NEUROPROTECTIVE STRATEGIES FOR MULTIPLE SCLEROSIS

EU – INTEGRATED PROJECT

PUBLISHABLE FINAL REPORT

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Project Coordinator: Francesca Aloisi

Project Coordinator organization: Istituto Superiore di Sanità, Italy

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NEUROPROMISE CONSORTIUM

'Neuroprotective Strategies for Multiple Sclerosis' – NeuroproMiSe - was established in 2005 as an Integrated Project under the Life Sciences, Genomics and Biotechnology for Health Thematic Priority Area of the 6th European Framework Programme. The Consortium activities started on November 1, 2005 and the activities ended on October 31, 2010.

Dr Francesca Aloisi, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, served as Project Coordinator, Dr. Harald Neumann, University of Bonn, as Project Co-coodinator, and Mrs Estella Sansonetti, Istituto Superiore di Sanità, as Project Manager. The Coordinator and Co-coordinator have been supported by a Steering Committee including: Dr. Tomas Olsson, Dr. Rikard Holmdahl, Dr. Hans Lassmann, Dr. Kenneth Smith, Dr. Lesley Probert, and Dr. Giovanni Cozzone, to overview the progress of the project tasks and provide solutions when issues have arisen.

The purpose of the Consortium was to investigate in depth the genetic and mechanistic pathways involved in multiple sclerosis (MS), the most common chronic inflammatory disease of the central nervous system, and exploit this knowledge for the development of better therapeutic agents. With its 19 academic and 3 industrial partners, the project combined expertise in the fields of neurodegenerative diseases, human and animal genetics, molecular and cellular neuroscience, neuropathology, neuroimmunology, electrophysiology, proteomics, genomics, in vivo disease models, biotechnology of recombinant proteins, antibody production and nanotechnologies.

The Consortium has been assisted throughout its duration by an experienced Advisory Board: Prof. Alastair Compston (Department of Clinical Neurosciences, University Neurology Unit, Addenbrooke's Hospital, Cambridge, UK), Prof. Hartmut Wekerle (Max-Planck Insitute for Neurobiology, Martinsried, Germany; until 2007), Dr. Chris Linington (Glasgow Biomedical Research Centre, University of Glasgow, Scotland, UK) and Prof. Vijay Kuchroo (Center for Neurologic diseases, Brigham and Women's Hospital Boston, MA, USA), four world leading scientists in the fields of human genetics, T-cell mediated autoimmunity, antibody-mediated autoimmunity and T-cell immunology, respectively. The Advisory Board members have reviewed the annual scientific progress of the NeuroproMiSe project and provided feedback on tasks and achievements that derived from its 4 Subprojects and 20 Workpackages.

NEUROPROMISE PARTNERS

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PROJECT OBJECTIVES

The mission of NeuroproMiSe was to advance knowledge on the aetiology and pathogenesis of multiple sclerosis (MS), the most common chronic and disabling disease of the central nervous system (CNS), and develop novel therapeutic strategies to halt neurodegeneration and disease progression. The main project aims were to:

i) identify MS genetic risk factors;

ii) shed light on the mechanisms underlying immune dysregulation and neurodegeneration in MS;

iii) identify and test in preclinical models therapeutic compounds that target pathways involved in immunoregulation and neuroprotection.

PROJECT RESULTS

The organization of NeuroproMiSe into four scientific subprojects (Identification, Mechanisms, Validation, Horizontal Integration) and one Training subproject has promoted the successful development and integration of the planned research activities during the whole project duration. The knowledge and tools generated in the 5-year time period have allowed the field to:

i) gain a better understanding of genes, gene-environment interactions and pathogenetic mechanisms involved in MS development;

ii) improve knowledge of immunoregulatory, neurodegenerative and neuroprotective pathways and identify new targets for pharmacological intervention;

III) develop anti-inflammatory and neuroprotective compounds with potential therapeutic efficacy in a clinical setting (Subproject Validation).

IDENTIFICATION OF NEW GENES INFLUENCING THE SUSCEPTIBILITY TO MULTIPLE SCLEROSIS

The results of the genetic studies carried out in the 'Subproject Identification' are one of the most relevant deliverables of NeuroproMiSe. The comparative genetic approach in rodent platforms and MS patient-control cohorts together with validation studies of MS risk genes identified in genome-wide association analyses has greatly contributed to widen our understanding of the contribution of genes outside the major histocompatibility complex (MHC) locus (the main known determinant of MS genetic susceptibility) to disease development. Despite the low impact of individual non-MHC genes in determining MS risk, a better understanding of the pathways they are involved in is starting to provide new insights into the complex mechanisms involved in MS pathogenesis.

MICE AND RAT GENETIC STUDIES. The unique platform of mouse and rat congenic strains and heterogeneous stocks established at Lund University and Karolinska Institut has led to the identification of several genetic loci and to positional cloning of a number of genes that control autoimmunity, experimental MS-like disease and neurodegeneration. These include genes involved in the regulation of innate and adaptive immunity, like *ncf-1*, *vav-1*, *CIITA, Cd200, IL4R, IL21R*, *IL22RA* and chemokine encoding genes (*ccl1, ccl2 and ccl13*) as well as non immune genes like *Slc38a1* (encoding for an amino acid transporter) and *RGMA* (implicated in axon regeneration and growth). Most importantly, it has been demonstrated that variants of some of these genes, like *vav-1*, *IL22RA*, *IL21R* and the chemokine ligand cluster genes, are significantly associated with MS, thus confirming the validity of the comparative approach for the identification of genes influencing the susceptivbility to MS. The functional role of Ncf1 and Vav1 in the immune network and the molecular mechanisms underlying the effects of allelic variants of these genes on the development MS-like disease have been investigated in more detail in appropriate animal models. It was found that one variant of Vav1 promotes proinflammatory cytokine production and severe experimental autoimmune encephalomyelitis (EAE), while another variant (Arg63Trp polymorphism) is associated with an increased proportion and higher absolute numbers of T regulatory cells in thymus and peripheral lymphoid organs resulting in milder EAE clinical course. CD4+ regulatory T cells play a pivotal role in

maintaining peripheral tolerance by inhibiting the expansion and function of pathogenic conventional T cells. It was also found that the Arg63Trp polymorphism is responsible for Vav1 constitutive activation, as revealed by its tyrosine hyperphosphorylation and increased guanine nucleotide exchange factor activity. Moreover, this polymorphism induces a marked reduction in Vav1 protein levels, associated with a reduction of calcium mobilization after TCR engagement (Colacios et al., manuscript under revision). Together, these data reveal a key role for Vav1-dependent T cell antigen receptor signaling in the natural development of regulatory T cells and suggests that genetic or acquired alterations in Vav1-dependent signaling could impact on the susceptibility to immune-mediated diseases.

More general conclusions that can be drawn from the genetic studies performed in rodents are that: 1) F2 quantitative trait loci (QTLs) as a rule contain more than one QTLs, implying that the initial QTLs are built up by the effect of many genes; 2) many genes are shared between different organ specific inflammatory diseases such as experimental arthritis and EAE (e.g., ncf1 and a c-type lectin cluster); 3) gene-gene interactions are common; 4) some QTLs are detected in different strain combinations while others are unique for a given strain; and 5) the influence of each QTL is modest and the total number of QTLs detected in rodent EAE models is around 50. It is likely that all these features are also involved in the control of MS and have to be considered as the number of known MS risk genes increases.

Key publications

Olsson T, Jagodic M, Piehl F, Wallström E. Genetics of autoimmune neuroinflammation. 2006. Current Opinion in Immunology 18:1-7.

Johannesson M, Hultqvist M, Holmdahl R. Genetics of autoimmune diseases: a multistep process. Curr Top Microbiol Immunol. 2006;305:259-76. Review.

Swanberg M, Duvefelt K, Diez M, Hillert J, Olsson T, Piehl F, Lidman O. Genetically determined susceptibility to neurodegeneration is associated with expression of inflammatory genes. Neurobiol. Dis. 2006;24:67-88.

Gelderman KA, Hultqvist M, Pizzolla A, Zhao M, Nandakumar KS, Mattsson R, Holmdahl R. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. J Clin Invest. 2007;117:3020-8.

Ahlqvist E, Bockermann R, Holmdahl R. Fragmentation of two quantitative trait loci controlling collagen-induced arthritis reveals a new set of interacting subloci. J Immunol. 2007;178:3084-90.

Marta M, Stridh P, Becanovic K, Gillett A, Ockinger J, Lorentzen JC, Jagodic M, Olsson T. Multiple loci comprising immune-related genes regulate experimental neuroinflammation. Genes Immun. 2010;11:21-36.

Ahlqvist, E., M. Hultqvist, and R. Holmdahl. 2009. The value of animal models in predicting genetic susceptibility to complex diseases such as rheumatoid arthritis. Arthritis Res Ther. 2009;11:226. Öckinger J, Stridh P , Beyen A, Lundmark F, Seddighzadeh M, Padyukov L, Hillert J, Kockum I, Jagodic M*, Olsson T*. Genetic variants of CC chemokine genes in experimental autoimmune encephalomyelitis, multiple sclerosis and rheumatoid arthritis. Genes Immun. 2010;1:142-54. *equal contribution.

Jagodic M, Colacios C, Nohra R, Dejean A, Beyeen AD, Casemayou A, Lamouroux L, Sjoholm L, Bernard I, Lagrange D, Dahlman I, Lundmark F, Seddighzadeh M, Klareskog L, Alfredsson L, Padyukov L, Khademi M, Hillert J, Clanet M, Edan G, Fontaine B, Fournie GJ, Saoudi A, Kockum I, Olsson T. Variants of Vav1 affects TNF and inflammatory diseases. Science Translational Medicine Science Translational Medicine. 2009;1(10):10ra21.

Beyeen AD, Zeitelhofer-Adzemovic M, Öckinger J, Stridh P, Becanovic K, Lassmann H, Harris R, Hillert J, Alfredsson L, Kockum I, Jagodic M, Olsson T. IL22RA2 associates with multiple sclerosis and macrophage effector mechanisms in experimental neuroinflammation. J Immunol. 2010;185:6883-6890.

Harnesk K, Swanberg M, Öckinger J, Diez M, Lidman O, Lobell A, Wallström E, Olsson T, Piehl F. Vra4 congenic rats with allelic differences in the class II transactivator gene display altered susceptibility to experimental autoimmune encephalomyelitis. J Immunol 2008;180:3289-3296.

Huberle, A., Beyeen, A.D., Öckinger, J., Ayrua, M., Jagodic, M., de Graaf, K.L., Fissolo, N., Marta, M., Olofsson, P., Hultqvist, M., Holmdahl, R., Olsson, T. and R. Weissert. Advanced intercross line mapping suggests that Ncf1 regulates severity in an animal model of Guillain-Barré syndrome, J Immunol 2009;182:4432-4438.

Gillett A, Marta M, Jin Tao, Tuncel J, Leclerc P, Nohra R, Lange S, Holmdahl R, Olsson T, Harris RA, Jagodic M. TNF production in macrophages is genetically determined and regulates inflammatory disease in rats. J Immunol. 2010;185:442-50.

Stridh PM, Hedreul MT, Beyeen AD, Adzemovic M, Gillet A, Öckinger J, Marta M, Lassmann H, Becanovic K, Jagodic M, Olsson T. Fine mapping resolves Eae23 into two QTLs and implicates ZEB1 as a candidate gene regulating experimental neuroinflammation. PloS One 2010; e 12716.

GENETIC STUDIES IN HUMANIZED MICE. To address the issue of genetic interactions in MS development Lars Fugger's group has combined genetic studies in humans with functional studies in humanized mice to demonstrate a functional epistatic interaction between the two MS-associated HLA-DR alleles, -DR2b and -DR2a which are in almost complete linkage disequilibrium. This study showed that the DR2a allele modifies the T-cell response activated by the DR2b allele through activation-induced cell death. This functional epistasis is associated with a milder form of MS-like disease, which resembles relapsing-remitting MS. Fugger's group also investigated the MHC class I region. Their data demonstrate a role for MHC class I-restricted CD8+ T-cells in MS-like disease induction in humanized mice, a functional interaction between alleles at MHC class I and class II loci which leads to disease progression, and a functional interaction between MHC class I alleles that results in disease protection.

Key publications:

Gregersen, J. W., Kranc, K., Ke, X., Svendsen, P., Madsen, L., Randrup Thomsen A., Cardon, L. Bell, J. and Fugger L. Functional epistasis on a common MHC haplotype associated with multiple sclerosis. Nature 2006;443:574-577.

Friese M., Jakobsen, K., Friis, L., Etzensperger, R., Craner, M., McMahon, R. M., Torp Jensen,L., Huygelen, V., Jones, E. Y., Bell, J.I. and Fugger, L. Opposing effects of HLA class I molecules in tuning autoreactive CD8+ T-cells in multiple sclerosis. Nature Medicine 2008;14:1227-1235.

HUMAN GENETIC STUDIES. The discovery made in 2007 by Jan Hillert, Tomas Olsson and colleagues at Karolinska Institute that an allelic variant of the *IL7Ralpha* gene is associated with a higher risk of MS in the Swedish population was the first demonstration of a genetic influence outside the MHC locus, a finding that has been confirmed in two independent studies by other international groups and in the Finnish MS population by Leena Peltonen's group. Because the IL7/IL7R pathway is involved in the regulation of T- and B-cell growth and of T-cell memory, this finding represents an important step toward the comprehension of the mechanisms underlying immune dysregulation in MS. More recently, it was also found that the human homologue of rat Vav1 associates with both MS and rheumatoid arthritis, and that Vav1 expression correlates with expression of proinflammatory cytokines in peripheral blood and cerebrospinal fluid cells of MS patients. Some of the other mouse or rat genes positioned, like IL22RA, IL21R and the chemokine ligand cluster, also display associations to human MS.

The involvement of the NeuroproMiSe partners in the International Multiple Sclerosis Genetics Consortium (IMSGC) has allowed them to include DNA material from the Swedish and Finnish MS patient and control populations in a genome wide association study (GWAS) of a very large MS case-control cohort. Within the IMSGC study the association with IL2R and IL7R has been refined and 7 genes associated with type 1 diabetes were analyzed; allelic variants of three of these genes, *C-type lectin domain family 16* (also known as *CLEC16A*), *CD226* (a glycoprotein epxressed on some immune cell types and involved in cellular adhesion) and *SH2B adapter protein 3* (*SH2B3*), a molecule involved in T-cell receptor activation signalling, were found to associate strongly with MS suggesting that different autoimmune diseases may share common pathogenic mechanisms. The effects of other gene variants identified in the IMSGC GWAS, like *IL2Ralpha, C7, CD6, TNFRSF1A, IRF8,* and *TYK2,* have been replicated in the Swedish and Finnish MS cohorts. A new MS-associated gene, *STAT3,* that encodes a transcription factor involved in multiple pathways and functions, including the Jak-STAT pathway, neuron axonal guidance, apoptosis, activation of immune responses and Th17 cell differentiation, has been identified by Peltonen's group in an independent GWAS of 68 distantly related cases and 136 controls from a high-risk internal isolate of Finland. The protective haplotype for MS in STAT3 is a risk allele for Crohn disease, implying that STAT3 represents a shared risk locus for at least two autoimmune diseases.

Most of the currently validated (IL2RA, IL7R, CD58, CLEC16A, IRF8, TNFRSF1A, TYK2) and suggested (C7 [MIM *217070], CD6 [MIM *186720], IL12A [MIM *161560], OLIG3 [MIM *609323]–TNFAIP3 [MIM *191163], PTGER4 [MIM *601586], RGS1 [MIM *600323] non-HLA MS susceptibility loci have known functions in the immune system and particularly in T cell function. Although the independent contribution of these non-MHC genes to MS risk is modest, their combined effect might be larger, and a large-scale international study is required to estimate their combined effect toward MS predisposition. The Olsson's and Peltonen's groups are participating in the upcoming GWAS of the IMSGC on 10.000 MS cases; the data will be published soon and will reveal a total of more that 50 unequivocal MS risk genes. Taken together with the well established, major role of MHC class II alleles in determining MS risk, the increasing amount of data on the contribution of non-MHC genes and variants to MS is an essential step to perform in the near future more advanced analyses to integrate genetic information with functional data and address the role of gene-gene and gene-environment interactions in MS.

Key publications:

Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallström E, Khademi M, Oturai A, Ryder LP, Saarela J, Harbo HF, Celius EG, Salter H, Olsson T, Hillert J. (2007) Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet. 39(9):1108-13.Fugger L, International Multiple Sclerosis Genetics Consortium (IMSGC) Refining genetic associations in multiple sclerosis. Lancet Neurol. 2008;7:567-9.

International Multiple Sclerosis Genetics Consortium (IMSGC, numerous contributors, among those Tomas Olsson). The expanding genetic overlap between multiple sclerosis and type 1 diabetes. Genes Immun. 2009;10:11-4.

