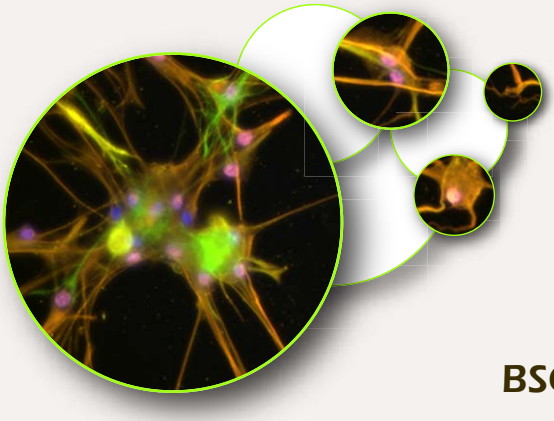
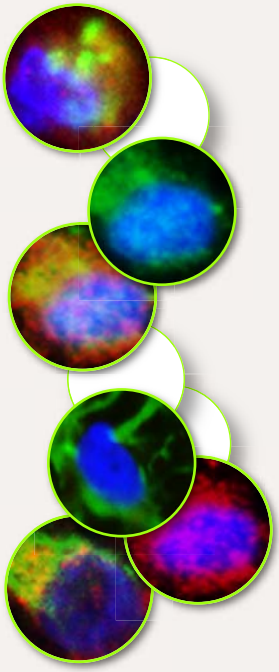


BSCDB Autumn Meeting

Neuroimmunology



October, 22th 2010
UHasselt - Building D - room H4

Local organisers: Niels Hellings
Sven Hendrix

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BIOMEDISCH ONDERZOEKSINSTITUUT

SBBCD BVCOB

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BIOMEDISCH ONDERZOEKSINSTITUUT

SBBCD BVCOB

Autumn Meeting 2010 of the Belgian Society of Cell and Developmental Biology (BSCDB)

Friday, October 22nd, 2010

Universiteit Hasselt, Campus Diepenbeek, Bldg. D,
BE-3590 Diepenbeek

Room H4

NEUROIMMUNOLOGY

Local Organizing committee: Niels Hellings & Sven Hendrix

8h30 - 9h15	Registration and display of posters
9h15 – 9h30	Welcome and introduction
MORNING SESSION	**Chairman: Sven Hendrix**
9h30 – 10h15	**Eva Peters** (Charité, Humboldt-University, Berlin, Germany) "Getting on my nerves – a new stress axis in the skin"
10h15 – 11h00	**Peter Ponsaerts** (University of Antwerp, Belgium) "Immunomodulatory functions of stem cells?"
11h00 – 11h30	***Coffee break and visit of stands***
11h30- 12h15	**Vincent van Pesch** (UCL, Brussels, Belgium) "The enigmatic role of antibodies in multiple sclerosis"
12h15 – 12h45	*Selected oral communications by young investigators* **Nina Swinnen** (UHasselt) "Microglia in the embryonic neocortex – maternal inflammation affects embryonic microglia" **Lila R. Avula** (UA) "Expression and distribution of MAS-related gene receptors in the non-inflamed and inflamed murine ileum" **Kato Deckers** (KULeuven) "Induced MOG-specific CD4+ T-cells as a therapeutic strategy in EAE"
12h45 – 14h15	***Sandwich lunch – Poster viewing – Visit of stands***

AFTERNOON SESSION	**Chairman: Niels Hellings**
14h15-14h30	General Assembly Board (open to all members)
14h30 – 15h15	*Selected oral communications by young investigators* **Joost Smolders (UM)** "Safety of modulating effects of high dose Vitamin D3 supplementation in relapsing remitting multiple sclerosis" **Bieke Broux (UHasselt)** "CX3CR1 drives senescent CD4+ T cells with cytotoxic properties into the multiple sclerosis brain" **Elke Ydens (VIB, UA)** "Acute neurodegeneration triggers an alternative macrophage response"
15h15 – 16h	**Catherine Lubetzki** (UMRS, Inserm 975, Paris, France) "Remyelination in multiple sclerosis – from cells to clinics"
16h – 16h45	**Geert Van Loo** (VIB, UGhent, Belgium) "NF-kB in CNS inflammation and demyelination"
16h45 – 17h	Closing remarks and proclamation of two poster prizes
END OF THE MEETING	

SPONSORS OF THE 2010 AUTUMN MEETING IN DIEPENBEEK

Invited Speakers

GETTING ON MY NERVES : A NEW STRESS AXIS IN THE SKIN.

Eva Milena Johanne Peters.

Psychoneuroimmunology, Dpts. of Psychosomatic Medicine and Psychotherapy,
University Medicine Charité, Berlin
and
Psychosomatic Medicine and Psychotherapy, Justus Liebig University, Gießen,
Germany.

Neuroendocrineimmune circuitry is long discussed in the generation, maintenance and aggravation of chronic inflammatory diseases. Strains imposed on the individual that require adaptive changes in the neurophysiology, immune response and behavior to adapt to the challenge are generally considered as stressors. Maladaptation of the classical stress axis, the hypothalamus pituitary adrenal axis and the sympathetic axis are well studied and their pathogenicity in the development and course of i.e. allergic inflammatory diseases is no longer discussed. We here report activation of a third stress axis in peripheral tissues. Along this axis neuropeptides and neurotrophins interact with the immune system to promote local pro-inflammatory circuits and induce systemic regulation. This third stress axis potently alters the course of chronic inflammatory disease and offers itself as a promising new target for the development of respective therapeutic strategies.

