induced elevation of the $(Ca^{2+})_i$ in cultured microglial cells from mouse brain. The $(Ca^{2+})_i$ and the $pH_i$ were measured using the ester forms of the $Ca^{2+}$- and pH-sensitive fluorescent dyes (BCECF and Fura-2 respectively). $pH_i$ in microglial cells was strongly dependent upon $pH_0$. Decreasing $pH_0$ to 6.0 resulted in the $pH_i$ 6.6. Increasing $pH_0$ to 8.3 led to an increase in $pH_i$ to 7.8. If a buffer with low (or high) pH was replaced by a buffer at physiological $pH_0$ (7.4), $pH_i$ very rapidly returned to the previous baseline level. In the majority (80%) of microglial cells decreasing extracellular pH to 6.0 resulted in a 60% decrease of ATP-induced calcium elevation. In the remaining 20% of cells ATP-induced calcium elevation was abolished. In contrary, intracellular alkalinization ($pH_0$ 8.3) resulted in a 30% increase of ATP-induced calcium elevation. To determine whether intracellular acidification and alkalinization also affect internal stores of calcium, the experiments were repeated in $Ca^{2+}$ - free solution. In the majority of cells (70%) lowering $pH_0$ to 6.0 resulted in a 50% decrease of ATP-induced $Ca^{2+}$ elevation. In the remaining 30% of cells pH 6.0 abolished ATP-induced $Ca^{2+}$ elevation. In contrary, increasing $pH_0$ to 8.3 resulted in a 30% increase of ATP-induced calcium signal. In conclusion, changing the $pH_0$ always led to changes in $pH_i$ in microglial cells, in the same direction as the change in $pH_0$. $pH_i$ can modulate ATP-induced cellular response of microglial cells, intracellular acidification has been shown to reduce whereas intracellular alkalinization has been shown to augment ATP-induced $(Ca^{2+})_i$ elevation.

## Microglial MHC class II expression in human astrocytomas

Graeber, M.B., Tran, C.-T., Egensperger, R., Kösel, S., Bise, K., Mehraein, P.
*Institute of Neuropathology, University of Munich, Thalkirchner Str. 36, 80337 Munich, Germany*

Astrocytic gliomas are the most common primary brain tumors. Current therapies are not curative and tumor recurrence is common mainly due to the diffuse infiltrative spread of these tumors. The possible role of microglia in the immune defense against human gliomas has so far not been addressed in a systematic manner. There is strong evidence that malignant gliomas actively suppress immune defense mechanisms both systemically and locally within the tumor. The goal of our study is to elucidate to what extent activation of microglia may limit astrocytoma spread and why microglia eventually fail to do so. As a first step, we have studied the expression of key immunoregulatory molecules, i.e., MHC class II molecules and the co-stimulator B7, on microglia in human astrocytomas of different degrees of malignancy.

More than 100 astrocytomas (WHO grades I-IV) were studied using the monoclonal antibodies CR3/43 (DAKO), which is directed against the $\beta$-chain of all products of the human class II gene subregions HLA-DR, -DP, and -DQ, Ki-M1P, a macrophage/microglia marker, and the RCA-1 lectin. Computer-assisted image analysis (Optimas 5.1) was employed for evaluating the number of labeled cell profiles in different tumor areas.

In "low grade" astrocytomas, MHC class II molecules were almost exclusively found on microglia and perivascular cells. Occasional tumor cells were also immunopositive but reactive astrocytes were consistently class II negative. Interestingly, activated microglia expressed the co-stimulatory molecule B7. A higher number of class II positive tumor cells was found in anaplastic astrocytomas. In cellular areas of glioblastomas, MHC class II expression appeared to be generally reduced. Studies are underway to determine whether MHC class II positive tumor cells in high grade astrocytomas co-express B7.

In summary, our data suggest that there is a downregulation of microglial immune competence in astrocytic gliomas and that this suppression together with aberrant MHC expression by tumor cells could contribute significantly to diffuse tumor spread.

## EXTRACELLULAR MATRIX MOLECULES IN THE FACIAL NUCLEUS AFTER AXOTOMY

Hager G., Schwaiger F. W., Reddington M., Hager G. H., Streif R., Kerber G. and Kreutzberg G. W.

*Max-Planck-Institute of Psychiatry, Department of Neuromorphology, D-82152 Martinsried, Germany*

Multifunctional cell surface molecules are a component of the extracellular matrix (ECM) in the CNS. During brain development they guide migrating cells to their targets. In the mature CNS extracellular matrix molecules also promote cell adhesion and thus the stabilisation of neuronal connectivity. After a remote lesion the extracellular matrix is rearranged along with the cellular reorganisation. Microglia are thought to play a major in this process. The aim of this study is to define the role of laminins, agrins and related molecules and their receptors after axotomy of the facial nucleus. In addition we are trying to identify new ECM molecules important for the regeneration process.

Immunohistological studies show an increase in immunoreactivity of antibodies against laminins in the facial nucleus 5-7 days after axotomy. Several antibodies against the whole laminin molecule and the γ1-chain of laminin show an increased labelling on a subpopulation of astrocytes and neurons. RT-PCR reveals an increase of the γ1-chain and the β2-chain of laminin. The γ1-chain of laminin is common for most laminin isoforms whereas the β2-chain codes for s-laminin. In situ hybridisation techniques demonstrate an increase of laminin mRNA in the motoneurons of the facial nucleus after axotomy. S-laminin is thought to be the laminin isoform taking part in synaptic organisation and stabilisation. Further we have recently discovered the up-regulation of a new glycoprotein called F-84.1 on the cell surface of motoneurons. F-84.1 glycoprotein belongs to the immunoglobulin superfamily and is thought to facilitate neurite-outgrowth.