De Jager PL, Baecher-Allan C, Maier LM, Arthur AT, Ottoboni L, Barcellos L, McCauley JL, Sawcer S, Goris A, Saarela J, Yelensky R, Price A, Leppa V, Patterson N, de Bakker PI, Tran D, Aubin C, Pobywajlo S, Rossin E, Hu X, Ashley CW, Choy E, Rioux JD, Pericak-Vance MA, Ivinson A, Booth DR, Stewart GJ, Palotie A, Peltonen L, Dubois B, Haines JL, Weiner HL, Compston A, Hauser SL, Daly MJ, Reich D, Oksenberg JR, Hafler DA. The role of the CD58 locus in multiple sclerosis, Proc Natl Acad Sci U S A. 2009;106:5264-9.

Fugger L, Friese MA, Bell JI. From genes to function: the next challenge to understanding multiple sclerosis. Nat Rev Immunol. 2009;9:408-417.

Kallio S, Jakkula E, Purcell S, Suvela M, Koivisto K, Tienari P, Elovaara I, Pirttilä T, Reunanen M, Bronnikov D, Viander M, Hillert J, Lundmark F, Harbo H, Lorentzen Å, De Jager P, Daly MJ, Hafler D, Palotie A, Peltonen L, Saarela J. Use of a genetic isolate to identify rare disease variants: C7 on 5p associated with MS. Hum Mol Genet. 2009;18:1670-1683.

Mero IL, Lorentzen AR, Ban M, Smestad C, Celius EG, Aarseth JH, Myhr KM, Link J, Hillert J, Olsson T, Kockum I, Masterman T, Oturai AB, Søndergaard HB, Sellebjerg F, Saarela J, Kemppinen A, Elovaara I, Spurkland A, Dudbridge F, Lie BA, Harbo HF. A rare variant of the TYK2 gene is confirmed to be associated with multiple sclerosis. Eur J Hum Genet. 2010;18:502-4.

International Multiple Sclerosis Genetics Consortium (IMSGC) (Olsson T among around 65 authors). IL12A, MPOSPH9/CDK2AP1 and RGS1 are multiple sclerosis susceptibility loci. Genes and Immunity, 2010;11: 397-405.

Jakkula E, Leppä V, Sulonen AM, Varilo T, Kallio S, Kemppinen A, Purcell S, Koivisto K, Tienari P, Sumelahti ML, Elovaara I, Pirttilä T, Reunanen M, Aromaa A, Oturai AB, Søndergaard HB, Harbo HF, Mero IL, Gabriel SB, Mirel DB, Hauser SL, Kappos L, Polman C, De Jager PL, Hafler DA, Daly MJ, Palotie A, Saarela J, Peltonen L. Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. Am J Hum Genet. 2010;86:285-91.

STUDIES OF GENE-ENVIRONMENT INTERACTIONS. Over the last few years there has been an increasing attention to the influence of environmental factors and life-style habits on MS development. EBV infection, sunlight exposure, Vitamin D levels and cigarette smoking have been confirmed as risk factors for the disease. This knowledge has prompted Tomas Olsson and colleagues at Karolinska Institute to perform a large scale epidemiological study aimed at understanding whether and how these environmental factors interact with the host genetic background to influence the risk of developing MS. The study, which involved a large Swedish case-control cohort, focused on EBV, smoking and sunlight exposure/vitamin D. It was shown that smoking, but not an oral moisture snuff, increases the risk for MS (OR=1.6). If stratified for the most important MS genes, HLA-A*02 (protective) and HLA-DRB1*15:01 (detrimental), a strong gene environment effect appeared with a 20-fold increased risk for smokers having both genetic risk factors (that is lack of HLA-A*02 and presence of HLA-DRB1*15:01). This finding leads to new testable hypothesis; for example, how lung irritations (perhaps not only smoking) could trigger an (auto)immune response ensuing in immune attack against the CNS as in MS.

Olsson's group also studied gene-environment interactions with regard to EBV, confirming that clinical infectious mononucleosis doubles the risk for MS, and establishing that there is an interaction with the HLA DRB1*15:01 in particular in relation to EBV serological parameters (e.g. anti-EBNA1 antibody titer). This would be consistent with the hypothesis that the HLA control of MS could be in part related to the way the immune system controls EBV infection (Sundqvist et al, manuscript in preparation). Olsson group also confirmed an increased risk for MS with low sunlight exposure habits and low Vitamin D levels; in this case, however, there was no interaction with HLA genes (Bäärnhielm et al, submitted).

Key publications:

Hedström A, Bäärnhielm M, Wallström E, Hillert J, Olsson T, Alfredsson L. Tobacco smoking, but not Nicotine increases the risk of Multiple Sclerosis. Neurology 2009;73:696-701.

Hedström AK, Sundqvist E, Bäärnhielm M, Hillert J, Kockum I, Olsson T, Alfredsson L .Smoking interacts with two HLA complex genes to dramatically increase the risk for multiple sclerosis. Brain, in press.

NEW INSIGHTS INTO NEURODEGENERATIVE MECHANISMS

It is now widely accepted that axon injury and degeneration is a significant component of MS pathology and that loss of axons underpins the irreversible progression of the neurological deficits. Knowledge of the molecular mechanisms underlying axon degeneration is therefore critical to design rational neuroprotective therapies for MS. Within NeuroproMiSe transcriptomics and proteomics approaches have been used to identify the early molecular changes occurring in axonal projections of defined neuronal populations as well as in whole brain tissue in different experimental models of neuroinflammation and neurodegeneration. Patterns of transcripts and proteins identified using these approaches were subjected to extensive bioinformatic analysis and validated using appropriate techniques. Furthermore, specific mechanisms of inflammatory neurodegeneration were investigated in post-mortem brain tissue from MS patients and in experimental models.

RESULTS OF THE PROTEOMICS ANALYSIS OF INJURED OPTIC NERVE AXONS IN A RAT MODEL OF WALLERIAN DEGENERATION: A novel method was developed using polymer chemistry to devise a poly-HEMA filter that would enrich for axoplasm proteins. Hugh Perry's group studied the protein changes in axoplasm enriched preparations of optic nerve fibres undergoing Wallerian degeneration after injury using an ITRAQ proteomics approach. It was found that some of earliest changes were associated with proteins regulating the dynamics of the actin cytoskeleton. In degenerating axons there was evidence of a shift towards a polymerized form of actin, F-actin, associated with the phosphorylation of cofilin (p-cofilin). Phosphorylation of cofilin inactivates cofilin and reduces its ability to depolymerise actin. Further in vitro studies showed that the blood components lysophosphatidic acid (LPA) and thrombin activate Rho in axons leading to the phosphorylation of cofilin which in turn leads to formation of enhanced F-actin foci regularly spaced along the tau positive axons. Shortly after the formation of the F-actin foci the processes degenerate. These data indicate that the actin cytoskeleton is a specific point of regulation following axonal injury, that the initiation of an actin stabilisation response is a protective response, and that blood components can modify the actin cytoskeleton suggesting that blood-brain disruption caused by persistent inflammation in the MS brain directly contributes to axon transection and degeneration.

Key publications:

Broom LJ, Perry VH (2010) Axon Degeneration: Mechanisms and Consequences "New Aspect of Axonal Structure and Function" Editors Dirk Feldmeyer and Joachin H R Lubke.

RESULTS OF THE TRANSCRIPTOMICS ANALYSIS OF SPECIFIC NEURONS AND WHITE MATTER TRACTS IN MICE WITH MUTATIONS IN MYELIN GENES (CNP1 AND PLP1 NULL MICE). Because demyelination is one of the pathological hallmarks of MS, myelin mutant mice were used by Klaus Nave's group to elucidate neuronal responses triggered by the loss of major myelin proteins. Cnp1 and Plp1 null mice exhibit no sign of dysmyelination but develop axonal swellings of myelinated neurons leading to axonal degeneration in adult animals. A new technical platform was developed which allowed the group to isolate defined neuronal populations and projections from the brains of mutant and control mice using fluorescence-directed laser microdissection and to analyze their gene expression profile with high

reproducibility, starting with as few as 100 singly isolated neurons. This approach allowed them to perform a global transcriptome analysis in a maximally unbiased approach, focussing on genetically labelled neurons and myelin from mutant mice with progressive neurodegeneration and other MS-like models. Differential expression of a large number of genes was detected during the process of neurodegeneration and a global picture of deregulated genes and pathways was generated using bioinformatic analysis. Both constitutive and transient changes in the expression of functionally grouped genes in cortical neurons were detected over time, key findings were validated by qRT-PCR and western blotting and systems-level functional validation strategies were developed for candidate genes. Molecular mechanisms that may underlie axonal degeneration and those that may contribute to neuronal survival have been proposed. This information now provides the basis for follow-up pre-clinical trials in MS-like disease models to finally identify novel targets and eventually substances that protect against axonal damage and neuronal death.

Key publications:

Rossner, M.J., Hirrlinger, J., Wichert, S.P., Boehm, C., Newrzella, D., Hiemisch, H., Eisenhardt, G., Stuenkel, C., von Ahsen, O., and Nave, K.-A. (2006) Global Transcriptome Analysis of Genetically Identified Neurons in the Adult Cortex. J. Neurosci 26, 9956-9966.

Nave KA, Myelination and the trophic support of long axons. Nat.Rev.Neurosci. 2010;11:275-283.

Botvinnik A, Wichert SP, Fischer TM, Rossner M. Integrated analysis of receptor activation and downstream signaling with EXTassays. Nature Methods. 2010;7:74-80.

RESULTS OF THE TRANSCRIPTOMICS ANALYSIS OF MOUSE BRAIN FROM DIFFERENT MODELS OF INFLAMMATORY NEURODEGENERATION. Bioinformatics tools have been applied by Lesley Probert's group to organize genome-wide cDNA microarray data obtained from mouse brain from different models of inflammatory neurodegeneration representing MS, Alzheimer's disease and stroke. The goal was to identify genes and molecular pathways involved in each individual disease, as well as to link commonly expressed genes into pathways that are likely to represent endogenous brain defense and repair mechanisms. Integration of the gene expression data from the different models representing stroke, MS and Alzheimer's disease revealed numerous genes and several pathways that are common between pathologies and are likely to represent common neurodegenerative (e.g. TRPM7) and brain defense/repair (e.g. oestrogen and TGF-β signaling pathways) mechanisms. Likewise disease-specific gene profiles were also identified. By comparing the genes differentially expressed in each independent mouse pathology with those published for the corresponding human pathologies this approach was also validated for gene discovery and the relevance of mouse models for the study of human disease (Tseveleki et al 2010).

Using the information from the microarray study a mini-panel of genes was developed whose expression changes would sensitively monitor the development of neuropathological changes associated with MS and which would be useful for evaluating the efficacy of novel therapeutics pre-clinically in the EAE model. A large number of genes were selected according to known or predicted involvement in pathological processes (mainly inflammation, neuronal function and death, myelin and remyelination, adaptive immune responses and CNS plasticity) and screened for their expression changes during the development of active MOG35-55-EAE in B6 mice by real-time RT-PCR. The current gene mini-panel comprises 10 genes that appear to sensitively monitor the main pathological processes in EAE and its use for predicting therapeutical effects of drugs has been successfully validated using Copaxone treatment in EAE (Vamvakas, Evangelidou et al, manuscript in preparation). Further tests of the panel in EAE using known and novel therapeutics intended for MS treatment, and further validation with neuropathological

analyses should determine its value as pre-clinical tool for predicting the therapeutic potential of novel drugs for MS.

Key publications:

Tseveleki V, Rubio R, Vamvakas SS, White J, Taoufik E, Petit E, Quackenbush J, Probert L. Comparative gene expression analysis in mouse models for multiple sclerosis, Alzheimer's disease and stroke for identifying commonly regulated and disease-specific gene changes. Genomics 2010;96:82-91.

DETRIMENTAL AND BENEFICIAL EFFECTS OF MICROGLIA ON NEURONS. Microglia, the innate immune cells of the CNS, show prominent activation in various neuropathological conditions and participate in both neurodegenerative and neuroprotective processes. Research carried out by Harald Neumann's group has advanced our understanding of the molecular basis of neuron-microglia bi-directional interactions by elucidating the effects of microglia-derived products on the axonal cytoskeleton and by identifying molecules that mediate signalling from dysfunctional neurons to microglia.

Axonal transport of mitochondria and synaptic vesicle precursors via kinesin motor proteins is essential to ensure the integrity of axons and synapses and disturbance of axonal transport is an early sign of most neuroinflammatory diseases including MS. Treatment of cultured neurons with the pro-inflammatory cytokine tumor necrosis factor (TNF), a major product of activated microglia, stimulated phosphorylation of c-Jun N-terminal kinase (JNK) in axons and induced dissociation of the motor protein kinesin KIF5B from tubulin in axons, but not cell bodies. Dissociation of KIF5B from tubulin after TNF treatment was dependent on JNK activity. Furthermore, TNF inhibited axonal transport of mitochondria and synaptophysin by reducing the mobile fraction via JNK.

Aggregated fibrillary microtubule-associated protein tau is a major component of the injured brain tissue in several neurodegenerative diseases, including MS. Co-culture of neurons with activated microglia induced aggregation of tau in neurites. Moreover, treatment of neurons with TNF resulted in accumulation of tau in neurites and stimulated reactive oxygen species generation. Aggregation of tau was inhibited by neutralization of TNF and the free radical inhibitor Trolox. These data demonstrate that activated microglia through release of TNF inhibits axonal mitochondria and synaptophysin transport via JNK and can induce aggregation of tau in neurites via reactive oxygen species. Fluorescence lifetime-based Förster resonance energy transfer (FRET) was applied to human brain tissue sections to detect pathogenic forms of tau molecules that had lost their normal spatial separation.

Key publications:

Unloading kinesin transported cargoes from the tubulin track via the inflammatory c-Jun N-terminal kinase pathway. Stagi M., Gorlovoy P., Larinov S., Takahashi K. and Neumann H. FASEB J. 2006;20:2573-5.

Accumulation of tau induced in neurites by microglial proinflammatory mediators. Gorlovoy P, Larionov S, Pham TT, Neumann H. FASEB 2009;23:2502-13.

Microglial molecules with a neuroprotective function were also identified. The effects of Triggering receptor expressed on myeloid cells-2 (TREM2), a DAP12-associated microglial innate immune receptor, were investigated in experimental MS-like disease. Using myeloid precursor cells genetically modified to express TREM2, it was observed that TREM2 has a beneficial effect in the recovery/resolution phase of EAE with amelioration of clinical symptoms, reduction in axonal damage, and prevention of further demyelination. The TREM2-transduced myeloid cells migrated in the inflammatory spinal cord lesions, showed increased

lysosomal and phagocytic activity, cleared damaged myelin, and created an anti-inflammatory cytokine milieu within the CNS. A method was also established for *in vitro* differentiation of mouse embryonic stem cells into microglial precursor cells which allows to generate high numbers of microglia for screening and cell therapy approaches.

Furthermore, the signal regulatory protein-beta1 (SIRPb1) was identified as a DAP12-associated microglial receptor. SIRPb1 was up-regulated and acted as a phagocytic receptor on microglia in APP transgenic mice and in EAE. Activation of SIRPb1 on cultured microglia increased phagocytosis of microsphere beads, neural debris, and fibrillary amyloid-beta, induced reorganization of the cytoskeleton protein beta-actin and suppressed lipopolysaccharide (LPS)-induced gene transcription of TNF and nitric oxide synthase-2. Thus, SIRPb1 has been identified as a microglial receptor that also supports clearance of neural debris, a primarily beneficial function in neuroinflammatory diseases.

The activatory DAP12-signalling via the immunoglobulin tyrosin-activation motif (ITAM) is counter-regulated by immunoglobulin tyrosine-inhibitory motif (ITIM). Siglec-11, belonging to the sialic acid-binding Ig superfamily lectins (Siglecs) which mainly signal via ITIM and recognize sialic acid residues of glycoproteins, was detected on microglia in human brain tissue and in a set of in vitro experiments it was shown that human Siglec-11 interacts with PSA on neurons and reduces the proinflammatory and phagocytic activity of microglia, leading to reduced microglial neurotoxicity.

Key publications:

Takahashi K., Prinz M., Stagi M., Chechneva O., and Neumann H. TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. PLoS Med. 2007;4(4):e124.

Gaikwad S, Larionov S, Wang Y, Dannenberg H, Matozaki T, Monsonego A, Thal DR and Neumann H. Signal regulatory protein-β1: a microglial modulator of phagocytosis in Alzheimer's disease. Am. J. Pathol. 2009;175:2528-39.

Wang Y, Neumann H. Alleviation of neurotoxicity by microglial human Siglec-11. J. Neurosci. 2010;30:3482–3488.

Larionvo S, Wielgat P, Wang Y, Thal RD, Neumann H. Spatially pathogenic forms of tau detected in Alzheimer's disease brain tissue by fluorescence lifetime-based Förster resonance energy transfer. J. Neurosci. 2010;192:127-37.