Short C.V. of the Speaker

Higher Education and Employment

2010 - Head of the *Psychoneuroimmunology Laboratory*, Department of Psychosomatic Medicine and Psychotherapy, Justus-Liebig-University, Giessen, Germany

2010 Specialisation in Dermatology, Medical Association Berlin, Berlin, Germany

2009 Specialisation in Psychooncology, Ruppiner Kliniken GmbH, Berlin, Germany

2004 - Head of the *Psychoneuroimmunology Laboratory*, Department of Psychosomatic Medicine and Psychotherapy, University-Medicine Charité, Berlin, Germany

2001 - 2004 Postdoctoral Fellow, Department of Psychosomatic Medicine and Psychotherapy, University-Medicine Charité, Berlin, Germany

1999 - 2001 Postdoctoral Fellow, Department of Dermatology, University of Hamburg, Hamburg, Germany

1998-1999 Postdoctoral Fellow, Department of Biomedical Sciences, University of Bradford, Bradford, UK

1990 - 1997 Study of Medicine at the Humboldt University Berlin, Berlin, Germany

1989-1990 Study of Philosophy, Theater Science and Anthropology at the Humboldt University Berlin, Berlin, Germany

Scientific Career

2007 - 2010 Vice speaker of the *Study Group Dermatoendocrinology*

2007 "Habilitation" and *Venia legendi* for the specialty "Psychoneuroimmunology", Humboldt University of Berlin, Berlin, Germany

2004-2006 Recipient of the *Rahel Hirsch Habilitation Research Fellowship* (transformed into position for a technician), Humboldt University Berlin, Berlin, Germany

2001 Doctoral thesis ("summa cum laude") in fulfillment for the title of Dr. med., Humboldt University Berlin, Berlin, Germany

1998-1999 Recipient of the *Herbert A. Stiefel Research Fellowship*, Stiefel Laboratories, Coral Gables, Florida, USA

1997 Guest scientist at the Department of Endocrinology, Nutrition and Diabetes, Vitamin D, Skin and Bone Research Laboratory, Boston University Medical Center, Boston, MA, USA

Awards & Honours

2006 Research On Skin Dryness Award (ROSA), La Roche Posay Laboratoire Pharmaceutique Germany (LRP Germany)

2001 Short listed for the Robert Koch Dissertation Price, Humboldt-University Berlin, Berlin, Germany

IMMUNOMODULATORY FUNCTIONS OF STEM CELLS ?

Peter Ponsaerts

Laboratory of Experimental Hematology
University of Antwerp

Currently, much attention is given to the development of cellular therapies for treatment of central nervous system (CNS) injuries. Diverse cell implantation strategies, either to directly replace damaged neural tissue or to create a neuro-regenerative environment, are proposed to restore impaired brain function. However, due to the complexity of the CNS, it is now becoming clear that the contribution of cell implantation into the brain will mainly act in a (immune-modulating) supportive manner. In addition, given the time-dependence of neural development during embryonic and post-natal life, cellular implants, either self or non-self, will most likely have to interact for a sustained period of time with both healthy and injured neural tissue. The latter also implies potential recognition of cellular implants by the brain's innate immune system.

During the past 7 years, our laboratory has actively investigated the potential regenerative effect of bone marrow mononuclear cells, bone marrow-derived mesenchymal stem cells and embryonic brain-derived neural stem cells in animal models of rat spinal cord injury and mouse experimental auto-immune encephalomyelitis. However, in none of these experimental set-ups we were able to demonstrate a beneficial effect of cell grafting to disease outcome. Therefore, our research was reoriented during the past 3 years in order to investigate the lack of therapeutic benefit and to eventually improve cellular strategies to promote regeneration of injured CNS tissue.

While many published cell therapy studies mainly report on functional outcome following cell transplantation, little is known about the actual fate of grafted cell populations. Therefore, we have developed a novel combined labelling strategy for cellular grafts, based on genetic modification with the reporter genes Luciferase and eGFP and physical labelling with blue fluorescent micron-sized iron oxide particles, in order to unambiguously identify transplant localization, survival and differentiation following engraftment in CNS of mice by in vivo bioluminescence and magnetic resonance imaging and post-mortem histological analysis. Using these techniques, we have demonstrated that lack of therapeutic benefit following intravenous cell administration is due to cell retention in lung capillaries as soon as one-minute post injection. With regard to cell grafting directly into the CNS tissue, our results demonstrate that both autologous and allogeneic cell grafts are recognized by the brain's innate immune system. While allogeneic cell grafts are readily eliminated by Iba1+CD11b+ immune-activated microglia, autologous cell grafts become highly infiltrated by (immune-suppressed ?) Iba1+CD11b- microglia. Moreover, we additionally observe a strong astroglial reaction against grafted cell populations in the CNS of immune-competent rodents.

In conclusion, having performed many autologous and allogeneic cell grafting experiments in the CNS of immune-competent hosts, we question the direct immune-modulating properties of stem cells populations in vivo and suggest that a better understanding of the brain's innate immune reactions towards cellular grafts will be needed before cell transplantation in the CNS can be performed safely and successfully.

References: (i) Ronsyn et al., BMC Biotechnology 2007; 7:90. (ii) Bergwerf et al., BMC Biotechnology 2009; 9:1. (iii) Tambuyzer et al., Immunology and Cell Biology 2009; 87:267. (iv) Reekmans et al., Cell Transplantation 2010; (in press).