Microglia may contribute in different ways to the function of extracellular matrix molecules. The remodelling of the ECM following injury is thought to involve proteases such as the urokinase-type plasminogen activator which has recently been shown to be released from microglia. As microglia are the most motile cells of the CNS after lesion they may use laminin as a substrate for their migration. This is suggested by recent results showing several integrin receptors for laminin and other cell surface molecules on microglial cells (G. Raivich, pers. com.).

## MICROGLIAL CELLS EXPRESS INTERLEUKIN-15 AND ITS COGNATE RECEPTOR COMPLEX

U.-K. Hanisch, F. Kirchhoff, S. Lyons, C. Nolte, and H. Kettenmann

Department of Cellular Neurosciences, Max Delbrück Center (MDC) for Molecular Medicine Berlin, 13122 Berlin-Buch, Germany

We present evidence that mouse microglia in culture synthesize mRNAs for the novel cytokine, interleukin-15 (IL-15), and all the subunits composing a specific IL-15 receptor (IL-15R) system. RT-PCR results from these cultures, supported by partial sequencing, revealed the expression of IL-15 and the receptor components, IL-15Rα, IL-2Rß and IL-2Rγ, necessary to build up a heterotrimeric high-affinity IL-15R complex. RT-PCR analyses were also performed on mRNA material harvested from electrophysiologically identified single cells to support the *in vitro* data. In addition, regional and time expression patterns were investigated by RT-PCR based on brain tissues. mRNA was sampled from various regions of virtually blood-free brains prepared from buffer-perfused mice at different ages, i.e., P5, P20 and P63. Together, the results point to the presence of an IL-15/IL-15R system in the mouse brain with microglia being a source. In functional experiments, IL-15 supported the growth of microglia *in vitro* at doses of 0.1 to 10 ng/ml, indicating that the microglial IL-15R could transmit signals. The IL-15 effect was demonstrated with complementary experimental protocols estimating cell death and survival (LDH and MTT-3 assays), respectively.

These findings are highly suggestive of a role for IL-15 as an autocrine growth factor for microglial cells. Microglial IL-15 may also have effects on other neural cells throughout the brain. It is known that IL-15 and the T cell growth factor, IL-2, share the subunits, IL-2Rß and IL-2Rγ, for signaling and have overlapping immune activities. Therefore, IL-15 could be responsible *in vivo* for several CNS effects currently ascribed to IL-2. Under pathophysiological conditions, microglial IL-15 could act as a chemoattractant and stimulator for invading T cells. In turn, T cell- or astroglia-derived IL-2 could affect (activated) microglia. Together, the data illustrate a role of the IL-15/IL-15R system in the regulation of microglial physiology and communication in the CNS. Supported by the BMBF and DFG of Germany.

## Molecular changes in neurons and glia during neuronal regeneration - a search for novel genes

Hol, E.M., Schwaiger, F.-W., Schmitt, A. and Kreutzberg, G.W.
*Max-Planck-Institute for Psychiatry, Department of Neuromorphology, Martinsried, Germany.*

Peripheral nerve injury leads to extensive morphological, metabolic and molecular changes in the severed neurons and the surrounding astrocytes and microglial cells. Neuron-glia interactions and changes in gene expression in these cells take part in the program that controls successful neuronal regeneration. It is most likely that a number of so far unknown genes play an important role during neuronal regeneration. The development of the differential display PCR (dd-PCR) technique has considerably simplified the identification of regeneration associated genes. These genes can either be novel genes or genes not previously implicated in neuronal regeneration.

Changes in gene expression were analyzed in rat spinal cord 72h after sciatic nerve crush and in rat facial nucleus 72h after facial nerve axotomy. The DNA fragments obtained with dd-PCR, were cloned, sequenced and their expression pattern was screened by in situ hybridization. Up to now 63 DNA fragments are isolated and sequenced from spinal cord and facial nucleus. About 70% of these gene fragments showed no homology at all with sequences in the gene bank. Other gene fragments isolated showed a moderate to high homology with genes like stearyl CoA desaturase, S3 ribosomal protein, 12S rRNA, human pBX2 gene, chicken c-maf, hox4.4/4.5, NADH ubiquinone oxidoreductase, connexin 43, mouse elongation factor 2, human colon mucosa protein, ribosomal protein L7, homeobox protein R1a and pyruvate kinase. In situ hybridization revealed that 20% of the novel gene fragments were differentially expressed. We found neuron specific as well as glial specific genes.

Thus, we successfully identified novel regeneration associated genes by means of the dd-PCR technique.

## REGULATION OF THROMBOSPONDIN IN THE REGENERATING FACIAL MOTOR NUCLEUS.

Jones, L.L., Möller, J.C., Klein, M.A., Haas, S., Kreutzberg, G.W. and Raivich G.

*Department of Neuromorphology, Max-Planck Institute for Psychiatry, 82152 Martinsried, Germany*

Thrombospondin is a multifunctional extracellular matrix protein which plays a role in neuronal survival, migration and axonal outgrowth in the developing central nervous system.

In the current study we have examined the localization and regulation of thrombospondin-like immunoreactivity (TSPli-IR) during neuronal regeneration in the axotomized facial motor nucleus using Western blotting and light- and electron-microscopic techniques. Transection of the facial nerve led to a gradual increase in TSPli-IR in the regenerating motoneurons, peaking 4-7 days after injury (4-7DAI). In addition to regenerating neurons, axotomy also led to a biphasic upregulation on adjacent microglia: a brisk upregulation of TSPli-IR on activated microglia throughout the facial nucleus, with a maximum 2-3 DAI, and a second increase 14-21 DAI on microglial nodules surrounding degenerating motoneurons and in phagocytic microglia removing neuronal debris.

In summary, injury leads to the induction of thrombospondin on axotomized neurons and activated microglia, peaking at the time point of maximal posttraumatic microglial proliferation and later, during the phagocytosis of degenerating neurons. Since thrombospondin is a multimodal extracellular protein with a variety of cell-attachement sites, current data suggest that thrombospondin may serve as a linker between these cell-types, in the microglial adhesion to injured neurons, followed by microglial proliferation and removal of the neuronal debris.