Beutner C, Roy K, Linnartz B, Napoli I and Neumann H. Generation of microglial cells from mouse embryonic stem cells. Nature Protocols 2010;5:1481-94.

In order to better understand how MS-associated neurodegeneration progresses, Hugh Perry's group asked whether systemic infections, which exacerbate clinical symptoms during the relapsing remitting phase of the disease, may have an impact on microglia activation and enhance neurodegeneration. The role of systemic inflammation on the macrophage and microglia populations responding to axon degeneration was investigated in the optic nerve Wallerian degeneration model. It was shown that systemic intraperitoneal challenge with LPS, to mimic a systemic infection, leads to a switch in the macrophage/microglia along the degenerating nerve from an anti-inflammatory to a pro-inflammatory and pro-phagocytic state. A dramatic increase in the number of injured axons was also observed in the MS-like model of EAE upon challenge with LPS. The CNS lesions with the greatest degree of axon injury correlated with the highest levels of inducible nitric oxide synthase. These data clearly demonstrate that systemic inflammation may have a profound effect on the macrophage/microglia phenotype and promote axon injury in the MS brain and spinal cord contributing to disease progression.

Key publications:

Palin K, Cunningham C, Forse P, Perry VH, Platt N. Systemic inflammation switches the inflammatory cytokine profile in CNS Wallerian degeneration. Neurobiol Dis. 2008;30:19-29.

ROLE OF N-TYPE VOLTAGE DEPENDENT CALCIUM CHANNELS (VDCCS) IN NEURODEGENERATION IN AUTOIMMUNE OPTIC NEURITIS: In a study aimed at shedding light into the neurodegenerative events occurring during autoimmune optic neuritis, Ricarda Diem's group found that pathological Ca^{2+} influx into optic nerves is mediated via N-type VDCCs. By analyzing the expression of VDCCs in the inflamed optic nerve, up-regulation of α1B, the pore-forming subunit of N-type VDCCs, in demyelinated axons was observed. High expression levels were also found on macrophages/activated microglia and lower levels were detected on astrocytes. The relevance of N-type VDCCs for inflammation-induced axon degeneration and the severity of optic neuritis was corroborated by treatment with ω-conotoxin. This blocker led to decreased axon and myelin degeneration in the optic nerves together with a reduced number of macrophages/activated microglia. These protective effects were confirmed by analyzing the spinal cords of the same animals. From these findings, it was concluded that N-type VDCCs play an important role in inflammation-induced axon degeneration via two mechanisms: firstly, they directly mediate toxic Ca2+ influx into the axons; secondly, they promote macrophage/microglia function, thereby promoting secondary axonal damage.

Key publications:

Gadjanski I, Boretius S, Williams SK, Lingor P, Knöferle J, Sättler MB, Fairless R, Hochmeister S, Sühs KW, Michaelis T, Frahm J, Storch MK, Bähr M, Diem R. The role of N-type voltage-gated calcium channels in autoimmune optic neuritis. Ann Neurol 2009;66:81-93.

ROLE OF AN ENERGY DEFICIT IN NEUROINFLAMMATORY LESIONS IN PROMOTING NEURODEGENERATION. Using a new experimental model in which an inflammatory lesion is induced that causes muscle weakness in the hindlimbs, Kenneth Smith's group have found that the weaknes is due to a reduction in the excitability of the motor neurons, which is in turn due to a reversible dysfunction of the motor neuronal mitochondria. The dysfunction has been traced to damage of the complex IV molecule of the mitochondrial electron transport chain. It was found that the complex remains present within the mitochondria, but it becomes modified in such as way as to render it non-functional. It was also found that the complex IV dysfunction is part of a wider spectrum of damage within the motor neurons associated with oxidative and nitrative stress with evidence of oxidized lipids and oxidised DNA within these specialized cells (Desai et al., manuscript in preparation). By developing a new method of labelling superoxide production within tissues during life, it was found that inflammation causes the excessive production of the reactive oxygen species, superoxide, within the motor neuron mitochondria. Superoxide is the first component of a cascade of reactive oxygen species resulting in oxidative stress and the structural change of many proteins within the mitochondria and cell. These findings shed new light on how inflammation causes neurological deficits in patients with MS, and simultaneously revealed the mechanisms underlying the energy deficit leading to neurodegeneration.

A separate line of research has revealed that the energy deficit appears to be exacerbated by a lack of oxygen within the inflamed brain tissue. Using a new method for measuring hypoxia in vivo, it was found that the brain tissue appears to be hypoxic during the peak of inflammation. This finding applies whether the inflammation is due to activation of the innate immune system by the intraspinal injection of the pro-inflammatory agent lipopolysaccharide, or due to the

induction of experimental MS-like disease, autoimmune encephaloymyelitis (EAE) (McIntosh et al., manuscript in preparation)..

Key publications:

Marik C, Felts P, Bauer J, Lassmann H, Smith KJ. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity. Brain 2007;130:2800-2815.

NEW INSIGHTS INTO NEURODEGENERATIVE MECHANISMS FROM NEUROPATHOLOGICAL STUDIES IN POST-MORTEM MS BRAIN TISSUE. Studies carried out by Hans Lassmann's and Wolfgang Bruck's groups in post-mortem and biopsy brain specimens from MS patients provided several basic insights into the mechanisms of neurodegeneration. It was shown that active demyelination and neurodegeneration in MS are invariably associated with inflammation and that stable remyelination is only present in patients where inflammation had subsided to levels seen in age matched controls. In 20% of the patients, the extent of remyelination was extensive with 60-96% of the global lesion area remyelinated. Extensive remyelination was found not only in patients with relapsing multiple sclerosis, but also in a subset of patients with progressive disease. Older age at death and longer disease duration were associated with significantly more remyelinated lesions or lesion areas. Furthermore, it was observed that failure of remyelination in MS is associated with a differentiation blockade of oligodendrocytes.
Wallerian degeneration was found to be a major component of axonal pathology in the periplaque white matter in early MS while chronic meningeal inflammation was associated with profound demyelination in the cerebral cortex, which not only involves the forebrain, but also the cerebellum. In MS, the cerebral cortex is exposed to severe inflammation for decades. Despite that, such chronic inflammatory exposure does not modify the appearance or extent of amyloid or neurofibrillary pathology, the hallmarks of Alzheimer's disease. A new experimental model of cortical demyelination, in which lesions were stereotactically targeted to the cerebral cortex by injection of pro-inflammatory mediators in rats that were immunized subclinically with myelin oligodendrocyte glycoprotein allowed to generate highly reproducible demyelinated lesions in the neocortex with remarkable histological similarities to cortical MS lesions. The focal cortical EAE model led to the typical pattern of intracortical and subpial demyelination, infiltration with inflammatory cells, complement deposition, acute axonal damage and neuronal cell death. Extensive cortical inflammation largely resolved indicate that remyelination of cortical inflammatory-demyelinating lesions may occur rapidly once inflammation has subsided.
In addition, RNA was extracted from autopsy paraffin-embedded brain tissue of MS patients and controls (archives of the University of Göttingen and the Medical University of Vienna) and analyzed using cDNA microarrays. The initial data indicate that such an approach is feasible, confirm previous observations that innate immunity mechanisms play a major role in the pathogenesis of MS lesions, and point to oxidative damage with subsequent mitochondrial injury as one of the major driving forces in the pathogenesis of active MS lesions.

Key publications:

Merkler D, Ernsting T, Kerschensteiner M, Brück W, Stadelmann C. A new focal EAE model of cortical demyelination: MS-like lesions with rapid resolution of inflammation and extensive remyelination. Brain, 2006;129:1972-1983.

Metz I, Lucchinetti CF, Openshaw H, Garcia-Merino A, Freedman M, Lassmann H, Brück W. Multiple sclerosis pathology after autologous stem cell transplantation: ongoing demyelination and neurodegeneration despite suppressed inflammation Brain 2006;130:1254-1262.

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Lursen H, Sorensen PS, Brück W, Lucchinetti C, Lassmann H. Remyelination is extensive in a subset of multiple sclerosis patients. Brain 2006;129:3165-3172.

Kutzelnigg A, Faber Rod JC, Bauer J, Lucchinetti CF, Sorensen PS, Laursen H, Stadelmann C, Brück W, Rauschka H, Schmidbauer M, Lassmann H. Widespread demyelination in the cerebellar cortex in multiple sclerosis. Brain Pathology 2006;17:38-44.

Dal Bianco A, Bradl M, Frischer J, Kutzelnigg A, Jellinger K, Lassmann H. Multiple Sclerosis and Alzheimer's Disease. Ann Neurol 2008;63:174-183.

Kuhlmann T, Mirron V, Cuo Q, Antel J, Brück W. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic MS. Brain 2008;131:1749-1758.

Frischer JM, Bramow S, Dal Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 2009;132:1175-1189.

Goldschmidt T, Antel J, König FB, Brück W, Kuhlmann T. The remyelination capacity of the MS brain decreases with disease chronicity. Neurology 2009;72:1914-1921.

Dziedzic T, Metz I, Dallenga T, König FB, Müller S, Stadelmann C, Brück W. Wallerian degeneration: a major component of early axonal pathology in multiple sclerosis. Brain Pathol. 2010;20:976-85.

Sharma R, Fischer MT, Bauer J, Smith KJ, Felts PA, Misu T, Fujihara K, Bradl M, Lassmann H. Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropath (Berl) 2010;120:223-236.

Bramow S, Frischer JM, Lassmann H, Koch-Henriksen N, Lucchinetti CF, Sørensen PS, Laursen H. Demyelination versus remyelination in progressive multiple sclerosis. Brain 2010;133:2983-98.

NEW INSIGHTS INTO MULTIPLE SCLEROSIS IMMUNOPATHOGENESIS

Although MS is the prototypic inflammatory disease of the CNS and considerable information has been gathered on immune cells and molecules that may contribute to CNS damage the antigenic stimuli and immunopathologic mechanisms causing inflammation, demyelination and neurodegeneration in the CNS have remained elusive. NeuroproMiSe has addressed this complex issue in preclinical models and through investigations of patients' tissues and cells. The results obtained suggest new pathogenic models for MS and the strategies adopted represent a major advancement to pinpoint critical immune events in the CNS and in the peripheral blood.

CONTRIBUTION OF CD8+ T CELLS TO BRAIN DAMAGE IN MS. The role of CD8+ T cells in CNS autoimmunity was investigated by Roland Liblau's group using double transgenic mice that were developed in collaboration with Pr. P. Chambon in Strasbourg and Dr. A. Waisman in Mainz and allowed to investigate the specific contribution of CD8+ T cells to inflammatory CNS tissue damage against either oligodendrocyte/myelin or neuron/axon. In collaboration with Hans Lassmann, a thorough neuropathological analysis of the effects of cytotoxic T cells was performed in double transgenic mice in which the CD8+ T cell response was targeted to oligodendrocytes. The study demonstrated the potential of CD8+ T cells to induce oligodendrocyte lysis in vivo as a likely consequence of direct antigen recognition. In another set of experiments, targeting the CD8+ T cell response to neurons led to induction of diabetes insipidus. The diabetes insipidus was clearly of central origin as the circulating vasopressin levels were very low in all sick animals and the polyuria treatable by exogenous administration of vasopressin. The almost complete elimination of hypothalamic vasopressin-secreting neurons

in the supraoptic and paraventricular nuclei supported this conclusion. It was found that MHC class I expression is strongly inducible on these neurons rendering them vulnerable to a CD8 T cell-mediated attack.

A key role for antigen presentation to CD8+ T cells by endothelial cells forming the blood-brain barrier was also highlighted in experimental work performed by Hugh Perry and Roland Liblau groups. Together, the above studies have provided evidence that recognition of antigen on CNS resident cells can cause marked neuropathological and functional alterations, suggesting that specific targeting of pathological CD8 T cells could represent a potentially useful strategy for CNS autoimmune diseases.

Key publications

Galea I, Bernardes-Silva M, Forse PA, van Rooijen N, Liblau RS, Perry VH. An antigen-specific pathway for CD8 T cells across the blood-brain barrier. J Exp Med. 2007;20:2023-30.

Saxena A, Bauer J, Scheikl T, Zappulla J, Audebert M, Desbois S, Waisman A, Lassmann H, Liblau RS, Mars LT Cutting edge: Multiple sclerosis like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. J Immunol 2008;181:1617-1621.

DEVELOPMENT OF STRATEGIES TO SEARCH FOR CNS INFILTRATING AUTOAGGRESSIVE T CELLS AND B CELLS. The most direct way to identify the antigens (self or non-self) that support the inflammatory process in MS is to pull out lymphocytes from the lesioned areas of the MS brain and study their antigenic specificities. Successful reconstruction of functional T-cell receptors (TCR) expressed by CD8+ T cells infiltrating the MS brain was achieved by K. Dornmair in Prof. Wekerle's group using a combination of: i) immunohistochemical techniques to identify expanded T-cell clones in MS lesions; ii) laser capture microdissection to isolate single T cells from biopsy or autopsy brain specimens; and iii) optimization of the procedures to clone and express matching alpha and beta chains in T-cell hybridomas and examine their antigen recognition properties. Matching TCR alpha- and beta-chains were identified from 20 independent T cells. Full length TCR cDNA clones were reconstructed from 12 chains (8 alpha- and 4 beta-chains), cloned into expression plasmids and transfected into T hybridoma cells. It was shown that the TCRs in the transfectants are functional, i.e. they are capable of secreting interleukin-2 upon stimulation. All clones were supertransfected with human CD8alpha and beta-chains. The cDNAs of all HLA alleles that were candidates as restriction elements were cloned and expressed in appropriate antigen presenting cells. It was found that MAIT (mucosal associated T) cells were present at high frequencies in the brain of one MS patient. A TCR with a characteristic MAIT alpha-chain was cloned and expressed. Preliminary evidence was provided that these MAIT cells may recognize peptides. All available TCR transfectants were tested for recognition of syngeneic EBV transformed B cell lines but none of them was activated. Attempts to identify the antigens of these clones using an unbiased method are currently ongoing.

In parallel, the technology to analyze oligoclonal band (OCB) antibodies from the cerebrospinal fluid of MS patients was improved further. Matching heavy and light chains from OCB spots have been identified on non-reducing 2-dimensional gels and five antibodies that represent individual OCBs have been cloned and expressed. All these antibodies were negative for recognition of EBV-related antigens and two of them recognize lipids (Obermeier et al., submitted). One antibody strongly binds sulfatides, either alone of in combination with other lipids. The other antibody binds galactosylcerebroside, although with somewhat lower affinity. Recognition of these lipids is particularly interesting because they are major components of the myelin sheath, which is the putative target of the immune attack in MS.

The issue of antigen recognition by encephalitogenic T cells was also addressed by Hartmut Wekerle's and Roland Liblau's groups in a transgenic mouse model of MS. It was found that 'molecular mimicry' at the CD4 T cell level exists between two distinct endogenous neural antigens, namely myelin-oligodendrocyte glycoprotein (MOG) and neurofilament-medium (NF-M), a component of cytoskeleton in neurons. By analogy with microbial molecular mimicry, it was observed that self-mimicry could aggravate MS-like disease by permitting a clonal $CD4^+$ T cell response to target more than one cognate auto-antigen simultaneously. The identification of self-mimicry in CNS autoimmunity defines a novel mechanism that permits the spreading of antigenic and cellular targets due to the plasticity of the T-cell receptor.

Key publications:

Dornmair, K., Meinl, E., and Hohlfeld, R. Novel approaches for identifying target antigens of autoreactive human B and T cells. Sem. Immunopathol. 2009;31:467-477.

G. Krishnamoorthy, A. Saxena, L.T. Mars, H.S. Domingues, R. Mentele, A. Ben-Nun, H. Lassmann, K. Dornmair, F.C. Kurschus, R. Liblau & H. Wekerle. Myelin-specific T cells also recognize neuronal autoantigen in a transgenic mouse model of multiple sclerosis. Nature Medicine. 2009;15:626-32.