Short C.V. of the Speaker

Peter Ponsaerts graduated in 1999 at the Department of Biochemistry of the University of Antwerp. From 1999-2003 he completed his PhD-research on dendritic cell biology and immunotherapy within the Laboratory of Experimental Hematology of the University of Antwerp. During this period, he published several manuscripts on mRNA electroporation as a non-viral alternative for efficient gene transfer in dendritic cells. Next, a first post-doctoral project from 2003-2006 was completed at the University of Antwerp where he studied the potential of mesenchymal stem cell transplantation to treat spinal cord injury in rat. This was followed by a second post-doctoral project from 2006-2009 aiming to develop multimodal imaging techniques to non-invasively monitor survival, migration and differentiation of grafted stem cells in vivo. During his post-doctoral career he published several manuscripts on genetic modification of stem cells and in vivo cell transplantation in the central nervous system of rodents. Currently, he has become group leader within the Laboratory of Experimental Hematology, where his research focuses on the interaction of autologous and allogeneic stem cells grafts with the brain's innate immune system.

THE ENIGMATIC ROLE OF ANTIBODIES IN MULTIPLE SCLEROSIS.

Vincent van Pesch.

UCL, Brussels, Belgium.

Multiple Sclerosis (MS) is an auto-immune inflammatory disease of the central nervous system (CNS), characterized by focal demyelination and neurodegeneration. It is one of the main causes of disability in young adults, with a prevalence estimated at 88 per 100.000 inhabitants in Belgium for example. Disease pathogenesis is multifactorial, implicating environmental, genetic and immunological factors. MS has long been considered to be a CD4+ Th1-mediated disease, as well as its animal model, Experimental allergic encephalomyelitis (EAE). This oversimplistic view has come to be challenged in view of emerging data concerning the implication of other cellular subsets such as Th17 cells, CD8+ T cells or T regulatory cells [1]. Interestingly, since the seminal finding by Kabat et al. of elevated IgG levels in the cerebrospinal fluid (CSF) of MS patients published in 1948, humoral immunity is also increasingly being recognized as a player in disease initiation and maintenance. The presentation will focus on recent experimental and clinical findings highlighting the possible role of B cells and antibodies in the setting of demyelinating disease [2].

Antibodies to myelin are not sufficient to initiate by themselves EAE, but they can enhance demyelination and inflammation, notably by acting as T-cell adjuvants or by inducing cytokine production. In Neuromyelitis optica, an MS variant, autoantibodies against Aquaporin 4 have been shown to be directly linked to disease pathogenesis.

Neuropathological studies have identified clonally expanded B lymphocytes in acute MS plaques, mirroring the status of B cells found in the CSF. Functional characterization of these cells suggest a persistent antigenic stimulation, responsible for the production of oligoclonal bands, a CSF diagnostic marker used in the clinic. The triggers for this sustained humoral response, as well as its antigenic targets remain controversial: antibodies against infectious agents (such as Epstein Barr virus, for example) or myelin antigens (such as Myelin Oligodendrocyte Glycoprotein) have been studied in relationship with MS [3]. Moreover, B cells could also be implicated in disease progression, by forming ectopic meningeal follicles, responsible for cortical demyelination and axonal damage, through pro-inflammatory cytokine production or antibody-mediated mechanisms [4].

Finally, B-cell depleting monoclonal antibodies (such as Rituximab) have shown efficacy in preliminary MS clinical trials.

In conclusion, immunological, pathological and therapeutic studies suggest diverse mechanisms by which B cells and antibodies could play a role in MS pathogenesis, although their contribution is likely to vary according to the stage of the disease [5]. Future studies will have to address the need for reliable disease-related biomarkers, to identify patients who would eventually benefit from B-cell targeted therapies.

Selected references

1. Kasper, L.H. and J. Shoemaker, Multiple sclerosis immunology: The healthy immune system vs the MS immune system. Neurology, 2010. 74 Suppl 1: p. S2-8.

2. Weber, M.S., B. Hemmer, and S. Cepok, The role of antibodies in multiple sclerosis. Biochim Biophys Acta, 2010.

3. Fraussen, J., et al., B cell characterization and reactivity analysis in multiple sclerosis. Autoimmun Rev, 2009. 8(8): p. 654-8.

4. Magliozzi, R., et al., Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain, 2007. 130(Pt 4): p. 1089-104.

5. Yanaba, K., et al., B-lymphocyte contributions to human autoimmune disease. Immunol Rev, 2008. 223: p. 284-99.

Short C.V. of the Speaker

1999: Medical Degree, Université Catholique de Louvain

2003: PhD thesis in Biomedical Science

Orientation: immuno-virology, at the Christian de Duve Institute of Cellular Pathology

"Type I-Interferon response in the mouse and inhibition of this response by the leader protein of Theiler's virus".

2006: End of Postgraduate training in Neurology

Current Position: Adjunct Clinical Head, Dpt. of Neurology, Cliniques Universitaires Saint-Luc, Brussels.

Orientation: Clinical Neuroimmunology (Multiple Sclerosis). In charge of the Neurophysiology Unit.

Member of the Belgian Multiple Sclerosis Study Group

Member of the Executive Committee of the Belgian Neurological Society

Member of BioMS-eu: Consortium for CSF Biomarker Research in MS

Scientific activity:

Neurochemistry Unit, Institute of NeuroScience, Catholic University of Louvain. (Prof. C. Sindic). Main project: clinical research on cytokine expression and functional analysis of Thelper subsets in CIS and relapsing-remitting MS patients.

neurons and nerve fibres, and the presence of MrgB10 on MMCs during inflammation support the hypothesis that Mrg's are involved in neuronal and immune responses.