**Controlling the response of brain microglia and intrinsic macrophages of the peripheral nerve to injury: role of transforming growth factor-ß1**

Kiefer, R., Schweitzer, T., Funa, K.*, Jung, S., Toyka, K.V. and Hartung, H.-P.
*Dept. of Neurology, Univ. of Würzburg, Würzburg, Germany, and *Ludwig Institute for Cancer Research, Uppsala, Sweden*

Microglial activation following injury and other disturbances of brain homeostasis is graded depending on the kind of stimulus and is terminated by mechanisms just evolving to be understood. The macrophage deactivating cytokine transforming growth factor-ß1 (TGF-ß1) has been shown to be expressed by activated microglial cells, suggesting an autocrine feed-back mechanism controlling microglial activity. Intrinsic endoneurial macrophages of the peripheral nerve, bearing morphological and immunophenotypic similarities with perivascular cells of the brain and possibly microglia, were also found to express low levels of TGF-ß1 mRNA already in normal rat sciatic nerve. However, during Wallerian degeneration following nerve transection or during experimental inflammation, intrinsic endoneurial macrophages are overwhelmed by a massive influx of extrinsic macrophages also expressing TGF-ß1 mRNA and protein at levels manyfold higher than intrinsic macrophages. Interestingly, these macrophages express TGF-ß1 only during active stages and the early recovery phase of experimental inflammation, with little expression in postphagocytic macrophages. Preliminary studies in human sural nerve biopsies from patients with autoimmune neuropathies suggest similar expression patterns. In the rat brain, studying experimental autoimmune encephalomyelitis, infiltrating macrophages were also found to express much higher levels of TGF-ß1 than resident activated microglia identified by their ramified morphology and appropriate immunocytochemical markers. In this model, despite the massive lesions, local microglia express considerably less TGF-ß1 than in other experimental models such as ischemic stroke, possibly due to the inhibitory effect of TGF-ß1 released from infiltrating macrophages. Extrinsic brain and nerve macrophages may utilize TGF-ß1 to control their cytotoxicity much as it has been suggested for intrinsic microglia, and may have inhibitory effects on local microglial responses. Comparing microglial responses with those of intrinsic endoneurial macrophages may provide further insight into the physiological roles and control mechanisms of these cells.

**The role of microglia in nerve cell death and astrocytic gliosis in spongiform encephalopathies (prion diseases)**

Kretzschmar H., Brown D.R., *Schmidt B., Herms J., Giese A.,
*Institut für Neuropathologie, *Abteilung Biochemie II, Universität Göttingen, Germany*

The scrapie isoform of the prion protein (PrP) induces pathological changes such as spongiform degeneration, nerve cell loss and gliosis in prion diseases (Creutzfeldt-Jakob disease (CJD), scrapie and bovine spongiform encephalopathy or BSE). A fragment of human PrP consisting of amino acids 106-126 is neurotoxic and induces gliosis *in vitro*. Using cell cultures from wild-type and PrP gene-ablated mice ($PrP^{0/0}$ mice) we have shown that the neurotoxic effect of PrP106-126 depends on the PrP genotype of the host cells and the action of microglia. PrP106-126 is only toxic for cells which express $PrP^{C}$, the cellular isoform of the prion protein. In addition, microglia are required in this process. Microglia respond to treatment with PrP106-126 by increasing their oxygen radical production. In mixed glial cultures PrP106-126 induces astroglial proliferation which is dependent on $PrP^{C}$ expression. In purified cultures of glial subtypes only microglia proliferate in response to PrP106-126. This effect is independent of PrP expression. Destruction of microglia in mixed glial cultures by L-leucine methyl ester (LLME) treatment abolishes enhanced proliferation caused by PrP 106-126. This proliferative effect can be restored by co-culturing LLME-treated astrocytes with microglia. Microglia therefore seem to mediate the effects of PrP106-126 which lead to nerve cell death by apoptosis as well as to astrocytic proliferation *in vitro*. Our findings suggest that changes in microglial behavior are a direct response to $PrP^{Sc}$, the scrapie isoform of the prion protein. Microglia seem to be the mediators of major pathological changes in cell culture systems of prion diseases. Further experiments will aim at elucidating the mechanisms of microglia activation as well as the role of microglia in pathogenesis of prion disease *in vivo*.

## MACROPHAGES AND MICROGLIA AS EFFECTOR CELLS IN ACTIVE MULTIPLE SCLEROSIS LESIONS

H. Lassmann, Institute of Neurology, University of Vienna, Austria

A central role of macrophages and/or microglia in the process of demyelination and myelin degradation has first been postulated by Babinski (1885). In actively demyelinating lesions phagocytes can be found in close contact with degenerating myelin sheaths. Myelin fragments are taken up by these cells and the immunocytochemical profile of myelin degradation products in macrophages is at present the most reliable marker for staging of lesional activity. Activation of these cells is reflected by their surface expression of histocompatibility antigens, adhesion molecules, Fc-receptors, complement receptors and LDL-receptors. Furthermore, the expression of macrophage activation antigens correlates well with lesional activity in multiple sclerosis. At least in a subpopulation of multiple sclerosis cases, degenerating myelin sheaths are dressed on their surface with immunoglobulins and complement, including the C9neo antigen. C9neo and immunoglobulin immunoreactivity is also present - together with myelin antigens - in the lysosomal degradation products in phagocytic cells. Sometimes also dying oligodendrocytes - identified by the fragmentation of their nuclear DNA - are found in the cytoplasm of phagocytes. It is yet unresolved, to what extent macrophages and microglia cells respectively contribute to myelin destruction in MS. However, using either morphological criteria or immunocytochemical markers, it is suggested that both cell types are involved. Their relative contribution may depend upon the stage of lesion formation and the subtype of the MS lesion.