INVOLVEMENT OF EPSTEIN-BARR VIRUS IN MS IMMUNOPATHOLOGY. Increasing seroepidemiological evidence supports an association between EBV and MS but the mechanisms linking viral infection to brain pathology remain unclear. EBV is a ubiquitous herpesvirus that infects about 95% of the world population and establishes a persistent, usually asymptomatic infection in the host's B cells. The starting point for investigating EBV in post-mortem MS brain tissue was the discovery made by Francesca Aloisi's group that large B-cell aggregates resembling lymphoid B-cell follicles develop in the meninges of a substantial proportion of patients with progressive disease. Having found that such abnormal structures are associated with a more severe cortical pathology and disease course and are infiltrated by immune cells that are involved in antiviral immune responses, like CD8 T cells and plasmacytoid dendritic cells, the subsequent step was to ask whether B- and T-cell activation within the MS ectopic B follicles was the manifestation of a perturbed EBV infection. It was shown that a high proportion of B cells within the ectopic follicles but also in the classical demyelinated lesions showed evidence of EBV latent infection. Lytically infected B cells were also detected although at a lower frequency and in the most acute MS lesions and cases. It was also found that CNS infiltrating CD8+ T cells show signs of cytotoxicity toward EBV infected B cells, suggesting that persistence of abnormal EBV deposits in the CNS could promote an immunopathological response in MS. Presence of EBV in the MS brain was confirmed using different techniques (in situ hybridization, immunohistochemistry, RT-PCR combined with laser capture microdissection of intracerebral immune infiltrates). Despite the extensive evidence provided by Aloisi and colleagues, other groups, including Wolfgang Bruck's group, were unable to detect EBV presence in most post-mortem MS brains analyzed. These discrepancies highlighted the need to rigorously assess the sensitivity of the techniques used as well as the characteristics (tissue processing, integrity, inflammatory activity) of the MS brain samples analyzed. To this purpose, a technical workshop organized by Hans Lassmann in Vienna in July 2010 gathered together 9 research groups from the USA and EU that have been involved in EBV search in MS tissues and body fluids in the last 2-3 years. Solving the issue of whether EBV establishes a persistent infection in the MS brain is critical to understand why MS patients have enhanced immune responses to the virus and can open new avenues for disease prevention and cure.

Within NeuroproMiSe, the involvement of EBV in MS was also investigated by Luca Battistini's group through immunological studies. This partner joined the NeuroproMiSe consortium at the

beginning of the 4th year with the aim to monitor EBV-specific T-cell responses in the peripheral blood obtained from MS patients and evaluate a possible correlation with disease activity. It was found that the frequency of EBV-specific CD8+ T cells (identified using the pentamer technology) was slightly higher in MS patients than in healthy donors, but this difference did not reach statistical significance. However, when patients were stratified based on the phase of the disease, interesting differences became evident. The presence of relapses or reactivation of the disease was monitored both clinically and with frequent MRIs to scan for active lesions. MS patients in the active phase of the disease showed a significantly higher frequency of CD8+ T cells specific for antigens of the EBV lytic phase, while patients in remission showed frequencies of EBV-specific CD8+ T cells comparable to those of healthy individuals. CD8+ T cells specific for antigens of the latent phase of the infection were present at comparable levels in MS and control groups; however, patients in the stable phase of the disease did show a consistent increase of these cells, albeit not at statistically significant levels. The functional phenotype of the EBV-specific CD8+ T cells detected in the blood of MS was carefully analyzed and compared with that of control subjects. Furthermore, the frequency of EBV-specific CD8+ T cells in the blood was found to be modified in patients undergoing therapy with interferon-beta, the most widely used immunomodulatory therapy for relapsing-remitting MS, and with natalizumab, a monoclonal antibody that binds to an adhesion molecule used by immune cells to migrate in inflamed tissues (Angelini et al., unpublished data). In conclusion, the data obtained indicate that studying the frequency and phenotype of EBV-specific T cells in the blood of MS patients can contribute to elucidate the link between EBV infection and disease activity, understand the role of EBV in disease progression and open new avenues to monitor the effects of therapeutic drugs.

Key publications:

Aloisi, F., Pujol-Borrell, R. Lymphoid neogenesis in chronic inflammation. Nature Rev. Immunol. 2006;6:205-217.

Magliozzi, R., Howell, O., Vora, A., Serafini B., Nicholas, R., Puopolo, M., Reynolds, R., Aloisi, F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007;130:1089-1104.

Serafini, B., Rosicarelli, B., Franciotta, D., Magliozzi, R., Reynolds, R., Cinque, P., Andreoni, P., Trivedi, P., Salvetti, M., Faggioni, A., Aloisi, F. Dysregulated Epstein Barr virus infection in the multiple sclerosis brain. J. Exp. Med. 2007; 204:2899-2912.

Serafini B, Columba-Cabezas S, Severa M, Rosicarelli B, Veroni C, Chiappetta G, Magliozzi R, Reynolds R, Coccia E, Aloisi F. Expression of Epstein-Barr virus latent membrane protein 2A and BAFF in B cells in the multiple sclerosis brain: implications for viral persistence and intrathecal B-cell activation. J Neuropathol Exp Neurol. 2010;69:677-93

Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, Roughan JE, Almendinger SE, Blewett MM, Brück W, Hafler DA, O'Connor KC. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain 2009;132:3318-28.

Aloisi F, Serafini B, Magliozzi R, Howell OW, Reynolds R Detection of Epstein-Barr virus and B-cell follicles in the multiple sclerosis brain: what you find depends on how and where you look. Brain 2010 Aug 25. [Epub ahead of print].

In a different approach to understand the role of infections in the development of CNS autoimmunity Lars Fgger's group asked whether commensal bacteria may play a role in triggering autoimmune T cell responses in MS. It was demonstrated that a microbial peptide, common to several major classes of bacteria, can induce MS-like disease in humanized mice by

crossreacting with a T cell receptor (TCR) that also recognizes a peptide from myelin basic protein, a candidate MS autoantigen. Structural analysis demonstrates this crossreactivity is due to structural mimicry of a binding hotspot shared by self and microbial antigens, rather than to degenerate TCR recognition. Biophysical studies reveal that the autoreactive TCR binding affinity is markedly lower for the microbial (mimicry) peptide than for the autoantigenic peptide. Thus, these data suggest a possible explanation for the difficulty in incriminating individual infections in the development of MS.

Key publications:

Harkiolaki, M., Holmes, S., Svendsen, P., Gregersen, J., Torp Jensen, L., McMahon, R., Friese, M.A., van Boxel, G., Etzensperger, R., Tzartos J.S., Kranc, K., Sainsbury, S., Harlos, K., Mellins E.D., Palace, J., Esiri, M., van der Merwe, P.A., Jones, E.Y. and Fugger, L. A mechanism for T-cell-mediated autoimmune disease resulting from highly specific but low affinity cross-reactivity to common microbial peptides. Immunity 2009;30;348-357.

DEVELOPMENT AND PRECLINICAL VALIDATION OF COMPOUNDS WITH IMMUNOMODULATORY AND NEUROPROTECTIVE ACTIVITY.

Within the Subproject Validation, the study of anti-inflammatory and neuroprotective pathways has proceeded in parallel with the development and validation of novel therapeutic compounds and evaluation of the direct neuroprotective effects of licensed drugs in preclinical models of MS.

DEVELOPMENT OF ANTI-INFLAMMATORY REAGENTS THAT TARGET THE NADPH OXIDASE COMPLEX. This work was carried out by Redoxis in collaboration with Rikard Holmdahl. Redoxis entered NeuroProMiSe in November 2007 with the plan to develop a screening method to detect cellular production of reactive oxygen species (ROS) and identify and validate NADPH oxidase complex activating compounds as a new tool for anti-inflammatory treatment. With FP6 support Redoxis has developed as a SME holding 6 employed researchers whose work focuses on target identification and development of novel treatments for autoimmune diseases. The following major results were obtained:

1. Development of congenic and transgenic mice or rat strains with altered NADPH oxidase complex function to validate the importance of redox regulation in animal models of arthritis and MS.
2. Two gene expression profiling studies where influence of ROS on the immune system are targeted with different setups, covering both human, rat and mouse gene expression.
3. Development of an assay for high throughput screening methods to detect cellular production of ROS. The method is based on luminescence detection or fluorescence based flow cytometer techniques in 96- or 384-well formats.
4. High throughput screening campaign of 60,000 small molecules leading to the identification of six different NADPH oxidase comple activator compound classes for further validation and mechanism characterisation.
5. Development of identified hits using medicinal chemistry based on in vitro, ex vivo, metabolism, physiochemical and ADME (Absorption, Distribution, Metabolism and Elimination) properties of the compounds.
6. Validation of selected lead compounds for effect on autoreactive T cells in both experimental arthritis and MS-like disease.

7. Development and validation of an ex vivo system based on adoptive T-cell transfer for characterization and screening of ROS-inducing substances in autoimmune T cells.
8. Validation studies of one lead compound in the treatment of experimental arthritis and MS-like disease.
9. Submission of patent applications to cover compositional matter of developed NADPH activating compounds.
10. Attraction of venture capital from two Swedish University holding companies and creation of the subsidiary company ProNoxis for preclinical drug development and immaterial properties of developed lead compounds.

The current lead development focuses on three substance classes, each including 5-8 model compounds with the goal of taking one-two compounds through preclinical documentation and initial clinical validation. The pharmacological and biological properties of the selected model drug will be studied to ensure that the compound is suitable for toxicological documentation and pre-clinical evaluation. Redoxis also plans to produce new molecules around the selected model compounds to modify and improve pharmacological properties and suitability as therapeutic drugs. The hope and intention is to initiate preclinical documentation in 2012 and Proof-of-concept trials in man in 2014.

Key publications:

Gelderman KA, Hultqvist M, Olsson LM, Bauer K, Pizzolla A, Olofsson P, Holmdahl R. Rheumatoid arthritis: the role of reactive oxygen species in disease development and therapeutic strategies. Antioxid Redox Signal. 2007;9:1541-67.

Olofsson P, Nerstedt A, Hultqvist M, Nilsson EC, Andersson S, Bergelin A, Holmdahl R. Arthritis suppression by NADPH activation operates through an interferon-beta pathway. BMC Biol. 2007;9:5:19.

Hultqvist M, Bäcklund J, Bauer K, Gelderman KA, Holmdahl R. () Lack of reactive oxygen species breaks T cell tolerance to collagen type II and allows development of arthritis in mice. J Immunol. 2007;179:1431-7.

DEVELOPMENT OF REAGENTS THAT TARGET SELECTIVELY HUMAN TUMOR NECROSIS FACTOR RECEPTORS (TNFR1 AND TNFR2). TNF is a master pro-inflammatory cytokine that has been implicated in different autoimmune diseases as well as a pleiotropic cytokine that regulates the function of different non-immune cell types, including neural cells. Because different TNF actions are mediated by activation of two distinct receptors, TNFR1 and TNFR2, generation of tools that interfere with specific TNF signalling pathways was deemed important in order to modulate TNF inflammatory and neuroprotective activities. The assessment of the differential contribution of the two TNFRs to distinct cellular responses in vitro and in disease models was made possible through availability of mouse TNFR specific antibodies provided by HyCult Biotechnology.

DEMONSTRATION OF A NEUROPROTECTIVE ROLE FOR TNFR2. A neuroprotective role of TNFR2 in neuroinflammation was demonstrated by Uli Eisel's group using genetically engineered mouse models. Furthermore, treatment with lovastatin, a member of the drug class of statins used for lowering cholesterol, was found to increase neuronal expression of TNFR2 resulting in neuroprotection against excitotoxic insults. It was also shown that TNFR2 is involved in downregulating immune infiltration into the CNS in experimental MS-like disease and induces the expression of anti-inflammatory and neurotrophic factors in microglia. Conversely, absence of TNFR1 protected mice against MOG-induced EAE, establishing a critical role for this receptor in the development of inflammatory neurodegenerative disease. In

line with this result, blocking of mouse TNFR1 by an antagonistic antibody (Hbt) ameliorated disease.

GENERATION OF NEW GENETICALLY ENGINEERED MOUSE MODELS FOR DRUG VALIDATION PROCESSES. Transgenic mice expressing humanized TNFR1 and TNFR2 have been generated. These receptors consist of the extracellular domain of the human receptor and the intracellular domain of the corresponding mouse receptor. These mice represent excellent new tools to validate the therapeutic potential of new species specific biological reagents targeting the TNFR system. A cell model selectively expressing human TNFR2 in mouse embryonic fibroblasts with a TNFR1 / TNFR2 double knockout was also established as a new tool for in vitro studies on TNFR2 signaling pathways mediating neuroprotection.

DEVELOPMENT OF TNF RECEPTOR TARGETING BIO-THERAPEUTICS FOR APPLICATION IN MULTIPLE SCLEROSIS.

TARGETING TNFR1: An antagonistic, humanized huTNFR1 selective antibody (ATROSAB) was developed by Klaus Pfizenmaier's group and is now developed by Celonic as a new therapeutic to target the pro-inflammatory action of TNF. Proof of concept of therapeutic activity of this antibody has been obtained in an acute inflammatory model (collagen-induced arthritis) in non-human primates. This reagent will be the first TNFR1 specific TNF antagonist to enter clinical trials; a Phase I trial is scheduled for 2011.

TARGETING TNFR2: To exploit the neuroprotective function of TNFR2, several agonists, selective for human TNFR2, were generated by Klaus Pfizenmaier's group based on the recently developed single-chain format of TNF. Of particular value is TNC-scTNF$_{R2,}$ which makes use of the trimerization domain of tenascin C to generate a soluble, human TNFR2 selective agonist that shows high intrinsic bioactivity. This molecule shows high serum stability, lacks systemic toxicity in vivo and displays favorable pharmacokinetic properties. Importantly, it was shown that TNC-scTNF$_{R2}$ displays neuroprotective activity in vitro protecting both oligodendrocytes and neurons from hydrogen peroxide-induced cell death.

Key publications:

Dolga AM, Blank T, Luiten PGM, Eisel ULM, Nijholt IM. TNF-alpha induces neuroprotection via NF-kB-dependent up-regulation of small conductance calcium-activated potassium channels. J Neurochem. 2008;107:1158-67.

Dolga AM, Nijholt IM, Ostroveanu A, Ten Bosch Q, Luiten PG, Eisel UL. Lovastatin induces neuroprotection through tumor necrosis factor receptor 2 signaling pathways. J Alzheimers Dis. 2008;13:111-22.

Zettlitz KA, Lorenz V, Landauer, KH, Münkel S, Herrmann A, Scheurich P, Pfizenmaier K, Kontermann RE. ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one-specific antibody. MAbs 2010;2:639-647.

Veroni C, Gabriele L, Canini I, Castiello L, Coccia E, Remoli ME, Columba-Cabezas S, Aricò E, Aloisi F, Agresti C. Activation of TNF receptor 2 in microglia promotes induction of anti-inflammatory pathways. Mol Cell Neurosci. 2010;45:234-44.

Fischer R, Maier O, Naumer M, Krippner-Heidenreich A, Scheurich P, Pfizenmaier K. Ligand-induced internalization of TNF receptor 2 mediated by a di-leucin motif is dispensable for activation of the NFκB pathway. Cell Signal 2011;23:161-170.

DEVELOPMENT OF CELL-PENETRATING REAGENTS FOR ENHANCING TNFR1 NEUROPROTECTION: Following the failure of non-selective TNF inhibitors in MS patients, Lesley Probert's group were interested in defining the CNS protective effects of TNF. They focused on the protective function of neuronal TNFR1, showing that caspase 8 is a dominant mediator of neuron death following excitotoxic and ischemic injury, and developed several cell-penetrating peptides, including dominant negative caspase 8 molecules and the anti-apoptosis protein FLIP, that were sufficient to protect neurons against damage in pre-clinical models for MS. They also showed that the two molecular forms of TNF, transmembrane TNF and cleaved soluble TNF, exert opposing beneficial and deleterious effects in preclinical MS models and that the selective inhibition of soluble TNF might be a promising therapeutic approach for MS.

Key publications:

Taoufik E, Valable S, Müller GJ, Roberts ML, Divoux D, Tinel A, Voulgari-Kokota A, Tseveleki V, Altruda F, Lassmann H, Petit E, Probert L. FLIP(L) protects neurons against in vivo ischemia and in vitro glucose deprivation-induced cell death. J Neurosci. 2007; 20;27:6633-46.

Taoufik E, Petit E, Divoux D, Tseveleki V, Mengozzi M, Roberts ML, Valable S, Ghezzi P, Quackenbush J, Brines M, Cerami A, Probert L. TNF receptor I sensitizes neurons to erythropoietin- and VEGF-mediated neuroprotection after ischemic and excitotoxic injury. Proc. Natl. Acad. Sci. USA 2008; 105: 6185-6190 .

Emmanouil M, Taoufik E, Tseveleki V, Vamvakas SS, Tselios T, Karin M, Lassmann H, Probert L. Neuronal I kappa B kinase beta protects mice from autoimmune encephalomyelitis by mediating neuroprotective and immunosuppressive effects in the central nervous system. J.Immunol. 2009;183:7877-89.

Taoufik E, Tseveleki V, Chu SY, Tselios T, Karin M, Lassmann H, Szymkowski DE, Probert L. Opposing beneficial and deleterious effects of transmembrane and soluble TNF in experimental autoimmune encephalomyelitis. Submitted for publication in September 2010.