REMYELINATION AS A TARGET FOR MS THERAPY

Catherine Lubetzki

INSERM UMRS 975, Hôpital de la Salpêtrière, UPMC, Paris France

Spontaneous myelin repair occurs in MS, and is mostly achieved by oligodendrocyte progenitor cells, which persist in the adult CNS. This newly formed myelin is characterized by a thin myelin sheath with short internodes. In addition to restoring the rapid conduction of the nerve influx, myelin repair prevents axonal damage and loss, as demonstrated recently. Although remyelination might be extensive in some cases, it is most often insufficient, limited to the periphery of the demyelinated lesions. Therefore, one major therapeutic goal in demyelinating pathologies is to favour myelin repair to prevent secondary neurodegeneration

Many different mechanisms are leading to this repair failure. In some lesions, demyelination coexists with the presence of oligodendroglial precursor cells within the lesion, suggesting the existence of local inhibitors of oligodendroglial differentiation or maturation. Some of these inhibitors, either axonal, astrocytic or oligodendroglial, have been identified recently. In contrast, other demyelinated lesions are characterized by an oligodendroglial depopulation. This might be related to an exhaustion of the oligodendroglial pool related to successive attacks of demyelination. Alternatively, this lack of oligodendroglial cells may correspond to the defect of recruitment of oligodendroglial progenitors to the demyelinated lesion, possibly related to a dysregulation of guidance molecules.

Experimental strategies have emerged, aimed at stimulating remyelination. Different strategies of exogenous repair are actively studied, with different cell candidates (neural stem cells, olfactory ensheathing cells ....). Other strategies are aimed at favouring endogenous myelin repair. In this context, our group demonstrated that guidance molecules of the semaphorin (Sema) family , Sema 3A and Sema 3F were dysregulated in the MS brain, as well as in experimental demyelination. These guidance cues are known to act on oligodendrocyte precursor cells migration during development, with a repulsive effect of Sema 3A and an attractive effect of Sema 3F. This raised the possibility that, in the adult CNS, dysregulation of these guidance cues after a demyelinating injury might impair the recruitment of oligodendrocyte progenitor cells, the remyelinating cells of the CNS, towards the demyelinated area, hence the myelin repair capacity. Recently, using gain and loss of function experiments, in an experimental model of demyelination in the adult mouse spinal cord, we demonstrated that overexpression of Sema 3A impairs oligodendrocyte precursor cells recruitment whereas overexpression of Sema 3F or suppression of Sema 3A accelerates this recruitment. Furthermore, this Sema 3F-induced earlier oligodendrocyte precursor cells recruitment towards the demyelinated lesion leads to an accelerated remyelination, hence neuroprotection. These data might open new avenues to promote endogenous remyelination in MS, therefore limiting disability progression.

Short C.V. of the Speaker

Catherine Lubetzki studied Medecine at Paris V University, then became specialized in Neurology, with a specific interest for Multiple Sclerosis. During the same period, she undertook a scientific cursus and obtained a PhD in Neurosciences at Pierre and Marie Curie University (Paris VI).

Since 1993, she is professor of Neurology at Pierre and Marie Curie University, and works in Hôpital de la Salpêtrière, Paris, France.

She is involved in several committees and funding boards, including the scientific committee of ARSEP (French multiple sclerosis association for research) where she is coordinator, ECTRIMS (European Congress on Treatment and Research In Multiple Sclerosis), and the board of ENP (Neuroscience school of Paris). She has been member of INSERM and ANR (national research agency) neuroscience study sections. She is currently in charge of the development of a structure dedicated to translational research in neurosciences in France. She is involved in different editorial boards such as Multiple Sclerosis, Neuron/glia Biology and Brain.

Her clinical activity is mainly dedicated to the management of multiple sclerosis patients, the department of Neurology being the most important clinical center for multiple sclerosis in France, with more than 5000 patients yearly. She coordinates the Salpêtrière Multiple Sclerosis clinical research center, in which both patho-physiological projects, imaging studies and therapeutic trials are developed. In this context, she recently participated to the development of an innovative method enabling myelin imaging using Pet-scan, which is currently investigated in Multiple Sclerosis patients. She is investigator of several international therapeutic trials.

Her research activity is focused on the cellular and molecular mechanisms involved in central nervous system myelination and remyelination, using different in vitro models as well as in vivo approaches and post-mortem analysis. The general aim of her research projects is to understand why some multiple sclerosis lesions remyelinate whereas others do not. This with the perspective of developing, through activation of promoting cues, or suppression of inhibitory pathways, strategies aimed at stimulating endogenous remyelination, hence preventing axonal damage and limiting disability progression in Multiple Sclerosis patients.

NF-KB IN CNS INFLAMMATION AND DEMYELINATION.

Geert van Loo.

VIB Department for Molecular Biomedical Research and UGent Vakgroep Biomedische Moleculaire Biologie, Gent.

NF-κB is essential in innate and adaptive immunity, inflammation and development and defects in the regulation of NF-κB-dependent gene expression and apoptosis contribute to a variety of diseases including inflammatory and autoimmune diseases, neurological disorders and cancer. NF-κB proteins are kept inactive by association with inhibitory factors belonging to the IκB family. NF-κB activating stimuli induce the phosphorylation, polyubiquitination and proteasome degradation of IκBs allowing NF-κB to accumulate in the nucleus and activate target genes. The inducible phosphorylation of IκB is mediated by the IκB kinase (IKK) complex, composed of two catalytic subunits termed IKK1 (or IKKα) and IKK2 (or IKKβ) and a regulatory protein named NEMO (or IKKγ).

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS). Nerve cells in our brain and spinal cord communicate with each other using electrical signals. This communication is so fast and efficient because the axons of our nerves are surrounded by an insulating sheath of myelin produced by oligodendrocytes. In MS this protective myelin sheath gets destroyed by cells of our own immune system and the communication between nerve cells gets disrupted. Although MS is widely considered as an autoimmune disease caused by an autoaggressive T cell response against myelin, its etiology is not fully understood. A complex inflammatory reaction involving both the adaptive and the innate immune system seems to govern disease progression during the first inflammatory phase, and a central player in the molecular mechanisms behind MS and in the inflammatory reaction involved, is NF-κB.