References:
Ozawa et al (1994) Brain 117:1311-1322
Brück et al (1994) Ann Neurol 35:65-73
Brück et al (1995) Ann Neurol 38:788-796

The study was funded by Austrian Science Foundation, Project P 10608MED

**Leucocyte responses to bacillus Calmette-Guérin sequestered in the CNS.** Matyszak, M.K. and Perry, V.H. *Dept. Pharmacology, Oxford University, Mansfield Road, Oxford.*

Immune responses play a part in many diseases in the CNS. It is therefore imperative that the mechanisms underlying these responses are well understood. We have recently developed an experimental model to study responses to non-CNS antigens sequestered in the brain. We showed that heat-killed BCG injected into the CNS parenchyma persists for months, undetected by the immune system. Electron microscopy study revealed BCG organisms in lysosomes and lipofucsin granules in a small number of mononuclear phagocytes in the parenchyma and in the perivascular spaces. Unlike in the CNS parenchyma, BCG injected into the lateral ventricle induced an immune response in the choroid plexus which was comparable both quantitatively and qualitatively to that seen in the skin after intradermal injection of BCG.

Although a single intracerebral injection of BCG fails to stimulate immune responses, subsequent subcutaneous injection of BCG results in recognition of BCG in the brain, and the induction of chronic focal lesions. Immunohistochemical and ultrastructural studies revealed changes in the cellular composition of these lesions with time. Early lesions (up to 1 month after a subcutaneous injection of BCG) were characterised by a conspicuous infiltration of leucocytes in which macrophages and lymphocytes were the dominant populations. There was also an extensive activation of microglia within the inflammatory lesion. In the later lesions, macrophages were the predominant cell type and there were fewer small lymphocytes. Later lesions also contained numerous plasma cells, which were not present in earlier lesions. In both early and late lesions we detected numerous dendritic cells. These cells were often seen in close contact with lymphocytes.

**MICROGLIAL AND ASTROCYTE PRODUCTION OF TNF AND NITRIC OXIDE: THE IMPLICATIONS IN MS AND CNS-AIDS.**

Merrill, J.E.[o], Koka, P.*, Mitrovic, B.[o], Ignarro, L.J.[+], He, K.*, and Ding, M*.
*Departments of Neurology*and Pharmacology[+], UCLA School of Medicine, Los Angeles, CA, BERLEX Bliosciences[o], Richmond, CA.*

There is growing evidence that inducible nitric oxide synthase (iNOS) and nitric oxide (NO) are involved in the pathology of Multiple Sclerosis (MS) and the animal model of MS, Experimental Allergic Encephalomyelitis (EAE). Induction of iNOS by HIV-1 glycoproteins suggest that NO may be important in central nervous system AIDS (CNS-AIDS) as well. In an *in vitro* model of MS pathology, we demonstrated that rat microglia kill oligodendrocytes by an NO-dependent mechanism. Rat glia produce NO in response to LPS and Interferon gamma (IFNγ); while astrocytes and oligodendrocytes produce NO, microglia are the major contributors to NO production. The induction occurs rapidly with peak production by 24-48 hrs. Human fetal astrocytes and microglia produce iNOS and NO in response to the cytokines IFNγ and Interleukin 1 beta (IL1β) as well as to well defined epitopes of HIV-1 gp41 and gp120. LPS did not induce NO in human glia. Adult human glia could only be induced to produce NO after stimulation with a cocktail of cytokines and immune complexes. iNOS induction in human fetal glia is rapid (2 hrs) with mRNA levels back down to baseline within 24 hrs and the enzyme itself persistent in cells for over one week. However, in contrast to rodent glia, human glial cells do not have functional iNOS enzyme until day three after cytokine stimulation. Hence, no significant levels of NO are detected in culture supernatants before that time. Likewise, citrulline production is detected at day 3 and peaks between day 4 and 5. Evidence of cGMP and nitrotyrosine is seen only after the peak in NO production, suggesting the existence of nonfunctional enzyme in human glia. The cofactor tetrahydrobiopterin ($BH_4$) is responsible for the conformational change in iNOS which is required for functional enzyme. Addition of $BH_4$ to the human glial cultures demonstrated an earlier appearance of NO in the supernatants suggesting that this cofactor is limiting in these cells *in vitro*. In summary, human fetal astrocytes and microglia make NO in response to some but not all of the same stimuli which induce rodent glia. These data further suggest that posttranslational regulation of iNOS is different in human glia compared to rodent glia. The biochemistry of the posttranslatonal regulation of iNOS function is currently under investigation and may prove important for understanding how to therapeutically intervene in NO production in MS and CNS-AIDS.

**Multiple biological responses induced in cultured microglia by epidermal growth factor: evidence for EGF-receptor expression.**

*Nolte, C., Kirchhhoff, F. and Kettenmann, H.*

Cellular Neurosciences, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str.10, D-13125 Berlin, FRG.

Within the central nervous system epidermal growth factor (EGF) modulates a variety of neural functions[1]. Here, it is shown that EGF acts directly on cultured microglial cells. Microglial cells are rapidly activated in brain diseases and after injury, and their activation involves morphological changes, proliferation and recruitment to the lesion site by chemotactic migration. In this study the role of EGF as a motogenic and/or mitotic signal for microglial cells in vitro was investigated.

Using primary microglial cells from newborn mouse cortex in multiwell microchemotaxis assays, we demonstrate a dose-dependent effect of EGF on microglial chemotaxis. The maximal chemotactic response is achieved by 10 µg/ml EGF in the lower compartment and reaches 300% ± 35 (mean ± S.D.) of the unstimulated control. The EGF-response is about 50% as that induced by C5a (10 nM), a previously described microglial chemoattractant. Checkerboard-analysis shows that the effect of EGF is not purely chemotactic but also involves stimulation of chemokinesis. As demonstrated by BrdU-incorporation and a colorimetric cell-ELISA, EGF (0.01 - 10 ng/ml for up to 36 h) is not a mitotic signal for microglia whereas CSF-1 containing supernatant of L929 fibroblasts stimulates microglial proliferation. The stimulation of chemotaxis strongly indicates that microglia possess binding sites for EGF. This is corroborated by RT-PCR results which show the presence of EGF-R mRNA in purified microglial cultures.