DEMONSTRATION OF THE BENEFICIAL EFFECTS OF AMILORIDE IN EXPERIMENTAL MS-LIKE DISEASE. Lars Fugger's group tested the hypothesis that the acid-sensing ion channel-1 (ASIC1) contributes to axonal degeneration in inflammatory lesions of the CNS. Initial experiments indicated that the acid-sensing ion channel-1 (ASIC1) contributes to axonal degeneration in inflammatory lesions of the CNS. After induction of EAE, mice lacking Asic1 (Asic1 –/–) showed both a markedly reduced clinical deficit and reduced axonal degeneration compared to wild-type mice. A role for ASIC1 activation in acidosis-mediated axonal injury in CNS was supported by observing a protective effect of Asic1 disruption in nerve explants in vitro and in vivo after induction of EAE. Genetic disruption and pharmacological blockade of ASIC1 also significantly reduced acidosis-induced oligodendrocyte injury in vitro and demyelination in vivo. Most importantly, it was found that amiloride, a licensed and clinically safe blocker of ASICs, had a beneficial effect on acute MS-like disease when treatment was initiated at disease onset and reduced disease progression seen by a significant reduction in permanent disability as well as mean peak neurological deficit. Due to its translational potential, another aim was to investigate if amiloride treatment could cause a similar reduction in clinical score and improve neurological status of the mice if treatment was initiated post the acute inflammatory phase when the mice are about to experience their first relapse. It was found that therapeutic amiloride treatment also resulted in improved outcome on the final disease score reflecting non-remitting deficits in the mice. The difference in clinical disease was more modest compared to when amiloride is given earlier, suggesting that amiloride would be most efficient as therapy for patients with acute/early

MS. The effect of amiloride treatment was also investigated in chronic-relapsing EAE. Post immunization with MOG peptide, the mice developed a reproducible relapse-remitting disease pattern, which was significantly reduced in mice receiving daily amiloride treatment. Amiloride reduced disease progression seen by a significant reduction in permanent disability and mean peak deficit. Neuropathological correlates of the significant difference in clinical disease were found to be a protection of axonal loss in amiloride treated mice in both corticospinal tract and dorsal column. In addition, quantification of myelination demonstrated less demyelination in amiloride treated mice. These results suggest that ASIC1 blockers could promote neuroprotection in MS and have provided the rationale for planning of a clinical trial.

Key publications:

Friese, M., Craner, M., Etzensperger, R., Vergo, S., Wemmie, J. A., Welsh, M. J., Vincent, A. and Fugger, L. Acid-sensing ion channel 1 contributes to axonal degeneration in autoimmune inflammation of the central nervous system. Nature Medicine 2007;12:1483-1489.

Vergo S, Craner MJ, Etzensperger R, Attfield K, Friese MA, Newcombe J, Esiri M, Fugger L. Acid-sensing ion channel 1 is involved in both axonal injury and demyelination in multiple sclerosis and its animal model. Brain 2010, in press.

IMPACT OF LICENSED DRUGS ON AUTOIMMUNE-MEDIATED NEURODEGENERATION A model of autoimmune-induced optic neuritis, which mimics some of the neurodegenerative aspects of MS, was used by Mathias Bähr's and Ricarda Diem's groups to evaluate the interaction between autoimmune inflammation and neurodegeneration. Optical coherence tomography was established as a method to detect retinal nerve fiber layer in rodents and to reliably monitor neurodegeneration in this model. By performing several neuroprotective treatment studies it was found that a strong anti-inflammatory activity is not sufficient to exert neuroprotective effects. Therefore, a neuronal and axonal-protective effect in autoimmune CNS pathology might be expected from substances which target inflammation and neurodegeneration simultaneously. This property was at least in part demonstrated for glatiramer acetate and minocycline in MS-like disease. Also, combination therapy with an immunomodulatory drug (interferon-beta) and a neuroprotective drug (flupirtine, a nonopioid analgesic used in treatment of chronic pain) was shown to be sufficient in improving visual function in the model of autoimmune optic neuritis. Studies in different subtypes of autoimmune optic neuritis have highlighted substantial differences in the level of neurodegeneration despite a similar degree of inflammatory infiltration and demyelination indicating that mechanisms other than those directly induced by autoimmune inflammation may contribute to neuronal loss. These findings have contributed to promote investigations on the role of altered expression of ion channels in neurodegenerative processes (see above).

Key publications:

Sättler MB, Demmer I, Williams SK, Maier K, Merkler D, Gadjanski I, Stadelmann C, Bähr M, Diem R Effects of Interferon-beta-1a on neuronal survival under autoimmune inflammatory conditions. Exp Neurol 2006;20:172-81.

Maier K, Merkler D, Gerber J, Taheri N, Kuhnert AV, Williams SK, Neusch C, Bähr M, Diem R. Multiple neuroprotective mechanisms of minocycline in autoimmune CNS inflammation. Neurobiol Dis. 2007;25:514-25.

Sättler MB, Williams SK, Neusch C, Otto M, Pehlke J, Bähr M, Diem R. Flupirtine as neuroprotective add-on therapy in autoimmune optic neuritis. Am J Pathol. 2008;173:1496-507.

Sättler MB, Togni M, Gadjanski I, Sühs KW, Meyer N, Bähr M, Diem R Strain-specific susceptibility for neurodegeneration in a rat model of autoimmune optic neuritis. J Neuroimmunol 2008;193: 77-86.

BLOCKERS OF SODIUM CHANNELS AND OF THE NA/CA EXCHANGER AS POTENTIAL NEUROPROTECTIVE AGENTS. Based on the finding that an energy deficit may be relevant to the mechanisms underlying axonal and neuronal degeneration in MS and on the consideration that protection of energy balance might promote axonal survival during CNS inflammation it was hypothesized that sodium channel blocking agents should be effective neuroprotective agents, and, indeed, Kenneth Smith's group and others have proven this to be the case. However, a clinical trial that was conducted based on this finding (lamotrigine in secondary progressive MS) revealed ambiguous results with regard to neuroprotection. What was clear was that the more severely affected MS patients did not easily tolerate the therapy, in contrast to people with other conditions, such as epilepsy. To protect neurons from energy imbalance, a novel sodium channel blocking agent that might avoid the complications experienced with lamotrigine, was used and proved very effective in axonal protection in EAE and a model of Parkinson's disease. Kenneth Smith's group also explored the use of nicotinamide to promote mitochondrial function. It was found that in EAE this agent is very effective in axonal protection, but that it might increase the extent of damage by demyelination, negating its use in MS. The use of blockers of the Na/Ca exchanger (KB-R7943 and SEA0400) was explored and it was found that whereas KB-R7946 was quite effective in neuroprotection in EAE, SEA0400 was not (Bei and Smith, manuscript in preparation). In contrast, both KB-R7943 and SEA0400 were both effective in axonal protection in a model of ischaemia. Although effective, there was also evidence that KB-R7943 might impair renal function, and so this compound was not pursued as a neuroprotective agent. SEA0400 is a more selective blocker of the exchanger and it may show promise in stroke. The failure in EAE vs. ischaemia may be due to differences in route of administration required for dosing over a month rather than a few days.

CONCLUSIONS

The results of the NeuroproMiSe project have significantly advanced our understanding of MS and of neuroinflammatory processes in general. With the knowledge acquired on genes and mechanisms involved in inflammatory neurodegeneration and neuroprotection and with the compounds developed on the basis of this knowledge, more reliable models of MS pathogenesis have been proposed and candidate therapeutic molecules have been developed and will be proposed for subsequent clinical evaluation.

USE AND DISSEMINATION OF KNOWLEDGE

EXPLOITABLE KNOWLEDGE AND ITS USE

Exploitable knowledge	Exploitable product(s)	Sector of application	Time table for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved
TNFR1 as target in neuro-degeneration	TNFR1 selective TNF-antagonist (ATROSAB)	Inflammatory disorders including neurological diseases	2017	Several international patent applications	Univ. Stuttgart, Celonic (licensee)
Drug candidates activating NAPDH oxidase	Pharmaceutical drug	Pharmaceutical industry	After 2014	Patent applications	ProNoxis AB Redoxis AB

BLOCKING TNF ACTION IN NEURODEGENERATIVE DISEASES.

Based on the demonstration of TNFR1 selective antagonistic activity of a humanized scFv, a genetically engineered IgG has been developed that is entering clinical trials in 2011 as a new class of therapeutics targeting TNF-mediated pathology including neurodegeneration. Several international patent applications have been filed. Kontermann, R, Münkel, S, Scheurich, P and Pfizenmaier, K. (2007) A human TNFR1 selective antagonist. European and US patent applications.

DEVELOPMENT OF NADPH OXIDASE ACTIVATORS FOR TREATMENT OF AUTOIMMUNE CONDITIONS.

Activation of radical oxygen species (ROS) from the NADPH oxidase complex was identified by Dr. Olofsson (Redoxis, P23) and Prof Holmdahl (Karolinska Institute, P8b) as a new mechanism for treatment of autoimmune conditions, based on the positional cloning of Ncf1 (Nature Gen. 2003). Redoxis have during recent years developed small molecule activators of the NADPH oxidase complex with drug-like properties and shown potential treatment effect in models of MS. For further preclinical development and commercialisation of developed drug candidates, Redoxis have funded Redoxis subsidiary company, ProNoxis AB, together with Swedish University investment holding companies. Hence, ProNoxis is the owner of the IPR of the project (inventors of current patents from Redoxis; i.e. Olofsson, Hultqvist and Wallner) and will together with Redoxis continue the preclinical development, including safety documentation, and pursue the development of this project with the aim of proof-of principle clinical trials in humans in 2014.

MEETINGS AND WORKSHOPS

The scientific programmes of the meetings and workshops listed below are published in the NeuroproMiSe website (www.neuropromise.eu).

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NEUROPROMISE ANNUAL MEETINGS

During its 5 years of activity the NeuroproMiSe Consortium has organized a yearly conference for all its participants and Advisory Board members. The programme of the NeuroproMiSe meetings typically included 2 days of scientific presentations by the different groups, followed or preceeded by lectures of world leading researchers on topics related to the project. The NeuroproMiSe meetings also provided opportunities for meetings of the Governing Bodies and Advisory Boards and for plenary sessions where ideas and proposals for the following years could be discussed.

FIRST NEUROPROMISE MEETING, Rome, November 7-8 2006. Organizer: Francesca Aloisi; 56 participants. The meeting programme included one lecture on 'Ethical issues in animal experimentation' given by Dr Vitale (Istituto Superiore di Sanità), a world leading expert in the field, and one lecture on 'Gender issues in science' given by Dr F. Zucco, President of the Italian Association Women and Science.

SECOND NEUROPROMISE MEETING, Bonn, November 11-12 2007. Organizer: Harald Neumann; 59 participants.

THIRD NEUROPROMISE MEETING, Toulouse, November 4-5, 2008. Organizer: Roland Liblau; 56 participants.

FOURTH NEUROPROMISE MEETING, Athens, 8-10 November 2009. Organizer: Lesley Probert; 38 participants.

FIFTH NEUROPROMISE MEETING, Rome, 21 October 2010. Organizer: Francesca Aloisi; 35 participants.

WORKSHOPS

**"NEUROPROTECTIVE STRATEGIES FOR MULTIPLE SCLEROSIS", ** Rome, November 6, 2006. Organizer: Francesca Aloisi. The aim of the workshop was to present the NeuroproMiSe goals and activities to the public; 125 participants attended the meeting; the audience comprised scientists and physicians within and outside the MS field as well as lay people.

"VIRAL TRIGGERS OF AUTOIMMUNITY: FOCUS ON EPSTEIN-BARR VIRUS AND MULTIPLE SCLEROSIS", Rome, May 19-20 2008. Organizer Francesca Aloisi; 210 participants. This international workshop was organized in collaboration with the COST Action Neurinfnet. The scientific programme included talks of NeuroproMiSe participants and of many invited experts with the aim to bridge the virology and autoimmunity fields and discuss the role of infectious agents, particularly EBV, in MS, rheumatoid arthritis and systemic lupus erythematosus.

"INFLAMMATION IN THE CENTRAL NERVOUS SYSTEM", Toulouse, France, November 3, 2008. Organizer: Dr. Roland Liblau; participants: 100. Five speakers (A. Compston, H. Wekerle, A. Prat, A. Saoudi and C. Linington) provided an overview of topics of major interest for the project

(genetics, immune regulation, animal models, antibodies in autoimmunity, and leukocyte migration to the CNS). The workshop was attended by NeuroproMiSe partners and local students and researchers..

"CD8 T CELLS IN CENTRAL NERVOUS SYSTEM INFLAMMATION", Rome, March 5-6, 2009. Organizers: Roland Liblau, Francesca Aloisi; 170 participants This international workshop was organized in collaboration with the COST Action Neurinfnet. Talks of NeuroproMiSe participants and of many invited experts focussed on the most recent achievements in CD8+ T-cell biology, CD8 T-cell involvement in experimental and human viral and autoimmune diseases, and involvement of CD8+ T cells in MS.

"NEW DEVELOPMENTS IN MULTIPLE SCLEROSIS RESEARCH", Groningen, The Netherlands, June 2, 2009. Organizer: Uli Eisel; 60 participants. The meeting was intended to connect partners of the NeuroproMiSe consortium with MS research groups in the Netherlands and Belgium and to discuss emerging new ideas in the field.

"ION CHANNELS AND MULTIPLE SCLEROSIS", Düsseldorf, Germany, September 10 2009. Workshop organized Kenneth Smith and Ricarda Diem during the 26th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS).

"EPSTEIN-BARR VIRUS IN THE MULTIPLE SCLEROSIS BRAIN", Vienna, July 14-15, 2010. Organizer: Hans Lassmann; 25 participants. This meeting gathered together research groups from Europe and the USA in a timely attempt to discuss one of the most controversial issues in MS research.

"FINAL NEUROPROMISE MEETING", Rome, October 22, 2010. Organizer: Francesca Aloisi; 95 participants. During this meeting the NeuroproMiSe partners presented the most relevant results achieved over 5-years time period and a final discussion addressed the impact of the project products on current thinking of and future research developments.

TRAINING COURSES

"GENETIC ANALYSIS AND BIOINFORMATICS", University of Lund, Sweden, January 8-12, 2007. Organizers: Dr. Rikard Holmdahl, Dr. Tomas Olsson. The programme of this course included lessons and practical sessions on: Genetic strategies in experimental populations; Bioinformatics; Statistics in genetical analysis; Use of data bases. Faculty members: 10; participants: 31 (of which 19 were from the Neuropromise Consortium).

"FUNCTIONAL IMAGING OF THE LIVING CELL", Institute of Cell Biology and Immunology, University of Stuttgart, Germany, October 9-12, 2007. Organizers: Dr Gertrude Bunt, Prof. Klaus Pfizenmaier. The goal of this course was to give scientists within and outside the NeuroproMiSe consortium a deeper understanding of the applications of modern methods of microscopy. Faculty members: 5, Tutors :7; participants: 40, of which 12 were from the NeuroproMiSe Consortium).

"INFLAMMATION IN NEUROIMMUNOLOGY: BASIC MECHANISMS" A 1-day educational course was organized by NeuroproMiSe during the 7th Course of the European School of Neuroimmunology (www.esni.org) held on September 20-23, 2007 in Oxford, UK. Organizers: Lesley Probert, Francesca Aloisi. The program included 10 talks given by partners of the NeuroproMiSe consortium on topics developed within the project. The ESNI course was attended by 250 participants.

"IDENTIFICATION OF GENES PREDISPOSING TO COMPLEX DISEASES, MS DISEASE AS AN EXAMPLE" Department of Medical Genetics and Center of Excellence in Disease Genetics

,University of Helsinki, Helsinki, Finland, May 7, 2008. Organizer: Dr Leena Peltonen. The workshop was attended by 100 participants from Finland, mainly researchers.

"**BASIC AND CLINICAL ASPECTS OF NEUROINFLAMMATION**" A 1-day educational course was organized by NeuroproMiSe during the 9th Course of the European School of Neuroimmunology (www.esni.org) held on September 1-4, 2007 in Istanbul, Turkey. Organizers: Lesley Probert, Roland Liblau. The program included 8 talks given by partners of the NeuroproMiSe consortium on topics developed within the project. The ESNI course was attended by 300 participants.

The NeuroproMiSe Partners also contributed as faculty members or organizers to numerous other training courses held in and outside Europe, like the FENS-IBRO European Neuroscience Schools, the Baltic Summer School, the pre-congress teaching courses of the Annual European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) and the pre-congress teaching courses of the bi-annual Congress of the International Society of Neuroimmunology (ISNI).

PUBLICATIONS

2006

1. Aloisi, F. Neuroprotective strategies for multiple sclerosis. Notiziario dell'Istituto Superiore di Sanità 2006; 19:1-7.

2. Aloisi, F., Pujol-Borrell, R. Lymphoid neogenesis in chronic inflammation. Nature Rev. Immunol. 2006; 6:205-217

3. Cassan, C., Piaggio, E., Zappulla, J., Mars, MT, Bucciarelli, F., Desbois, S., Bauer, J., Gonzalez-Dunia, D., Liblau, R.S. Pertussis toxin reduces the number of splenic Foxp3$^+$ regulatory T cells. J. Immunol. 2006; 177: 1552-60.