Short C.V. of the Speaker

Geert van Loo was born in 1971 in Gent, Belgium. He obtained a Master in Bioengineering in Chemistry in 1994, a Master in Biotechnology in 1996, and a PhD in Biotechnology in 2002, in the Department of Biomedical Molecular Biology at Gent University and the Department of Molecular Biomedical Research at the VIB.

During his PhD, which he performed in the research group of Dr. Peter Vandenabeele, Geert focussed on signalling pathways and molecular mechanisms leading to apoptotic and necrotic cell death, and the involvement of mitochondria in both processes. After his PhD, he moved to Italy for a postdoc at the European Molecular Biology Laboratory (EMBL), Mouse Biology Unit, in the research group of Dr. Manolis Pasparakis, for which he was granted a Marie Curie intra-European fellowship. During this postdoc he used “state of the art” gene targeting technology to dissect genetically in vivo the function of signalling pathways controlling cell survival and death. The main focus of his research concerned the study of NF-κB and apoptotic signalling in central nervous system (CNS) inflammation, with particular emphasis on the autoimmune inflammatory disease multiple sclerosis (MS). In 2006, Geert returned to Gent University and VIB where he joined the research group of Dr. Rudi Beyaert. His current research is also focused on in vivo mechanisms regulating inflammation and degeneration using genetically modified mice in combination with mouse models of human diseases. His research was/is financed by a FWO postdoctoral fellowship, a Marie Curie European Reintegration Grant, grants from the Charcot Foundation and an FWO Odysseus Grant.

Short Communications

MORNING SESSION

Poster abstract 26:

MICROGLIA IN THE EMBRYONIC NEOCORTEX – MATERNAL INFLAMMATION AFFECTS EMBRYONIC MICROGLIA.

Nina Swinnen (1,2,3,4), Chiara Rigato (2,3,4), Bert Brône (1), Pascal Legendre (2,3,4) and Jean-Michel Rigo (1).

1: BIOMED, Brain Protection And Repair, Hasselt University, Diepenbeek;
2: Institut National de la Santé et de la Recherche Médicale, U952, Université Pierre et Marie Curie, Paris, France;
3: Centre National de la Recherche Scientifique, UMR 7224, Université Pierre et Marie Curie, Paris, France;
4: UMPC Université Paris 06, Paris, France.

Poster abstract 1:

EXPRESSION AND DISTRIBUTION OF MAS-RELATED GENE RECEPTORS IN THE NON-INFLAMED AND INFLAMED MURINE ILEUM.

Leela R. Avula (1), Luc Van Nassauw (1,2), Edith Stuyven (3), Roeland Buckinx (1), Katrien Alpaerts (1), Dirk Adriaensen (1), Herman W. Favoreel (3), Eric Cox (3) and Jean-Pierre Timmermans (1).

1: Lab. Cell Biology and Histology, Fac. Veterinary Medicine, and
2: Lab. Human Anatomy & Embryology, Fac. Medicine, University of Antwerp, Antwerp;
3: Lab. Immunology, Fac. Veterinary Medicine, Ghent University, Ghent.

Poster abstract 7:

INDUCED MOG-SPECIFIC CYTOLYTIC CD4+ T CELLS AS A THERAPEUTIC STRATEGY FOR EAE.

Kato Deckers, Luc Vander Elst, Vincent Carlier, Marc Jacquemin and Jean-Marie Saint-Rémy.

Center for Molecular and Vascular Biology, University of Leuven, B-3000 Leuven.

AFTERNOON SESSION

Poster abstract 24:

SAFETY AND T CELL MODULATING EFFECTS OF HIGH DOSE VITAMIN D3 SUPPLEMENTATION IN RELAPSING REMITTING MULTIPLE SCLEROSIS.

Joost Smolders (1-3), Evelyn Peelen (1-3), Mariëlle Thewissen (2), Jan Willem Cohen Tervaert (1,2), Paul Menheere (4), Raymond Hupperts (1,3) and Jan Damoiseaux (2,5).

1: School for Mental Health and Neuroscience, MUMC, Maastricht, The Netherlands;
2: Dept. of Internal Medicine, div. of Clinical and Experimental Immunology, MUMC, Maastricht, The Netherlands;
3: Academic MS Center Limburg, Orbis MC, Sittard, The Netherlands;
4: Dept. of Clinical Chemistry, MUMC, Maastricht, The Netherlands;
5. Laboratory for Clinical Immunology, MUMC, Maastricht, The Netherlands.

Poster abstract 4:

CX3CR1 DRIVES SENESCENT CD4+ T CELLS WITH CYTOTOXIC PROPERTIES INTO THE MULTIPLE SCLEROSIS BRAIN.

Bieke Broux (1), Kim Pannemans (1), Tom Broekmans (1), Bert Op 'T Eynde (1), Bart Van Wijmeersch (1,2), Veerle Somers (1), Piet Geusens (3) Piet Stinissen (1) and Niels Hellings (1).

1: Biomedical Research Institute, Hasselt University and School of Life Sciences, transnationale Universiteit Limburg, Diepenbeek;
2: Mariaziekenhuis Noord-Limburg and Revalidatie & MS-centrum, Overpelt;
3: Reumatologie, Genk.

Poster abstract 33:

ACUTE NEURODEGENERATION TRIGGERS AN ALTERNATIVE MACROPHAGE RESPONSE.

Elke Ydens, Sofie Goethals, Vincent Timmerman and Sophie Janssens.