Acute and chronic pathological processes within the brain stimulate the synthesis and release of immunoregulators and growth factors (including EGF) which play a major role in the brain´s response to injury[2]. EGF is secreted by activated microglia themselves in vivo[1] and might also act as an autocrine modulator of microglial cell function. Data obtained so far suggest an important role of EGF as an autocrine and/or paracrine motility factor stimulating microglial chemotaxis to direct them to the lesion site.

*References:* (1) Plata-Salaman (1991) *Peptides* **12**:653-63; (2) Benveniste (1995) Neuroglia Eds. Kettenmann and Ransom.

**SIGNALLING MOLECULES AND NEUROGLIAL ACTIVATION IN THE INJURED CENTRAL NERVOUS SYSTEM**

Raivich, G., Möller, J.C., Klein, M., Jones, L.L., Werner, A., Blüthmann,H.* and Kreutzberg, G.W.
*Dept Neuromorphology, MPI for Psychiatry, 82152 Martinsried*
**Dept Molecular Genetics, Hoffmann-LaRoche, Basel, Schweiz*

Growth factors and cytokines play a crucial role in regulating cellular activation, growth and differentiation. To understand the molecular mechanisms operating in the process of nerve regeneration, we are analysing the regulation of these signalling molecules and their contribution to posttraumatic growth and repair after injury. Previous studies have shown that injury causes massive, local and transient expression of many different growth factors in the lesioned nervous system, like NGF, TGF-ß, IL6, MCSF, GMCSF and their receptors.
Here we have focused on the effects of macrophage colony stimulating factor (MCSF) and interleukin-6 (IL6) deficiency in the axotomised facial motor nucleus using mouse strains which completely lack one of these molecules. Absence of MCSF led to an almost complete abrogation of posttraumatic microglial proliferation and a clear reduction in their activitation markers such as $\alpha_M\beta_2$-integrin, thrombospondin and the receptor for MCSF. It did not interfere with the astrocytic or neuronal response to facial nerve axotomy. Deficiency for IL6 led to a massive reduction in the activation of GFAP-positive astrocytes and moderate inhibition of microglial proliferation and activation. Surprizingly, absence of IL6 also slightly enhanced the neuronal response to injury.
In summary, current data help to define the physiological role of growth factors like MCSF and IL6 in the injured neural tissue. This combination of cytokine knockouts and nervous injury models provides a new approach to gain insight into the cellular and molecular activation cascade which regulates plasticity and regeneration in the lesioned nervous system.

**Membrane traffic and immune competence of microglia and astrocytes: a comparative analysis.**

Régnier-Vigouroux, A.[+*], Burudi, E.M.E.[+], Pereira, P.[+], Riese, S.[+]

[+]*Neurobiology, University of Heidelberg, IFN 364, 69120 Heidelberg, Germany.* [*]*Immunology Centre of Marseille-Luminy, Case 906, 13288 Marseille, France.*

The definitive and respective function of microglia and astrocytes in the initiation and/or regulation of the immune response that takes place in the brain is not yet known. The immune competence of these glial cells has been mainly evaluated with regard to their capacities to activate specific T lymphocytes and to secrete or respond to various cytokines. But we hardly know anything about some basic features of these cells despite their high relevance to immune competence of cells, such as the membrane traffic that underlies the processing of antigen and its association with MHC II class molecules. We therefore have undertaken a comparative analysis of the endocytic and exocytic pathways of primary mouse microglia with those of astrocytes and professional antigen presenting cells. In order to assess whether and to which extent the immune activation could affect these pathways, this analysis is performed using both resting and IFNγ-activated cells. We are currently investigating the endocytic/recycling pathway of these cells, the biosynthetic route and intracellular fate of MHC class II molecules as well as the intracellular traffic of antigen. We are analysing the capacity of microglia and astrocytes to generate a peptidic repertoire from a given antigen in comparison with a professionnal antigen presenting cell. In order to extent our investigation of a putative effect of the immune activation on the regulation of membrane traffic, we have recently started to analyse the pathway(s) of APP processing in both resting and immune competent cells. Results will be discussed in the context of the specific role of microglia and astrocytes in brain injury.

## ANALYSIS OF GLUCOCORTICOID AND cAMP SIGNALLING BY GENE TARGETING.

Schütz, G.
*German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany.*

To understand the role of glucocorticoid/mineralocorticoid signalling during development and in whole animal physiology, we have disrupted the mouse genes encoding the receptors for these steroids by gene targeting. Most of the mice with a disrupted glucocorticoid receptor gene die shortly after birth due to respiratory failure. The adrenal medulla is disorganized and severly reduced in size; no cells capable of adrenaline synthesis can be detected. These mice display impaired regulation of the hypothalamic-pituitary-adrenal axis. Mineralocorticoid receptor deficient mice die about ten days after birth. As a consequence of impaired water/sodium retention the renin-angiotensin-aldosterone system is dramatically turned on. Similarly, the genes for CREB, CREM and ATF1 which transduce the cAMP signal in the nucleus were mutated. Mice with a hypomorphic allele of CREB appear healthy and exhibit no impairment of growth and development. They, however, display impaired memory consolidation, an absence of microglia activation after nerve axotomy and reduced expression of the withdrawal syndrome following chronic morphine treatment. A null mutation of CREB leads to perinatal lethality due to respiratory distress resulting from severe atelectasis. Mice without a functional ATF-1 gene are viable and display no abnormal features to date. Mice lacking the CREM gene are sterile due to a blockade in spermatogenesis at the level of round spermatids indicating that signalling via CREM is an essential step in spermatogenesis. These mutations will now be intercrossed in the hope that their involvement in other processes will thus become apparent.