4. Columba-Cabezas, S., Griguoli, M., Rosicarelli, B., Magliozzi, R., Ria, F., Serafini, B., Aloisi, F. Suppression of established experimental autoimmune encephalomyelitis and formation of meningeal lymphoid follicles by lymphotoxin βreceptor-Ig fusion protein. J. Neuroimmunol. 2006; 179:76-86.

5. Gregersen, J. W., Kranc, K., Ke, X., Svendsen, P., Madsen, L., Randrup Thomsen A., Cardon, L. Bell, J. and Fugger L. Functional epistasis on a common MHC haplotype associated with multiple sclerosis. Nature 2006; 443, 574-577.

6. Johannesson M, Hultqvist M, Holmdahl R. Genetics of autoimmune diseases: a multistep process. Curr Top Microbiol Immunol. 2006;305:259-76. Review.

7. Liu Y., Hao W., Letiembre M., Walter S., Kulanga M., Neumann H. and Fassbender K. Suppression of microglial inflammatory activity by myelin phagocytosis: role of p47-PHOX-mediated generation of reactive oxygen species.J Neurosci. 2006;26:12904-13.

8. Mastronardi FG, Wood DD, Mei J, Raijmakers R, Tseveleki V, Dosch HM, Probert L, Casaccia-Bonnefil P, Moscarello MA. Increased citrullination of histone H3 in multiple sclerosis brain and animal models of demyelination: a role for tumor necrosis factor-induced peptidylarginine deiminase 4 translocation. J Neurosci. 2006¸26:11387-96.

9. Merkler D, Ernsting T, Kerschensteiner M, Brück W*, Stadelmann C*. A new focal EAE model of cortical demyelination: MS-like lesions with rapid resolution of inflammation and extensive remyelination. Brain, 2006; 129: 1972-1983. * Shared senior authors.

10. Neumann H. Microglia: A cellular vehicle for CNS gene therapy (invited commentary) J Clin Invest. 2006; 116:2857-60.

11. Olsson T, Jagodic M, Piehl F, Wallström E. Genetics of autoimmune neuroinflammation. Current Opinion in Immunology 2006; 18:1-7.

12. Rossner, M.J., Hirrlinger, J., Wichert, S.P., Boehm, C., Newrzella, D., Hiemisch, H., Eisenhardt, G., Stuenkel, C., von Ahsen, O., and Nave, K.-A. Global Transcriptome Analysis of Genetically Identified Neurons in the Adult Cortex. J. Neurosci 2006; 26, 9956-9966.

13. Saarela, J., Kallio, S.P., Chen, D., Montpetit, A., Jokiaho, A., Choi, E., Asselta, R., Bronnikov, D., Lincoln, M.R., Sadovnick, A.D., Tienari, P.J., Koivisto, K., Palotie, A., Ebers, G.C., Hudson, T.J., Peltonen, L. PRKCA and Multiple Sclerosis: Association in two independent populations. PLoS Genetics 2006¸2:364-375

14. Sättler MB, Demmer I, Williams SK, Maier K, Merkler D, Gadjanski I, Stadelmann C, Bähr M, Diem R Effects of Interferon-beta-1a on neuronal survival under autoimmune inflammatory conditions. Exp Neurol 2006; 20:172-81.

15. Stagi M., Gorlovoy P., Larionov S., Takahashi K. and Neumann H. Unloading kinesin transported axonal cargoes from the tubulin track via the inflammatory c-Jun N-terminal kinase pathway. FASEB J. 2006; 20:2573-5.

16. Swanberg M, Duvefelt K, Diez M, Hillert J, Olsson T, Piehl F, Lidman O. Genetically determined susceptibility to neurodegeneration is associated with expression of inflammatory genes. Neurobiol. Dis. 2006; 24:67-88.

2007

1. Ahlqvist E, Bockermann R, Holmdahl R. Fragmentation of two quantitative trait loci controlling collagen-induced arthritis reveals a new set of interacting subloci. J Immunol. 2007;178:3084-90.

2. Aloisi, F., Serafini, B., Rosicarelli, B., Magliozzi R., Vora, A., Reynolds, R. Dendritic cells and lymphoid microenvironments in the inflamed CNS. Curr Topics Neuroimmunol., 2007, Tabira T., Yamamura , Kira J. Medimont International Proceedings, Bologna, Italy. Pp-103-107.

3. Biber K, Neumann H, Inoue K, Boddeke HW. Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci. 2007; 30:596-602.

4. Birdsall Abrams M, Josephson A, Dominguez C, Oberg J, Diez M, Spenger C, Olson L, Piehl F, Lidman O. Recovery from spinal cord injury differs between rat strains in a major histocompatibility complex-independent manner. Eur J Neurosci. 2007; 26:1118-27.

5. Cassan C, Liblau RS. Immune tolerance and control of CNS autoimmunity: from animal models to MS patients. J Neurochem. 2007;100:883-92. Review.

6. Friese MA, Craner MJ, Etzensperger R, Vergo S, Wemmie JA, Welsh MJ, Vincent A, Fugger L. Acid-sensing ion channel-1 contributes to axonal degeneration in autoimmune inflammation of the central nervous system. Nat Med. 2007;13:1483-9.

7. Galea I, Bernardes-Silva M, Forse PA, van Rooijen N, Liblau RS, Perry VH. (2007) An antigen-specific pathway for CD8 T cells across the blood-brain barrier. J Exp Med. 2007;204:2023-30.

8. Gelderman KA, Hultqvist M, Olsson LM, Bauer K, Pizzolla A, Olofsson P, Holmdahl R. Rheumatoid arthritis: the role of reactive oxygen species in disease development and therapeutic strategies. Antioxid Redox Signal. 2007;9:1541-67. Review.

9. Gelderman KA, Hultqvist M, Pizzolla A, Zhao M, Nandakumar KS, Mattsson R, Holmdahl R. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. J Clin Invest. 2007;117:3020-8.

10. Hultqvist M, Bäcklund J, Bauer K, Gelderman KA, Holmdahl R. Lack of reactive oxygen species breaks T cell tolerance to collagen type II and allows development of arthritis in mice. J Immunol. 2007; 179:1431-7.

11. Kiialainen A, Veckman V, Saharinen J, Paloneva J, Gentile M, Hakola P, Hemelsoet D, Ridha B, Kopra O, Julkunen I, Peltonen L. Transcript profiles of dendritic cells of PLOSL patients link demyelinating CNS disorders with abnormalities in pathways of actin bundling and immune response. J Mol Med. 2007; 85:971-83.

12. Kutzelnigg A, Faber-Rod JC, Bauer J, Lucchinetti CF, Sorensen PS, Laursen H, Stadelmann C, Brück W, Rauschka H, Schmidbauer M, Lassmann H. Widespread demyelination in the cerebellar cortex in multiple sclerosis. Brain Pathol. 2007; 17:38-44.

13. Lennart T. Mars, Jan Bauer, David A. Gross, Florence Bucciarelli, Huseyin Firat, Denis Hudrisier, Francois Lemonnier, Kostas Kosmatopoulos, and Roland S. Liblau. CD8 T Cell Responses to Myelin Oligodendrocyte Glycoprotein-Derived Peptides in Humanized HLA-A *0201-Transgenic Mice. J. Immunol. 2007; 179:5090-8.

14. Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallström E, Khademi M, Oturai A, Ryder LP, Saarela J, Harbo HF, Celius EG, Salter H, Olsson T, Hillert J. Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet. 2007; 39:1108-13.

15. Magliozzi, R., Howell, O., Vora, A., Serafini B., Nicholas, R., Puopolo, M., Reynolds, R., Aloisi, F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007; 130:1089-1104.

16. Maier K, Merkler D, Gerber J, Taheri N, Kuhnert AV, Williams SK, Neusch C, Bähr M, Diem R. Multiple neuroprotective mechanisms of minocycline in autoimmune CNS inflammation. Neurobiol Dis. 2007; 25:514-25.

17. Marik C, Felts PA, Bauer J, Lassmann H, Smith KJ. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? Brain 2007; 130:2800-15.

18. Mars LT, Bauer J, Gross DA, Bucciarelli F, Firat H, Hudrisier D, Lemonnier F, Kosmatopoulos K, Liblau RS. CD8 T cell responses to myelin oligodendrocyte glycoprotein-derived peptides in humanized HLA-A*0201-transgenic mice. J Immunol. 2007; 179:5090-8.

19. Metz I, Lucchinetti CF, Openshaw H, Garcia-Merino A, Lassmann H, Freedman MS, Atkins HL, Azzarelli B, Kolar OJ, Brück W. Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. Brain. 2007;130:1254-62.

20. Neumann H, Takahashi K. Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. J Neuroimmunol. 2007;184:92-9.

21. Olofsson P, Nerstedt A, Hultqvist M, Nilsson EC, Andersson S, Bergelin A, Holmdahl R. Arthritis suppression by NADPH activation operates through an interferon-beta pathway. BMC Biol. 2007;9:5:19.

22. Palin K, Cunningham C, Forse P, Perry VH, Platt N. Systemic inflammation switches the inflammatory cytokine profile in CNS Wallerian degeneration. Neurobiol Dis. 2008;30:19-29.

23. Serafini, B., Rosicarelli, B., Franciotta, D., Magliozzi, R., Reynolds, R., Cinque, P., Andreoni, P., Trivedi, P., Salvetti, M., Faggioni, A., Aloisi, F. Dysregulated Epstein Barr virus infection in the multiple sclerosis brain. J. Exp. Med. 2007; 204:2899-2912.

24. Smith KJ. Sodium channels and multiple sclerosis: roles in symptom production, damage and therapy. Brain Pathol. 2007; 17:230-42. Review. Erratum in: Brain Pathol. 2007;17:345.

25. Takahashi K, Prinz M, Stagi M, Chechneva O, Neumann H. TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. PLoS Med. 2007; 4(4):e124.

26. Taoufik E, Valable S, Müller GJ, Roberts ML, Divoux D, Tinel A, Voulgari-Kokota A, Tseveleki V, Altruda F, Lassmann H, Petit E, Probert L. FLIP(L) protects neurons against in vivo ischemia and in vitro glucose deprivation-induced cell death. J Neurosci. 2007;27:6633-46.

27. Taoufik E, Tseveleki V, Euagelidou M, Emmanouil M, Voulgari Kokota A, Haralambous S, Probert L. Positive and negative implications of TNF neutralisation for the pathogenesis of multiple sclerosis. Neurodegenerative Diseases 2007; 5:32-37.

28. Tseveleki V, Tsagozis P, Koutsoni O, Dotsika E, Probert L. Cellular FLIP long isoform transgenic mice overcome inherent Th2-biased immune responses to efficiently resolve Leishmania major infection. Int Immunol. 2007; 19:1183-89.

29. Winau, F., G. Hegasy, R. Weiskirchen, S. Weber, C. Cassan, P. A. Sieling, R. L. Modlin, R. S. Liblau, A. M. Gressner & S.H.E. Kaufmann Ito cells are professional liver-resident APCs for activating T cell responses. Immunity 2007; 26:117-29.

2008

1. Aloisi F, Columba-Cabezas S, Franciotta D, Rosicarelli B, Magliozzi R, Reynolds R, Ambrosini E, Coccia E, Salvetti M, Serafini B. Lymphoid chemokines in chronic neuroinflammation. J Neuroimmunol, 2008;198:106-12.

2. Anderton SM and Liblau RS. Regulatory T cells in the control of inflammatory demyelinating diseases of the central nervous system. Curr. Opin. Neurol. 2008;21:248-54.

3. Aulchenko YS, Hoppenbrouwers IA, Ramagopalan SV, Broer L, Jafari N, Hillert J, Link J, Lundström W, Greiner E, Dessa Sadovnick A, Goossens D, Van Broeckhoven C, Del-Favero J, Ebers GC, Oostra BA, van Duijn CM, Hintzen RQ. Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. Nat Genet. 2008;40:1402-3.

4. Bereczky-Veress B, Lidman O, Sabri F, Bergström T, Spenger C, Olsson T, Piehl F, Diez M, Sköldenberg B. Host strain-dependent difference in susceptibility in a rat model of herpes simplex type 1 encephalitis. J Neurovirol. 2008;14:102-18.

5. Couturier N., Zappulla J.P., Lauwers-Cances V., Uro-Coste E., Delisle M-B., Clanet M., Montagne L., Van der Valk P., Bö L. and Liblau R. S.. Mast cell transcripts are increased within and outside multiple sclerosis lesions. J Neuroimmunol. 2008;195:176-85.

6. Dal Bianco A, Bradl M, Frischer J, Kutzelnigg A, Jellinger K, Lassmann H Multiple Sclerosis and Alzheimer's Disease. Ann Neurol 2008; 63: 174-183.

7. Dolga, AM, Blank, T, Luiten, PGM, Eisel, ULM, and Nijholt, IM. TNF-alpha induces neuroprotection via NF-kB-dependent up-regulation of small conductance calcium-activated potassium channels. J Neurochem. 2008;107:1158-67.

8. Dolga, AM; I M. Nijholt; A Ostroveanu; Q ten Bosch; P G M Luiten, and U L M Eisel Lovastatin induces neuroprotection through tumor necrosis factor receptor 2 signaling pathways J Alz Dis 2008; 13:1-12.

9. Dominguez CA, Li L, Lidman O, Olsson T, Wiesenfeld-Hallin Z, Piehl F, Xu XJ. Both MHC and non-MHC genes regulate development of experimental neuropathic pain in rats. Neurosci Lett. 2008;442:284-286.

10. Dominguez CA, Lidman O, Hao J-X, Diez M, Tuncel J, Olsson T, Wiesenfeld-Hallin Z, Piehl F, Xu XJ. Genetic analysis of neuropathic pain-like behavior following peripheral nerve injury suggests a role of the major histocompatibility complex in development of allodynia. Pain 2008;136:313-9.

11. Dominguez CA, Lidman O, Olsson T, Wiesenfeld-Hallin Z, Piehl F, Xu XJ.Contrasting genetic effects of major histocompatibility complex on ischemic peripheral nerve and spinal cord injury in female rats. Neurosci Lett. 2008;443:95-8.

12. Farmer C. E., Smith K.J., Docherty R.J. Low concentrations of tetrodotoxin interact with tetrodotoxin-resistant voltage-gated sodium channels. Brit. J. Pharmacol. 2008; 155:34-43.

13. Franciotta D, Salvetti M, Lolli F, Serafini B, Aloisi F B cells and multiple sclerosis. Lancet Neurol, 2008;7:852-8. Review.

14. Friese MA, Jakobsen KB, Friis L, Etzensperger R, Craner MJ, McMahon RM, Jensen LT, Huygelen V, Jones EY, Bell JI, Fugger L. Opposing effects of HLA class I molecules in tuning autoreactive CD8+ T cells in multiple sclerosis. Nat Med. 2008;14:1227-35.

15. Galea I, Felton LM, Waters S, van Rooijen N, Perry VH, Newman TA. Immune-to-brain signalling: the role of cerebral CD163-positive macrophages. Neurosci Lett. 2008;448:41-6.

16. Harnesk K, Swanberg M, Öckinger J, Diez M, Lidman O, Lobell A, Wallström E, Olsson T, Piehl F. Vra4 congenic rats with allelic differences in the class II transactivator gene display altered susceptibility to experimental autoimmune encephalomyelitis. J Immunol 2008;180:3289-3296.

17. Hochmeister S, Zeitelhofer M, Bauer J, Nicolussi EM, Fischer MT, Heinke B, Selzer E, Lassmann H, Bradl M. After injection into the striatum, in vitro differentiated microglia- and bone marrow derived dendritic cells can leave the central nervous system via the blood stream. Am J Pathol. 2008;173:1669-81.

18. Holmdahl R. IL-21 and autoimmune disease - hypothesis and reality? Eur J Immunol 2008;38:1800-2.

19. International Multiple Sclerosis Genetics Consortium (IMSGC) Refining genetic associations in multiple sclerosis. Lancet Neurol. 2008; 7:567-9.

20. International Multiple Sclerosis Genetics Consortium (IMSGC, numerous contributors, among those Tomas Olsson). The expanding genetic overlap between multiple sclerosis and type 1 diabetes. Genes Immun. 2009;10:11-4.

21. International Multiple Sclerosis Genetics Consortium (IMSG - 65 contributors including Tomas Olsson). Refining genetic associations in multiple sclerosis. Lancet Neurology 2008; 7:567-569.

22. Kemppinen A, Suvela M, Tienari PJ, Elovaara I, Koivisto K, Pirttilä T, Reunanen M, Baumann P, Hillert J, Lundmark F, Oturai A, Ryder L, Harbo HF, Celius EG, Palotie A, Daly M, Peltonen L, Saarela J. MYO9B polymorphisms in multiple sclerosis. Eur J Hum Genet. 2009;17:840-3.