Peripheral Neuropathy Group, VIB-Department of Molecular Genetics, University of Antwerp, B-2610 Wilrijk.

Poster Abstracts

1: Avula Leela
presenting author ; e-mail : leela.avula@ua.ac.be

EXPRESSION AND DISTRIBUTION OF MAS-RELATED GENE RECEPTORS IN THE NON-INFLAMED AND INFLAMED MURINE ILEUM.

Leela R. Avula (1), Luc Van Nassauw (1,2), Edith Stuyven (3), Roeland Buckinx (1), Katrien Alpaerts (1), Dirk Adriaensen (1), Herman W. Favoreel (3), Eric Cox (3) and Jean-Pierre Timmermans (1).

1: Lab. Cell Biology and Histology, Fac. Veterinary Medicine, and
2: Lab. Human Anatomy & Embryology, Fac. Medicine, University of Antwerp, Antwerp;
3: Lab. Immunology, Fac. Veterinary Medicine, Ghent University, Ghent.

Mas-related gene receptors (Mrg's) constitute a complex family of orphan G protein-coupled receptors. Some Mrg's are predominantly expressed in spinal sensory neurons and are implicated in nociception. Agonists of Mrg's are suggested to mediate mast cell-primary afferent nerve communication, and to participate in the so-called receptor-independent activation of mast cells during inflammation. There is a lack of data concerning the expression and function of Mrg's in the GI tract both during physiological and pathophysiological conditions.

Therefore, we aimed to unravel the expression and distribution of Mrg's in the non-inflamed and Schistosoma mansoni-infected murine ileum. The relative expression levels of all the 21 curated Mrg's were quantitatively analysed using real-time PCR. Custom-made polyclonal antisera directed against MrgA4, B8 and B10 were produced and used for immunohistochemical analyses on cryosections and whole-mounts.

Real-time PCR revealed significantly increased expression of MrgA1, A4, A5, A7, B1, B2, B4, B8, B10 and D in the inflamed ileum. MrgA7, B1, B10 and D were exclusively detected in the inflamed ileum, while the other 17 Mrg's were expressed in the non-inflamed and inflamed tissue. Immunohistochemistry revealed a faint MrgB10 immunoreactivity (IR) and a moderate MrgA4 and MrgB8 IR in a few neuronal cell bodies and nerve fibres in the non-inflamed ileum. In the inflamed ileum, MrgA4, MrgB8 and MrgB10 IR was clearly observed on an increased number of neuronal somata and nerve fibres in both enteric plexuses, and on an increased number of nerve fibres in the tunica muscularis and the lamina propria. Colocalisation studies using antibodies directed against several neurochemical markers, demonstrated that MrgB4-, MrgB8- and MrgB10-immunoreactive neurons were predominantly intrinsic primary afferent neurons. In the inflamed ileum, MrgB10 IR was also detected on a few mucosal mast cells (MMCs).

In conclusion, this study revealed the relative expression levels of the 21 curated Mrg's as well as the cellular distribution of MrgA4, MrgB8 and MrgB10 in the murine ileum during (patho)physiological conditions, suggesting a functional role of some Mrg's during intestinal inflammation. The increased expression of MrgA4, MrgB8 and MrgB10 in neurons and nerve fibres, and the presence of MrgB10 on MMCs during inflammation support the hypothesis that Mrg's are involved in neuronal and immune responses.

Supported by FWO-grant G.0179.08

2: Bogie Jeroen
presenting author ; e-mail : Jeroen.bogie@uhasselt.be

IMMUNE MODULATION BY MYELIN-LADEN MACROPHAGES.

Jeroen Bogie, Piet Stinissen, Niels Hellings and Jerome Hendriks.

Biomedical Research Institute (BIOMED), Hasselt University, B-3590 Diepenbeek.

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) in which macrophages play a pivotal role. Nonetheless, it remains largely unknown which macrophage subpopulations are induced during CNS inflammation, what underlying cellular mechanisms govern the induction of these macrophage subsets and how they contribute to MS pathology. Initially, macrophages where thought to be merely detrimental in MS, as they internalize myelin and secrete toxic and pro-inflammatory mediators. However, recent evidence suggests that they may also have protective effects, especially following myelin phagocytosis.

In this study, we demonstrate that peritoneal macrophages ingest myelin efficiently in vitro and acquire an unique phenotype following a subsequent activation. In addition, we demonstrate that myelin-phagocytosing macrophages inhibit lymphocyte proliferation in a TCR a-specific manner, mediated by an altered nitric oxide/arginase balance. These observations suggest that myelin phagocytosis leads to an altered macrophage phenotype that modulates lymphocyte responses in MS. More research is needed to further elucidate this immunomodulatory role of myelin-phagocytosing macrophages and is essential to increase our understanding of neuroinflammatory responses in diseases like MS.

3: Bottelbergs Astrid
presenting author ; e-mail : astrid.bottelbergs@pharm.kuleuven.be

RELATIONSHIP BETWEEN DEMYELINATION, AXONAL DEGENERATION AND NEUROINFLAMMATION IN THE NESTIN-PEX5 MOUSE MODEL.

Bottelbergs Astrid (1), Verheijden Simon (1), Devos Rita (2) and Baes Myriam (1).

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Inactivation of peroxisomes in neural cells in the Nestin-Pex5 knockout mouse model causes severe phenotypical abnormalities, including cataract, motoric and cognitive abnormalities and lethargy, finally leading to premature death before the age of six months. At the end stage of their disease, these mice present with severe demyelination, axonal degeneration and neuroinflammation. Until now, the onset and the relationship between axonal and myelin pathology and neuroinflammation is not clear. Therefore the Nestin-Pex5 model was examined at three weeks and three months of age by fluorescence and electron microscopy, by quantitative RT-PCR and microarray analysis.