**Functional Significance of Microgliosis.**

Streit, W.J. *Department of Neuroscience, University of Florida Brain Institute, Gainesville, FL 32610.*

Evidence from *in vivo* and *in vitro* studies suggests that microglial cells can be both beneficial and harmful. Microglia have been shown to produce neurotrophic substances, as well as cytotoxins. It remains a great challenge for future research to ascertain when neurotrophic or neurotoxic effects are exerted by microglia *in vivo*.

Our research is focused on studying microglia in the injured rat CNS, and based on the premise that microglial activation in the post-traumatic microenvironment is a beneficial process which helps to facilitate the limited regenerative potential of CNS tissue. This hypothesis is supported by lectin histochemical studies of reactive microgliosis after facial nerve section in both adult and newborn animals, after rubrospinal tractomy, after cerebral ischemia, and after trimethyltin intoxication. In addition, developmental studies and transplantation experiments involving allografts of fetal CNS cell suspensions depleted of microglial cells provide evidence for a beneficial function of microglia possibly related to angiogenesis.

We have also examined microglial activation in the aging human brain, and in age-matched patients with and without heart disease. Our results confirm data from rodents that there is increasing microglial activation with normal aging. In addition, the brains of patients with heart disease show a higher level of microglial activation than non-heart disease control subjects. This enhanced activation of microglia is coincident with increased presence of intra-and extracellular β-amyloid. We have also found increased microglial activation in hypercholesterolemic rabbits suggesting a link between hypercholesterolemia/heart disease and accelerated aging. These findings have led to speculations about how slowly progressive inflammation of the CNS may be a fundamental mechanism in aging.

## THE ROLE OF MICROGLIAL CELLS IN THE DE- AND REGENERATING RETINA

**Solon Thanos**

*Research Laboratory, Department of Ophthalmology, University of Tübingen, School of Medicine, 72076 Tübingen, Germany.*

Axotomized retinal ganglion cells respond immediately to the lesion with increased metabolism and the attempt to regenerate their cut axons, as this can be documented both *in vivo* and *in vitro*. The mechanisms of cell-signalling to neighboring microglial cells still remain to analyse. Experimental evidence shows, however, that the signalling occurs soon after lesion, and that microglial cells become neurophagic at prethanatic stages of neurons. This feature is offered as target of pharmacological intervention. Indeed, injection of microglial blockers (MIF, IL-4, protease inhibitors) during the time of optic nerve lesion delays the process of neuronal degradation and rescues a reasonable number of ganglion cells from death and disposal. The macrophage/microglial inhibiting factor (MIF) is a tripeptide (Thr-Lys-Pro), produced by proteolytic cleavage of the IgG. Its injection into the vitreous body results in transient and dose-dependent retraction of microglia branches, but not of neuronal or astrocytic processes. As a consequence, immobilized microglial cells have a reduced ability to engulf neurons during the posttraumatic phase. The cytokine IL-4 is produced by Th-2 leukocytes and has immunosupressive properties on the microglial cells. Its injection results in deactivation of the microglia, perhaps by binding on its osluble Il-4 receptor. Protease inhibitors block initial stages of microglia activity which involve proteolytic degradation of the lesioned neurons. Combined application of the different drugs has additive effects indicating that they use different mechanisms of deactivation. Most of the axotomized cells can be rescued (60 to 70% at 14 days after axotomy) and about 50% of the cells regrow their axons under optimal conditions of microglial blockade. Viability of the rescued cells can be tested *in vivo* and *in vitro* with significant numbers of neurons to regenerate their axons. Functionality of the neurons can be tested in the grafting paradigm of a simultaneous raplacement of the optic nerve with a sciatic nerve. Reconnection of the retina with the SC results in resynaptogenesis and restoration of deficits like the discrimination of light, simple spacial patterns and eliciting of visually evoked cortical potentials. The results suggest that a principal method to manipulate the posttraumatic neuronal ability for regeneration is the suppression of neurodestructive cascades, with the microglial cell to play a major role in determining whether the cells die or not.

# Microglial and neuronal reactions to inflammation and ischemia

E. Weihe
*Institute for Anatomy and Cell Biology, University of Marburg, Robert Koch Str. 6, D-35033 Marburg, Germany*

**LOCALIZATION AND REGULATION OF RECEPTORS FOR MACROPHAGE COLONY-STIMULATING FACTOR (MCSF) IN NORMAL AND INJURED MOUSE CENTRAL NERVOUS SYSTEM**

Werner, A., Haas, S., Raivich, G., and Kreutzberg, G.W.
*Department of Neuromorphology, Max-Planck Institute for Psychiatry, 82152 Martinsried, Germany*

Macrophage colony stimulating factor (MCSF) is an acidic, 40-76 kDa glycoprotein which stimulates the survival, proliferation and terminal differentiation of cells which belong to the monocyte/macrophage lineage. In the injured central nervous system (CNS), MCSF-deficiency leads to a very marked reduction in microglial proliferation. Here we have studied the localization and regulation of the receptor for this microglial growth factor in the normal and injured mouse nervous system. We have also looked into the effect of MCSF on the glial reaction in the MCSF deficient mouse.

Immunocytochemical staining with different antibodies specific to the MCSF receptor (MCSFR) revealed differential receptor expression on microglia throughout the CNS, with very low levels on the dentate gyrus, a moderate expression in the cerebral cortex, olfactory bulb, cerebellum and brainstem, and high levels in the substantia nigra and spinal cord dorsal horn. A particularly strong expression was found in the area postrema, a CNS region which lacks the blood-brain barrier.

Expression of the MCSFR was also induced by injury. In the axotomized facial motor nucleus model, there was a very rapid, massive increase in MCSFR immunoreactivity 1 day after injury (1 DAI), preceding microglial proliferation. This MCSFR upregulation was followed by a more gradual increase in microglial cell number 2-5 DAI. In the MCSF deficient, op/op mice, we see a major reduction in the expression of MCSFR and other microglial activation markers like Thrombospondin and $\alpha_M\beta_2$-integrin.