23. Khademi M, Stol M, Olsson T, Wallström E. Induction of TNF alphą Expression in Natalizumab treated Multiple Sclerosis. Eur J Neurol 2008;15:309-12.

24. Kontermann, R, Münkel, S, Neumeyer, J, Müller, D, Branschädel, M, Scheurich, P and Pfizenmaier, K. A humanized tumor necrosis factor receptor 1 (TNFR1)-specific antagonistic antibody for selective inhibition of tumor necrosis factor (TNF) action. J Immunother 2008;31: 225-234.

25. Kristjansdottir G, Sandling J, Sigurdsson S, Bonetti A, Roos I, Lundmark A, Tienari P, Hillert J, Olsson T, Alcina A, Fernandez O, Leyva L, Guerrero M, Lucas M, Izquierdo G Matesanz F, Syvönen A-C. Polymorphisms in the interferon regulatory factor 5 (IRF5) gene are associated with multiple sclerosis in three distinct populations. J Med Genet. 2008;45:362-9.

26. Kuhlmann T, Mirron V, Cuo Q, Antel J, Brück W. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic MS. Brain 2008;131:1749-1758.

27. Lande, R., Gafa, G., Serafini, B., Giacomini E., Visconti, A., Remoli, ME., Severa, M., Parmentier, M., Ristori, G., Salvetti, M., Aloisi, F., Coccia, E. Plasmacytoid dendritic cells in MS: intracerebral recruitment and impaired maturation in response to IFN-β. J. Neuropathol. Exp. Neurol. 2008;67:388-401.

28. Mars L, Gautron A-S, Novak J, Beaudoin L, Diana J, Liblau R, Lehuen A. Invariant NKT cells regulate EAE and infiltrate the CNS in a CD1d-independent manner. J. Immunol. 2008;181:2321-29.

29. Nave KA, Trapp BD. Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci. 2008;31:535-61.

30. Nijholt IM, Ostroveanu A, Luiten PG, Van der Zee EA and Eisel ULM Inhibition of PKA anchoring to A-kinase anchoring proteins impairs consolidation and facilitates extinction of contextual fear memories Neurobiol Learn Mem 2008;90: 223-229

31. Nijholt, IM, Dolga, AM, Vieth, T, Ostroveanu, A, de Bruyn, M, Luiten, PGM, Schmidt, M and Eisel, ULM (2008) Neuronal AKAP150 coordinates PKA and Epac2 mediated PKB/AKT phosphorylation. Cell Signal. 2008;20:1715-24.

32. Olsson T, Hillert J. The Genetics of Multiple Sclerosis and its Experimental Models. Current Opinion in Neurology, 2008; 21: 255-260.

33. Popovic M, Ahlqvist E, Rockenbauer E, Bockermann R, Holmdahl R. Identification of new loci controlling collagen-induced arthritis in mouse using a partial advanced intercross and congenic strains. Scand J Immunol 2008;68:405-13.

34. Sättler MB, Togni M, Gadjanski I, Sühs KW, Meyer N, Bähr M, Diem R Strain-specific susceptibility for neurodegeneration in a rat model of autoimmune optic neuritis. J Neuroimmunol 2008;193: 77-86.

35. Sättler MB, Williams SK, Neusch C, Otto M, Pehlke J, Bähr M, Diem R. Flupirtine as neuroprotective add-on therapy in autoimmune optic neuritis. Am J Pathol. 2008;173:1496-507.

36. Saxena A, Bauer J, Scheikl T, Zappulla J, Audebert M, Desbois S, Waisman A, Lassmann H, Liblau RS, Mars LT Cutting edge: Multiple sclerosis like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. J Immunol 2008; 181: 1617-1621.

37. Schwarting S., Litwak S., Hao W., Bähr M., Weise J. and Neumann H. Hematopoietic stem cells reduce postischemic inflammation and ameliorate ischemic brain injury. Stroke, Stroke. 2008;39:2867-75.

38. Serafini B, Magliozzi R, Rosicarelli B, Reynolds R, Zheng TS, Aloisi F Expression of TWEAK and its receptor Fn14 in the multiple sclerosis brain: implications for inflammatory tissue injury. J Neuropathol Exp Neurol, 2008 67:1137-48.

39. Taoufik E & Probert L. Ischemic neuronal damage. Current Pharmaceutical Design 2008;14: 3565-73.

40. Taoufik E, Petit E, Divoux D, Tseveleki V, Mengozzi M, Roberts ML, Valable S, Ghezzi P, Quackenbush J, Brines M, Cerami A, Probert L. TNF receptor I sensitizes neurons to

erythropoietin- and VEGF-mediated neuroprotection after ischemic and excitotoxic injury. Proc. Natl. Acad. Sci. USA 2008;105:6185-6190 .

41. Tárnok, K., Kiss, E., Luiten, P.G.M., Nyakas, Cs., Tihanyi, K., Schlett, K., Eisel, U.L.M. Effects of Vinpocetine on mitochondrial function and neuroprotection in primary cortical neurons. Neurochem Int. 2008;53:289-95.
42. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? Annu. Rev. Neurosci. 2008;31:247-69.

2009

1. Ahlqvist, E., M. Hultqvist, and R. Holmdahl. The value of animal models in predicting genetic susceptibility to complex diseases such as rheumatoid arthritis. Arthritis Res Ther. 2009;11:226.
2. Bradl M, Misu T, Takahashi T, Watanabe M, Mader S, Reindl M, Adzemovic M, Bauer J, Berger T, Fujihara K, Itoyama Y, Lassmann H. Autoantibodies against aquaporin 4 found in patients with neuromyelitis optica are pathogenic in vivo. Ann Neurol 2009;66:630-43.
3. Brynedal B, Bomfim IL, Olsson T, Duvefelt K, Hillert J. Differential expression and genetic association of CD58 in Swedish multiple sclerosis patients, Proc Natl Acad Sci U S A. 2009;106(23):E58.
4. De Jager PL, Baecher-Allan C, Maier LM, Arthur AT, Ottoboni L, Barcellos L, McCauley JL, Sawcer S, Goris A, Saarela J, Yelensky R, Price A, Leppa V, Patterson N, de Bakker PI, Tran D, Aubin C, Pobywajlo S, Rossin E, Hu X, Ashley CW, Choy E, Rioux JD, Pericak-Vance MA, Ivinson A, Booth DR, Stewart GJ, Palotie A, Peltonen L, Dubois B, Haines JL, Weiner HL, Compston A, Hauser SL, Daly MJ, Reich D, Oksenberg JR, Hafler DA. The role of the CD58 locus in multiple sclerosis, Proc Natl Acad Sci U S A. 2009;106:5264-9.
5. Diez M, Abdelmagid N, Harnesk K, Ström M, Lidman O, Swanberg M, Lindblom R, Al-Nimer F, Jagodic M, Olsson T, Piehl F. Identification of gene regions regulating inflammatory microglial response in the rat CNS after nerve injury. Journal of Neuroimmunology, 2009;212:82-92.
6. Dolga, AM, Granic, I, Nyakas, C, van der Zee, EA, Luiten, PGM and Eisel, ULM (2008) Pretreatment with Lovastatin prevents N-Methyl-D-Aspartate-induced neurodegeneration in the Magnocellular Nucleus Basalis and behavioral dysfunction. J Alzheimers Dis. 2009;17:327-36.
7. Dornmair, K., Meinl, E., and Hohlfeld, R. Novel approaches for identifying target antigens of autoreactive human B and T cells. Sem. Immunopathol. 2009;31:467-477.
8. Eisel, U. L. M., Dobos, N.,Dierckx, R., Luiten, P.G.M., Korf, J. (2010) Tumor Necrosis Factor as Neuroinflammatory Mediator in Alzheimer's Disease and Stroke. Molecular mechanisms and neuroinflammatory imaging. Inflammatory mediators, stroke and neurodegenerative disease. In Neuroimmune Biology (Vol. 9) The Brain and Host Defence (Ed. I. Berczi).
9. Emmanouil M, Taoufik E, Tseveleki V, Vamvakas SS, Tselios T, Karin M, Lassmann H, Probert. Neuronal I kappa B kinase beta protects mice from autoimmune encephalomyelitis by mediating neuroprotective and immunosuppressive effects in the central nervous system. J.Immunol. 2009;183:7877-89.
10. Friese MA, Fugger L. Pathogenic CD8(+) T cells in multiple sclerosis. Ann Neurol. 2009;66:132-41. Review.

11. Frischer J, Bramow S, Dal-Bianco A, Lucchinetti C, Rauschka H, Schmidbauer M, Laursen H, Soelberg Sorensen P, Lassmann H The Relation between Inflammation and Neurodegeneration in Multiple Sclerosis Brains. Brain 2009;132:1175-1189.

12. Fugger L, Friese MA, Bell JI. From genes to function: the next challenge to understanding multiple sclerosis. Nat Rev Immunol. 2009;9:408-17.

13. Gadjanski I, Boretius S, Lingor P, Knöfferle J, Sättler MB, Williams SK, Sühs KW, Michaelis T, Frahm J, Bähr M, Diem R. Axon degeneration in autoimmune optic neuritis depends on the N-type of voltage-gated calcium channels. Ann Neurol 2009;66:81-93.

14. Gaikwad S, Larionov S, Wang Y, Dannenberg H, Matozaki T, Monsonego A, Thal DR, Neumann H. Signal regulatory protein-β1: a microglial modulator of phagocytosis in Alzheimer's disease. Am. J. Pathol 2009;175:2528-39.

15. Gillett A, Maratou K, Fewings C, Harris RA, Jagodic M, Aitman T, Olsson T. Alternative Splicing and Transcriptome Profiling of Experimental Autoimmune Encephalomyelitis Using Genome-wide Exon Arrays. Plos One 2009;4:e7773.

16. Gjertsson, I., K. L. Laurie, J. Devitt, S. J. Howe, A. J. Thrasher, R. Holmdahl, and K. Gustafsson. Tolerance induction using lentiviral gene delivery delays onset and severity of collagen II arthritis. Mol Ther 2009; 17:632-640.

17. Goldschmidt T, Antel J, König FB, Brück W, Kuhlmann T. Remyelination capacity of the MS brain decreases with disease chronicity. Neurology 2009; 72:1914-1921.

18. Gorlovoy P, Larionov S, Pham TT, Neumann H. (2009) Accumulation of tau induced in neurites by microglial proinflammatory mediators. FASEB J. 2009;23:2502-13.

19. Granic, I., A.M. Dolga, I.M. Nijholt, G. van Dijk, U.L.M. Eisel (2009) Inflammation and NF-kB in Alzheimer's disease and Diabetes. J Alz Dis. 16:809-821.

20. Granic, I., K.C. Mulder, I.M. Nijholt, P.J.W. Naude, A. de Haan, B. Penke, P.G.M. Luiten, U.L.M. Eisel Beta-sheet breaker LPYFDa neutralizes Aβ-induced toxicity and memory impairment. J Alzheimers Dis. 2009;16:809-21. Review.

21. Harnesk K, Swanberg M, Diez M, Olsson T, Piehl F, Lidman O. Differential nerve injury-induced expression of MHC class II in the mouse correlates to genetic variability in the type I promoter of *C2ta*. J Neuroimmunology, 2009;212:44-52.

22. Harkiolaki M, Holmes SL, Svendsen P, Gregersen JW, Jensen LT, McMahon R, Friese MA, van Boxel G, Etzensperger R, Tzartos JS, Kranc K, Sainsbury S, Harlos K, Mellins ED, Palace J, Esiri MM, van der Merwe PA, Jones EY, Fugger L. T cell-mediated autoimmune disease due to low-affinity crossreactivity to common microbial peptides. Immunity. 2009;30:348-57.

23. Hedström A, Bäärnhielm M, Wallström E, Hillert J, Olsson T, Alfredsson L. Tobacco smoking, but not Nicotine increases the risk of Multiple Sclerosis. Neurology. 2009; 73:696-701.

24. Hochmeister, S., Bittner, R.E., Höger, H., Lassmann, H., and Bradl, M. The susceptibility to experimental autoimmune encephalomyelitis is not related to dysferlin-deficiency. Autoimmunity 2009;42: 235-241.

25. Huberle, A., Beyeen, A.D., Öckinger, J., Ayrua, M., Jagodic, M., de Graaf, K.L., Fissolo, N., Marta, M., Olofsson, P., Hultqvist, M., Holmdahl, R., Olsson, T. and R. Weissert. Advanced intercross line mapping suggests that Ncf1 regulates severity in an animal model of Guillain-Barré syndrome, J Immunol 2009;182:4432-4438.

26. Hultqvist, M., K. S. Nandakumar, U. Bjorklund, and R. Holmdahl. The novel small molecule drug Rabeximod is effective in reducing disease severity of mouse models of autoimmune disorders. Ann Rheum Dis 2009; 68:130-135.

27. Hultqvist, M., L. M. Olsson, K. A. Gelderman, and R. Holmdahl. 2009. The protective role of ROS in autoimmune disease. Trends Immunol. 30:201-8. Review.

28. International Multiple Sclerosis Genetics Consortium (IMSGC, numerous contributors, among those Tomas Olsson). The expanding genetic overlap between multiple sclerosis and type 1 diabetes. Genes Immun. 2009;10:11-4.

29. Jagodic M, Colacios C, Nohra R, Dejean A, Beyeen AD, Casemayou A, Lamouroux L, Sjoholm L, Bernard I, Lagrange D, Dahlman I, Lundmark F, Seddighzadeh M, Klareskog L, Alfredsson L, Padyukov L, Khademi M, Hillert J, Clanet M, Edan G, Fontaine B, Fournie GJ, Saoudi A, Kockum I, Olsson T. Variants of *Vav1* affects TNF and inflammatory diseases. Science Translational Medicine. 2009;1(10):10ra21.

30. Kallio S., E. Jakkula, S. Purcell, M. Suvela, K. Koivisto, P. Tienari, I. Elovaara , T. Pirttilä , M. Reunanen, D. Bronnikov, M. Viander, J. Hillert, F. Lundmark, H. Harbo, Å. Lorentzen, P. De Jager, M.J. Daly, D. Hafler, A. Palotie, L. Peltonen, J. Saarela: Use of a Genetic Isolate to Identify Rare Disease Variants: C7 on 5p associated with MS. Hum Mol Genet. 2009;18:1670-1683.

31. Kemppinen A., M. Suvela, P.J. Tienari, I. Elovaara, K. Koivisto, T. Pirttilä, M. Reunanen, P. Baumann, J. Hillert, F. Lundmark, A. Oturai, L. Ryder, H.F. Harbo, E.G. Celius, A. Palotie, M. Daly, L. Peltonen, J. Saarela: MYO9B polymorphisms in multiple sclerosis. Eur J Hum Genet. 2009;17:840-3.

32. Kierdorf K, Wang Y, Neumann H (2009) Immune-Mediated CNS Damage. Results Probl Cell Differ. 2010;51:173-96. Review.

33. Krishnamoorthy G, Saxena A, Mars LT, Domingues HS, Mentele R, Ben Nun A, Lassmann H, Dornmair K, Kurschus FC, Liblau RS, Wekerle H. Myelin-specific T cells also recognize neuronal autoantigen in a transgenic mouse model of multiple sclerosis. Nature Med 2009;15: 626-632.

34. Kuhlmann T, Goldschmidt T, Antel J, Wegner C, König F, Metz I, Brück W. Gender differences in the histopathology of MS? J. Neurol. Sci. 2009;286:86-91.

35. Lindvall, T., J. Karlsson, R. Holmdahl, and A. Andersson. 2009. Dissection of a locus on mouse chromosome 5 reveals arthritis promoting and inhibitory genes. Arthritis Res Ther 11:R10.

36. Mahad D.J., I. Ziabreva, G. Campbell, F. Laulund, J.L. Murphy, A.K. Reeve, L. Greaves, K.J. Smith, D.M. Turnbull. Detection of cytochrome c oxidase activity and mitochondrial protein immunoreactivity in single cells. J Neurosci Methods. 2009;184:310-9.

37. Mars L.T., L. Araujo, P. Kerschen, S. Diem, E. Bourgeois, L.P. Van, N. Carrié, M. Dy, R. S. Liblau and A. Herbelin. Invariant NKT cells inhibit development to the Th17 lineage. Proc Natl Acad Sci U S A. 2009; 106: 6238-43.

38. Mero IL, Lorentzen AR, Ban M, Smestad C, Celius EG, Aarseth JH, Myhr KM, Link J, Hillert J, Olsson T, Kockum I, Masterman T, Oturai AB, Søndergaard HB, Sellebjerg F, Saarela J, Kemppinen A, Elovaara I, Spurkland A, Dudbridge F, Lie BA, Harbo HF. A rare variant of the TYK2 gene is confirmed to be associated with multiple sclerosis. Eur J Hum Genet. 2010;18:502-4.