Already at the age of three weeks Nestin-Pex5 knockout mice show demyelination and axonal degeneration in certain brain areas, in particular in cerebellum, as evidenced by immunohistochemistry and electron microscopy (EM). Microglia proliferation is already present, but these cells do not yet display a swollen macrophage morphology. RT-PCR confirmed the upregulation of various neuroinflammatory markers. At the age of three months, EM analysis revealed regions of severe demyelination and axonal degeneration in the corpus callosum of Nestin-Pex5 knockout mice. Some axons exhibited an irregular or absent myelin sheet. The axons displayed swellings, a disrupted cytoskeleton or complete degeneration. At this age neuroinflammation is prominently present, with a massive proliferation of microglial cells in the corpus callosum, as visualised by F4/80 staining. Several microglial cells have a swollen morphology and are MAC-3 positive, which is a marker for activated microglial cells performing phagocytosis. By microarray and quantitative RT-PCR analysis it was confirmed that the expression of a multitude of neuroinflammatory markers was vastly increased. It can be concluded that demyelination, axonal degeneration and neuroinflammation have an early onset in the peroxisome deficient mouse brain and that these processes could thus far not be disentangled in time.

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CX3CR1 DRIVES SENESCENT CD4+ T CELLS WITH CYTOTOXIC PROPERTIES INTO THE MULTIPLE SCLEROSIS BRAIN.

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Premature immunosenescence has been linked to many autoimmune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis (RA). In particular, CD4+ T cells gain aberrant, possibly cytotoxic functions after repeated antigenic stimulation or homeostatic proliferation. Until now, the absence of CD28 has been used as a marker for these senescent CD4+ T cells. However, a marker which is present on the surface of these cells can greatly benefit the isolation and further characterization of this subset of T cells. Therefore, a phenotypic characterization of these cells was performed using flow cytometry. Several markers (CD11a, CD49d, CD54, CD56, NKG2D) were significantly upregulated ($p<0.05$) on CD4+CD28null T cells of either healthy controls (HC, n=9) and patients (MS: n=8, RA: n=23) in comparison to CD4+CD28+ T cells. Interestingly, CX3CR1 (fractalkine receptor) was present on the vast majority of CD4+CD28null T cells (HC: 88.2 ± 3.9%, MS: 82.4 ± 4.7%, RA: 90.4 ± 1.5%) and mostly absent on CD4+CD28+ T cells (HC: 2.6 ± 0.9%, MS: 1.5 ± 0.6%, RA: 2.4 ± 0.5%). We further demonstrated that only CD4+CD28null T cells migrate towards fractalkine in a transwell system, thereby proving that CX3CR1 is functional on these cells. Moreover, immunofluorescence stainings of MS brain lesions demonstrate the presence of CD4+CX3CR1+ T cells in the brain of 6 out of 17 donors tested. Once in the target tissue, these cells might exert their cytotoxic properties. Our results show that CD4+CX3CR1+ T cells degranulate after TCR and NKG2D stimulation, as well as after stimulation with the MS related antigens MBP and MOG. These results indicate that CD4+CD28null T cells contribute to the pathogenesis of autoimmune diseases such as MS and RA.

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THE INHIBITORY NEUROTRANSMITTER GABA MODULATES MACROPHAGE FUNCTION IN VITRO AND AFFECTS THE DISEASE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.

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The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) is able to modulate peripheral immune cell responses. Such an immunomodulatory role may be important in neuroinflammatory diseases like multiple sclerosis (MS). Although transcriptomic and proteomic analysis of MS lesions indicate alterations in the GABAergic inhibitory system, the exact contribution of GABA to the disease process is unclear. In this study, the in vitro effect of GABA on disease-contributing macrophage actions was explored, together with its role in CNS inflammation in vivo, using the animal model for MS, experimental autoimmune encephalomyelitis (EAE).

GABA reduced LPS-induced TNF-alpha production by peritoneal macrophages in a concentration-dependent manner. In contrast, IL-6 production was increased at low GABA concentrations (30-300 µM). Furthermore, the macrophage capacity to phagocytose myelin was significantly reduced after GABA treatment. The effects of GABA were mediated by GABAA and GABAB receptors, as their respective antagonists picrotoxin and saclofen reversed the GABA-evoked effects. In addition, GABA enhanced the activation of STAT-3 and ERK, intracellular signaling molecules known to be implicated in macrophage activity. To elucidate the influence of GABA on CNS inflammation in vivo, chronic myelin oligodendrocyte glycoprotein (MOG) EAE was induced in C57Bl/6J mice. Animals were treated daily from day 3 onwards with GABA (200 mg/kg) or vigabatrin (250 mg/kg), a GABA-transaminase inhibitor that increases endogenous GABA levels. GABA treatment significantly augmented disease severity which was associated with enhanced MOG-dependent proliferation of spleen-derived lymphocytes. In contrast, vigabatrin treatment prevented EAE development. When treatment was started in the chronic phase of the disease, vigabatrin again significantly reversed paralysis and protected mice against a second relapse.

These findings indicate that exogenous GABA has a detrimental influence on neuroinflammation, while increasing endogenous GABA concentrations ameliorates EAE outcome. This may be explained by dissimilarities in local GABA levels after GABA or vigabatrin treatment, which in turn may differentially affect macrophage function, as suggested by our in vitro experiments. It is also conceivable that GABA and vigabatrin vary in several aspects, such as peripheral versus central mediated effects, turnover time or timing of their accomplished effects. Future studies are needed to gain further insight into the exact role of GABA and its metabolic pathways in the development of inflammatory diseases affecting the CNS.