In summary, although normal levels of MCSFR in most microglia are low, microglial activation is accompanied by a rapid and massive increase of the receptor. This early upregulation of a receptor for a microglial growth factor may prepare these macrophage-related cells to take part in the cellular response to injury in the central nervous system.

**The role of microglial cells in classical and primitive plaque formation**

Wegiel, J., Wisniewski, H.M.
*NYS Institute for Basic Research in Developmental Disabilities, Staten Island, New York*

Extracellular amyloid-ß deposition in the vascular wall and brain parenchyma are the hallmarks of Alzheimer disease. Amyloid-ß deposition is associated with several types of cells. Diffuse, nonfibrillar amyloid deposition is associated with neurons. Smooth muscle cells are engaged in fibrillar amyloid deposition in the wall of leptomeningeal and cortical arteries and veins. Production of amyloid fibrils by single microglial cells or their clusters generates primitive and classical perivascular and neuropil plaques. The proportion of classical plaques in control group is significantly higher than in AD. They constitute more than 17% of all plaques in the brain cortex of nondemented elderly persons and about 3% in the late stage of AD; however, their total number increases from 4.3 million in control group to 6.8 million in the late stage of AD. Ultrastructural three-dimensional reconstruction allows distinguishing of the stages of plaque initiation, growth, and degradation. Staging is based on the amount and the morphology of amyloid in a central core, the number of degenerated neuronal processes, and the amount of amyloid wisps and astrocytic processes isolating them. Deposition of fibrillar amyloid by microglial cell/s initiates plaque formation. The reaction on amyloid deposition is neuronal degeneration and astrocytic proliferation. The increase in the number of microglia-positive plaques parallely with microglial cell necrosis in plaques suggests that plaque formation in AD is associated with accelerated microglial cell turnover. Production of amyloid in the capillary wall by perivascular cells and formation of numerous classical plaques in the vicinity of capillaries suggests enhanced recruitment of amyloid-producing cells from monocyte-perivascular cell lineage.

**Regulation of Microglia Phagocytosis in vitro**

v. Zahn, J., Nolte, C. and Kettenmann, H..

*Max-Delbrück-Centrum for Molecular Medicine, Cellular Neurosciences, Robert-Rössle-Str. 10, 13122 Berlin, Germany*

Microglia in various disease states react with a transformation from a resting state to an activated, highly motile and phagocytic state. In acute inflammations and in tissue damage after hypoxia and trauma, phagocytic microglia remove bacteria and debris but in EAE or multiple sclerosis microglia may exacerbate the disease by the release of cytotoxic substances and uncontrolled phagocytosis. This phagocytosis might be differentially regulated by pro- and anti-inflammatory cytokines like GM-CSF, TNF-alpha and TGF-beta, which are secreted by astrocytes, activated T-cells and microglia themselves during disease states of the CNS (S.J. Hopkins and N.J. Rothwell, *TINS* **18**, 2 (1995)).

We studied the effects of these cytokines on microglial phagocytosis in an *in vitro* model using flow cytometry (J. Steinkamp, *Science* **215**, 64 (1981)). In brief, microglia from primary mouse brain cultures were preincubated with cytokines from 2 to 18 hours and then allowed to phagocytose fluorescent latex spheres (2.2 µm in diameter). The cells were then extensively washed to remove unbound latex particles and the number of ingested latex particles per 1000 cells was determined by flow cytometry. Unstimulated controls were normalized to 100%. To evaluate the amount of unspecific binding of latex beads microglial phagocytosis was blocked by preincubation with cytochalasin D. Under these conditions the signal was reduced to 19.7% of control.

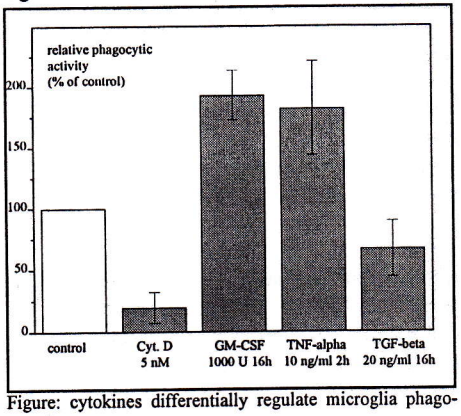



Figure: cytokines differentially regulate microglia phagocytosis

Treatment with 1000 U/ml GM-CSF for 16 hours almost doubled microglia's phagocytic activity to 193.0% of control (±20.3, n=2). A 2 hours treatment with TNF-alpha (10 ng/ml) increased microglia's phagocytic activity to 182.1% of control (±38.6, n=6). As shown in a quantitative immunoassay the same cytokines did not significantly influence the expression level of CR3 on microglial cells. TGF-beta (20 ng/ml for 16 hours) reduced microglia's phagocytosis to 66.6% of control (±22.6, n=6, $p<0.05$) (see figure).

Using a relatively simple and convenient in vitro assay we demonstrate phagocytosis enhancing and suppressing effects of 3 different cytokines which might also in vivo play an important role in modulating microglial activation and thereby keeping the balance between the protective, defensive and destructive/chronic-inflammatory properties of microglia.