39. Messerschmidt SK, Musyanovych A, Altvater M, Scheurich P, Pfizenmaier K, Landfester K, Kontermann RE. Targeted lipid-coated nanoparticles: Delivery of tumor necrosis factor-functionalized particles to tumor cells. J Control Release 2009:137, 69-77.

40. Napoli I, Kierdorf K, Neumann H. Microglial precursors derived from mouse embryonic stem cells. Glia 2009;57:1660-71.

41. Napoli I, Neumann, H. Microglial clearance function in health and disease. Neuroscience. 2009;158:1030-8.

42. Neumann H, Kotter MR, Franklin RJ Debris clearance by microglia: an essential link between degeneration and regeneration. Brain. 2009;132:288-95. Review.

43. Nicolussi, E.M., Huck, S., Lassmann, H., and Bradl, M. The cholinergic anti-inflammatory system limits T cell infiltration into the neurodegenerative CNS, but can not counteract complex CNS inflammation. Neurobiol. Dis. 2009;35:24-31.

44. Piehl F, Olsson T. Inflammation and susceptibility to neurodegeneration: The use of unbiased genetics to decipher critical regulatory pathways. Neuroscience. 2009;158:1143-50. Review.

45. Richter, C., Herrero San Juan, M., Will, J., Brandes, R.P., Kalinke, U., Akira, S., Pfeilschifter, J.M., Hultqvist, M., Holmdahl, R. and H.H. Radecke. Ncf1 provides a reactive oxygen species-independent negative feedback regulation of TLR9-induced IL-12p70 in murine dendritic cells. J Immunol 2009;182:4183-4191.

46. Salvetti M, Giovannoni G, Aloisi F. Epstein-Barr virus and multiple sclerosis. Curr Opin Neurol. 2009;22:201-6. Review.

47. Schoderboeck, L., Adzemovic, M., Nicolussi, E.M., Crupinschi, C., Hochmeister, S., Fischer, M.-T., Lassmann, H., and Bradl, M. The "window of susceptibility" for inflammation in the immature central nervous system is characterized by a leaky blood brain barrier and the local expression of inflammatory chemokines. Neurobiol. Dis. 2009; 35:368-375.

48. Sellebjerg F, Börnsen L, Khademi M, Krakauer M, Olsson T, Fredriksen JL, Sörensen PS. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. Neurology. 2009;73:2003-10.

49. Sulonen A-M., S.P. Kallio, P. Ellonen, M. Suvela, I. Elovaara, K. Koivisto, T. Pirttilä, M. Reunanen, P.J. Tienari, A. Palotie, L. Peltonen, J. Saarela: No evidence for shared etiology in two demyelinative disorders, MS and PLOSL. J Neuroimmunol. 2009; 206:86-90.

50. Thessen Hedreul M, Gillett A, Olsson T, Jagodic M, Harris RA. Characterization of Multiple Sclerosis Candidate gene expression in rat Experimental Autoimmune encephalomyelitis. J Neuroimmunol 2009;210:30-9.

51. van der Most PJ, Dolga AM, Nijholt IM, Luiten PG, Eisel UL. Statins: mechanisms of neuroprotection. Prog Neurobiol 2009;88:64-75.

52. Walter L, Neumann H.. Role of microglia in neuronal degeneration and regeneration. Semin Immunopathol. 2009;31:513-25.

53. Witan H, Gorlovoy P, Kaya AM, Koziollek-Drechsler I, Neumann H, Behl C, Clement AM. Wild-type Cu/Zn superoxide dismutase (SOD1) does not facilitate, but impedes the formation of protein aggregates of amyotrophic lateral sclerosis causing mutant SOD1. Neurobiol. of Disease 2009; 36:331-42.

54. Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, Roughan JE, Almendinger SE, Blewett MM, Brück W, Hafler DA, O'Connor KC. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain, 2009;132:3318-28.

2010

1. Aloisi F. Pathways leading to multiple sclerosis in the search for better treatments. 'Projects' Journal, Insight Publishers, 2010; vol. 18:86-88.

2. Aloisi F, Serafini B, Magliozzi R, Howell OW, Reynolds R Detection of Epstein-Barr virus and B-cell follicles in the multiple sclerosis brain: what you find depends on how and where you look. Brain 2010 Aug 25.

3. Annibali V, Ristori G, Angelini DF, Serafini B, Mechelli R, Cannoni S, Romano S, Paolillo A, Abderrahim H, Diamantini A, Borsellino G, Aloisi F, Battistini L, Salvetti M. CD161highCD8+T cells bear pathogenetic potential in multiple sclerosis. Brain, in press.
4. Bereczky-Veress B, Abdelmagid N, Piehl F, Bergström T, Olsson T, Birgit Sköldenberg B, Diez M. Influence of perineurial cells and Toll-like receptors 2 and 9 on Herpes simplex type 1 entry to the central nervous system in rat encephalitis. PLoS One. 2010 Aug 27;5:e12350.
5. Beutner C, Roy K, Linnartz B, Napoli I and Neumann H. Generation of microglial cells from mouse embryonic stem cells. Nat Protoc. 2010;5:1481-94.
6. Beyeen AD, Zeitelhofer-Adzemovic M, Öckinger J, Stridh P, Becanovic K, Lassmann H, Harris R, Hillert J, Alfredsson L, Kockum I, Jagodic M, Olsson T. IL22RA2 associates with multiple sclerosis and macrophage effector mechanisms in experimental neuroinflammation. J Immunol. 2010185:6883-6890.
7. Botvinnik A, Wichert SP, Fischer TM, Rossner M. Integrated analysis of receptor activation and downstream signaling with EXTassays. Nature Methods. 2010;7:74-80.
8. Bramow S, Frischer JM, Lassmann H, Koch-Henriksen N, Lucchinetti CF, Sørensen PS, Laursen H. Demyelination versus remyelination in progressive multiple sclerosis. Brain 2010;133:2983-98.
9. Broom LJ, Perry VH (2010) Axon Degeneration: Mechanisms and Consequences "New Aspect of Axonal Structure and Function" Editors Dirk Feldmeyer and Joachin H R Lubke.
10. Brzózka MM, Radyushkin R, Wichert SP, Ehrenreich H and Rossner M. Cognitive and sensorimotor gating impairments in transgenic mice overexpressing the schizophrenia susceptibility gene Tcf4 in the forebrain. Biological Psychiatry. 2010; 68: 33-40.
11. Cavalcante P§, Serafini B§, Rosicarelli B, Maggi L, Barberis M, Antozzi C, Berrih-Aknin S, Bernasconi P, Aloisi F*, Mantegazza R*. Epstein-Barr virus persistence and reactivation in myasthenia gravis thymus. Ann Neurol 2010;67:726-38. § These authors equally contributed to this work. *These authors share equal credit for senior authorship.
12. Dziedzic T, Metz I, Dallenga T, König FB, Müller S, Stadelmann C, Brück W. Wallerian degeneration: a major component of early axonal pathology in multiple sclerosis. Brain Pathol 2010;20:976-85,
13. Evangelidou M, Tseveleki V, Vamvakas SS, Probert L. TNFRI is a positive T-cell costimulatory molecule important for the timing of cytokine responses. Immunol Cell Biol. 2010; 88:586-95.
14. Fischer R, Maier O, Naumer M, Krippner-Heidenreich A, Scheurich P, Pfizenmaier K. Ligand-induced internalization of TNF receptor 2 mediated by a di-leucin motif is dispensable for activation of the NFκB pathway. Cell Signal 2010;23, 161-170.
15. Gillett A, Marta M, Jin Tao, Tuncel J, Leclerc P, Nohra R, Lange S, Holmdahl R, Olsson T, Harris RA, Jagodic M. TNF production in macrophages is genetically determined and regulates inflammatory disease in rats. J Immunol. 2010;185:442-50.
16. Gillett A, Thessen Hedreul M, Khademi M, Espinosa A, Beyen AD, Jagodic M, Kockum I, Harris RA, Olsson T. Interleukin 18 Receptor 1 expression distinguishes MS patients. Mult Scler. 2010;16:1056-65.
17. Goebbels S, Oltrogge J, Kemper R, Heilmann I, Bormuth I, Wolfer S, Wichert S, Moebius W, Liu X, Lappe-Siefke C, Rossner M, Groszer M, Suter U, Frahm J, Boretius S, and Nave KA. Elevated phosphatidylinositol-3,4,5-trisphosphate in glia triggers cell autonomous membrane wrapping and myelination. J Neurosci. 2010;30:8953-64.
18. Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, Vrethem M, Olsson T, Piehl F, Norgren N, Rosengren L, Svenningsson A, Lycke J. Axonal damage in multiple sclerosis is markedly reduced by natalizumab treatment. Ann. Neurol. 2010, in press.
19. Hultqvist M, Sareila O, Vilhardt F, Olsson LM, Olofsson P, Hellman U, et al. Positioning of a

Polymorphic Quantitative Trait Nucleotide in the Ncf1 Gene Controlling the Oxidative Burst Response and Arthritis Severity in Rats. Antioxid Redox Signal 2010; under revision.

20. Hultqvist, M., Nandakumar, K.S., Björklund, U., and R. Holmdahl. Rabeximod reduces arthritis severity in mice by decreasing activation of inflammatory cells. Ann Rhem Dis 2010;69;1527-1532.
21. International Multiple Sclerosis Genetics Consortium (IMSGC) (Olsson T among around 65 authors). KIF1B is not associated with multiple sclerosis, Nat Genet. 2010;42:469-70;
22. International Multiple Sclerosis Genetics Consortium (IMSGC) (Olsson T among around 65 authors). IL12A ,MPOSPH9/CDK2AP1 and RGS1are multiple sclerosis susceptibility loci. Genes and Immunity, 2010;11: 397-405.
23. Jakkula E, Leppä V, Sulonen AM, Varilo T, Kallio S, Kemppinen A, Purcell S, Koivisto K, Tienari P, Sumelahti ML, Elovaara I, Pirttilä T, Reunanen M, Aromaa A, Oturai AB, Søndergaard HB, Harbo HF, Mero IL, Gabriel SB, Mirel DB, Hauser SL, Kappos L, Polman C, De Jager PL, Hafler DA, Daly MJ, Palotie A, Saarela J, Peltonen L. Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. Am J Hum Genet. 2010;86:285-91.
24. Larionvo S, Wielgat P, Wang Y, Thal RD, Neumann H. Spatially pathogenic forms of tau detected in Alzheimer's disease brain tissue by fluorescence lifetime-based Förster resonance energy transfer. J. Neurosci. Methods 2010; 192: 127–137.
25. Linker RA, Lee D-H, Cetin S, Wiese S, Kruse N, Siglienti I, Gerhardt E, Neumann H, Sendtner M, Lühder F., Gold R. Functional role of brain derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. Brain 2010;133:2248-63.
26. Linnartz B, Wang Y, Neumann H. (2010). Microglial immunoreceptor tyrosine-based activation and inhibition motif (ITAM/ITIM) signaling in neuroinflammation. Review. International Journal of Alzheimer's disease, Volume 2010, Article ID 587463, doi:10.4061/2010/587463.
27. Magliozzi R, Howell O.W, Roncaroli F, Nicholas R, Serafini B, Aloisi F, Reynolds R Substantial neuronal loss in the grey matter accompanies the presence of meningeal inflammation with B-cell follicles in multiple sclerosis. Ann Neurol. 2010;68:477-93.
28. Mars LT, Saikali P, Liblau RS, Arbour N. Contribution of CD8 T lymphocytes to the immuno-pathogenesis of multiple sclerosis and its animal models. Biochim Biophys Acta. 2010 Jul 15. [Epub ahead of print].
29. Marta M, Stridh P, Becanovic K, Gillett A, Ockinger J, Lorentzen JC, Jagodic M, Olsson T. Multiple loci comprising immune-related genes regulate experimental neuroinflammation. Genes Immun. 2010;11:21-36.
30. Melchior B, Garcia AE, Hsiung B-K, Lo KM, Doose JM, Thrash JC, Stalder AK, Staufenbiel M., Neumann H. and Carson MJ. Dual induction of TREM2 and tolerance-related transcript, Tmem176b, in amyloid transgenic mice: implications for vaccine-based therapies for Alzheimer's disease. ASN NEURO 2010;2(3):art:e00037.doi:10.1042/AN20100010
31. Napoli I, Neumann H. Protective effects of microglia in multiple sclerosis. Exp Neurol. 2010;225:24-8.
32. Nave KA. Myelination and the trophic support of long axons. Nat.Rev.Neurosci. 2010; 11:275-283.
33. Öckinger J, Stridh P , Beyen A, Lundmark F, Seddighzadeh M, Padyukov L, Hillert J, Kockum I, Jagodic M*, Olsson T*. Genetic variants of CC chemokine genes in experimental autoimmune encephalomyelitis, multiple sclerosis and rheumatoid arthritis. Genes Immun. 2010;1:142-54. *equal contribution.
34. Ottervald J, Franzén B, Nilsson K, Andersson LI, Khademi M, Eriksson B, Kjellström S, Marko-Varga G, Végvári A, Harris RA, Laurell T, Miliotis T, Matusevicius D, Salter H, Ferm M, Olsson

T. Multiple Sclerosis: Identification and clinical evaluation of novel CSF biomarkers. J Proteomics. 2010;73:1117-32.

35. Sättler MB, Bähr M. Future neuroprotective strategies. Review. Exp Neurol. 2010;225:40-7.
36. Schwarting S and Neumann H. Immunoregulatory neuroprotection of cerebral ischaemia by haematopoietic stem and precursor cells. Review. EU Neurological Review 2010; 4: 42-45.
37. Serafini B, Columba-Cabezas S, Severa M, Rosicarelli B, Veroni C, Chiappetta G, Magliozzi R, Reynolds R, Coccia E, Aloisi F. Expression of Epstein-Barr virus latent membrane protein 2A and BAFF in B cells in the multiple sclerosis brain: implications for viral persistence and intrathecal B-cell activation. J Neuropathol Exp Neurol. 2010;69:677-93
38. Sharma R, Fischer MT, Bauer J, Smith KJ, Felts PA, Misu T, Fujihara K, Bradl M, Lassmann H. Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropath (Berl) 2010;120: 223-236.
39. Sharma R., M.T. Fischer, J. Bauer, K.J. Smith, T. Misu, K. Fujihara, M. Bradl, H. Lassmann. Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropathol. 2010;120:223-36.
40. Scheikl T. Pignolet B., Mars LT. Liblau RS. Transgenic mouse models of multiple sclerosis. Cell Mol Life Sci. 2010;67:4011-34.
41. Stridh PM, Hedreul MT, Beyeen AD, Adzemovic M, Gillet A, Öckinger J, Marta M, Lassmann H, Becanovic K, Jagodic M, Olsson T. Fine mapping resolves Eae23 into two QTLs and implicates ZEB1 as a candidate gene regulating experimental neuroinflammation. PloS One 2010; e 12716.
42. Sundqvist E, Bäärnhielm M, Alfredsson L, Hillert J, Olsson T, Kockum I. Confirmation of association between multiple sclerosis and CYP27B1. Eur J Hum Genet. 2010;18:1349-1352.
43. The International Multiple Sclerosis Genetics Consortium (IMSGC). The genetic association of variants in CD6, TNFRSF1A and IRF8 to multiple sclerosis. In press.
44. Tseveleki V, Rubio R, Vamvakas SS, White J, Taoufik E, Petit E, Quackenbush J, Probert L. Comparative gene expression analysis in mouse models for multiple sclerosis, Alzheimer's disease and stroke for identifying commonly regulated and disease-specific gene changes. Genomics 2010;96:82-91.
45. Tseveleki V, Rubio R, Vamvakas SS, White J, Taoufik E, Petit E, Quackenbush J, Probert L. Comparative gene expression analysis in mouse models for multiple sclerosis, Alzheimer's disease and stroke for identifying commonly regulated and disease-specific gene changes. Genomics 2010;96:82-91.
46. Vergo S, Craner MJ, Etzensperger R, Attfield K, Friese MA, Newcombe J, Esiri M, Fugger L. Acid-sensing ion channel 1 is involved in both axonal injury and demyelination in multiple sclerosis and its animal model. Brain 2010, in press.
47. Veroni C, Gabriele L, Canini I, Castiello L, Coccia E, Remoli ME, Columba-Cabezas S, Aricò E, Aloisi F, Agresti C. Activation of TNF receptor 2 in microglia promotes induction of anti-inflammatory pathways. Mol Cell Neurosci. 2010;45:234-44.
48. Wang Y, Neumann H. Alleviation of neurotoxicity by microglial human Siglec-11. J. Neurosci. 2010; 30:3482–3488.
49. Zettlitz KA, Lorenz V, Landauer, KH, Münkel S, Herrmann A, Scheurich P, Pfizenmaier K, Kontermann RE. ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one-specific antibody. MAbs 2010;2:639-647.

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