Richard Banati
Hammersmith Hospital
Cyclotron Unit
Ducane Road
London W12 OHS
UK

Franz Birke
Boehringer Ingelheim
ZNS-Pharmakologie
Binger Strasse 173
55216 Ingelheim/Rhein
Germany

Lidia Bonfanti
Scuola Normale Superiore
Classe di Scienze
Piazza dei Cavalieri
I-56126 Pisa
Italy

Alexandre Dobbertin
Chaire de Neuropharmacologie
INSERM U.114, Collège de France
11, pl. Marcelin Berthelot
F-75231 Paris Cedex 5
France

Henning Draheim
Boehringer Ingelheim
ZNS-Pharmakologie
Binger Strasse 173
55216 Ingelheim/Rhein
Germany

Lidia Faff
Zelluläre Neurowissenschaften
Max-Delbrück-Centrum für
Molekulare Medizin
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany

Ralf Gold
Julius-Maximilian-Universität
Neurologische Klinik
Josef-Schneider-Str.
97080 Würzburg
Germany

Manuel Graeber
Universität München
Thalkirchnerstr. 36
80337 München
Germany

Uwe Hanisch
Zelluläre Neurowissenschaften
Max-Delbrück-Centrum für
Molekulare Medizin
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany

Gerhard Hager
MPI für Psychiatrie
Neuromorphologie
Am Klopferspitz 18a
82152 Martinsried
Germany

Elly Hol
MPI für Psychiatrie
Neuromorphologie
Am Klopferspitz 18a
82152 Martinsried
Germany

Leonard Jones
MPI für Psychiatrie
Neuromorphologie
Am Klopferspitz 18a
82152 Martinsried
Germany

Helmut Kettenmann
Zelluläre Neurowissenschaften
Max-Delbrück-Centrum für
Molekulare Medizin
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany

Reinhard Kiefer
Neurologische Universitätsklinik
Josef-Schneider-Str. 11
97080 Würzburg
Germany

H. A. Kretzschmar
Institut für Neuropathologie
Universität Göttingen
Robert-Koch-Str. 40
D-37075 Göttingen
Germany

Georg W. Kreutzberg
MPI für Psychiatrie
Neuromorphologie
Am Klopferspitz 18 A
D-82152 Martinsried
Germany

Hans Lassmann
Neurologisches Institut
Universität Wien
Schwarzspaniergasse 17
A-1090 Wien
Austria

M. K. Matyszak
University Department of
Pharmacology
Mansfield Road
Oxford OX1 3QT
UK

Jean Merrill
BERLEX Biosciences
Department of Immunology
P.O. Bo 4099
Richmond CA 94804-0099
USA

Christiane Nolte
Zelluläre Neurowissenschaften
Max-Delbrück-Centrum für
Molekulare Medizin
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany

Bruno Oesch
Universität Zürich
Institut für Hirnforschung
August-Forel-Str. 1
CH-8029 Zürich
Schweiz

Gennadij Raivich
MPI für Psychiatrie
Neuromorphologie
Am Klopferspitz 18 a
82152 Martinsried
Germany

Anne Régnier-Vigouroux
Universität Heidelberg
Institut für Neurobiologie
Im Neuenheimer Feld 364
69120 Heidelberg
Germany

Franz-Josef Schneider
Boehringer Ingelheim KG
Biochemische Forschung
Binger Straße
D-55216 Ingelheim
Germany

Jutta Schnitzer
Zelluläre Neurowissenschaften
Max-Delbrück-Centrum für
Molekulare Medizin
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany

Günter Schütz
DKFZ
Im Neuenheimer Feld 280
69120 Heidelberg
Germany

Wolfgang J. Streit
College of Medicine
Department of Neuroscience
University of Florida
Gainsville FL 32610-0244
USA

Solon Thanos
Universität Tübingen
Augenklinik
Schleichstr. 12
72076 Tübingen
Germany

E. Weihe
Institut für Anatomie und
Zellbiologie
Universität Marburg
Robert Koch Str. 6
D-35033 Marburg
Germany

Alexander Werner
MPI für Psychiatrie
Neuromorphologie
Am Klopferspitz 18a
82152 Martinsried
Germany

Thomas Weiser
Boehringer Ingelheim
ZNS-Pharmakologie
Binger Strasse 173
55216 Ingelheim/Rhein
Germany

Henryk M. Wisniewski
Department of Pathology and
Neurobiology
New York State Institute for Basic
Res. Develop. Disabilities
1050 Forest Hill Road
Staten Island NY 10314
USA

Joachim von Zahn
Zelluläre Neurowissenschaften
Max-Delbrück-Centrum für
Molekulare Medizin
Robert-Rössle-Str. 10
D-13122 Berlin-Buch
Germany

To arrive at Castle Ringberg we recommend to come from Munich Main Station to Tegernsee by train/bus. Get of at the station „Tegernsee" and take a taxi to Castle Ringberg. The taxi fare amounts to approx. DM 35,-- to DM 40,-- and takes about 20 minutes.

Please find in the following all direct train/bus connections from Munich Main Station to Tegernsee on Wednesday, September 11, 1996:

| **Departure Munich** | **Arrival Tegernsee** |
|---|---|
| 08.53 a.m. | 10.01 a.m. |
| 10.54 a.m. | 12.01 p.m. |
| 13.30 p.m. | 14.35 p.m. |
| 15.26 p.m. | 16.33 p.m. |
| 16.28 p.m. | 17.34 p.m. |
| 17.28 p.m. | 18.36 p.m. |
| 20.55 p.m. (bus) | 22.20 p.m. (bus) |

And here are the connections back from Tegernsee to Munich on Saturday 14, 1996:

| **Departure Tegernsee** | **Arrival Munich** |
|---|---|
| 07.20 a.m. | 08.25 a.m. |
| 09.20 a.m. | 10.26 a.m. |
| 11.20 a.m. | 12.26 p.m |
| 13.20 p.m. | 14.26 p.m |
| 15.21 p.m | 16.26 p.m |
| 17.21 p.m | 18.27 p.m |
| 19.21 p.m | 20.26 p.m |
| 20.05 p.m (bus) | 21.40 p.m (bus) |

**Title Photograph:** „Abräumzellen verschiedenen Alters"
(Different stages of removing cells)
Ludwig Merzbacher (1910): Table I
Untersuchungen über die Morphologie und Biologie der Abräumzellen im Zentralnervensystem.
In. Franz Nissl & Alois Alzheimer (eds.)
*Histologische und histopathologische Arbeiten über die Grosshirnrinde*. Gustav Fischer, Jena.

**Content and Layout**: Meino Gibson