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IDENTIFICATION OF PUTATIVE NETWORKS INVOLVED IN BRAIN INFLAMMATION DURING EAE: POST-SYNAPTIC DENSITY PROTEIN 95, AND CALCIUM-ACTIVATED POTASSIUM CHANNEL ALPHA 1 AS CANDIDATE REGULATORY PROTEINS.

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Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system. To analyze the changes in the brain proteome during the disease course, a quantitative proteomics study, two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) analysis followed by MS protein identification was performed. The acute EAE model was selected to focus on the inflammatory pathways involved in the lesion development and regulation of EAE. Rats were analyzed over time (just before onset of the symptoms, at the top of the disease, and after recovery of the symptoms). We were able to identify 95 differentially expressed protein spots, representing 79 unique proteins. To find disease-related networks, all of these proteins were mapped to existing biological networks using Ingenuity Pathway Analysis (IPA) to disclose connections between proteins found in this study and their interaction with other proteins and known MS-markers. The analysis pointed at 54/77 proteins known earlier to take part in neurological disease. Furthermore, these proteins are located downstream of the known MS-markers and may represent cytoplasmic effectors or targets of the described MS-markers which are mainly located in the plasma membrane. In addition to the DLG4 post-synaptic density protein 95, a key player in neuronal signaling and an important node in our data, the KCNMA1 calcium-activated potassium channel alpha 1, an MS-marker with a relationship to our own data involved in neurotransmitter release and innate immunity, were selected as focus proteins. A vast amount of proteins (80% of the differences during EAE) seem to be functionally related to DLG4 and KCNMA1. Alterations in the enzyme complexes of mitochondria as also observed here and the concomitant cellular reaction to stress which has been described in literature seem to be regulated by both key players.

7: Deckers Kato
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INDUCED MOG-SPECIFIC CYTOLYTIC CD4+ T CELLS AS A THERAPEUTIC STRATEGY FOR EAE.

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Multiple sclerosis (MS) is a demyelinating disease affecting young adults. There is currently no cure for this highly debilitating disease. Pathogenesis results from activation of CD4+ T cells recognizing autoantigens in the brain. Switching off the activation of effector T cells is a strategic target for the cure of the disease.

We found that cytolytic CD4+ T cells (cCD4+ T cells) induce apoptosis in antigen presenting cells (APC) and suppression of activated bystander T cells in an antigen-specific manner (Janssen et al., 2003). These cCD4+ T cells are elicited by presentation of class II-restricted epitopes encompassing a thiol-disulfide oxidoreductase motif (CxxC format, Carlier et al, submitted).

Two models of EAE induced by immunization of C57Bl/6 mice with MOG peptide 35-55 were implemented in our lab, a transient model and a chronic progressive one. We tested the hypothesis that immunization with MOG 35-55 peptide containing a CxxC motif could prevent and/or suppress the development of disease. To this end, 4 SC injections of 50 microgram of CxxC-MOG peptide were made in alum at 10-day intervals, either prior to disease induction or 2 weeks after clinical signs were patent.

Complete prevention of disease development was observed in both the transient and chronic progressive models. Significant suppression of clinical signs was observed in the chronic progressive model. Histology carried out on spinal cord showed less inflammation in prevention and suppression groups when compared to the control group.

Altogether, these preliminary results indicate that cCD4+ T cells elicited by active immunization towards a single MOG epitope represent a potential novel therapeutic strategy for MS.

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MESENCHYMAL STEM CELLS INDUCE A TIME-DEPENDANT RECRUITMENT OF MICROGLIA AND ASTRCYTES FOLLOWING AUTOLOGOUS GRAFTING IN BRAIN TISSUE.

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Background

The use of stem cell transplantation as a therapeutic tool to treat neurodegenerative disorders has gained increasing interest over the last decade. However, a profound knowledge of cell implant behaviour, survival and differentiation will be necessary to understand potential therapeutic effects of stem cell transplantation.

Methods

In this study we aimed to follow up the survival of grafted bone marrow-derived mesenchymal stem cells (MSC) in the central nervous system (CNS) of mice by non-invasive bioluminescence imaging (BLI) combined with a post-mortem histological study of cell differentiation and recruitment of inflammatory cells towards the implant site.

Results

BLI analysis shows stable survival of MSC-Luc/eGFP in vivo. These results were further validated by histology demonstrating the presence of Sca1+ and eGFP+ cells at every time point investigated (day 1 to day 14). At a very early time point (day 1), histological analysis did not show recruitment of microglia and/or astrocytes. However, starting from day 3, MSC grafts are invaded by Iba1+/CD11b+ microglia (activated microglia) and surrounded by a glial scar of astrocytes. From day 10 on, activated Iba1+/CD11b+ microglia were found in the surrounding of the implant, while Iba1+/CD11b- microglia remain within the MSC graft, which suggests that MSC might have certain immune-suppressive characteristics to modulate the activation status of microglia.

Conclusions

Although the CNS has historically been considered to be immune-privileged, our data demonstrate that the CNS is not immune-ignorant to autologous cellular implants. Further research should be undertaken to understand the in vivo interaction between MSC, microglia and astrocytes.

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CASPASE-14 IS REQUIRED FOR FILAGGRIN DEGRADATION TO NATURAL MOISTURIZING FACTORS IN THE SKIN.

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Caspase-14 is mainly expressed in suprabasal epidermal layers and activated during keratinocyte cornification. Caspase-14 deficient mice display a reduced epidermal barrier function and an increased UVB radiation sensitivity. We found that although profilaggrin, a protein with a pivotal role in skin barrier function, is processed correctly to its functional filaggrin monomeric unit in caspase-14-/- mice, these mice accumulate proteolytic filaggrin fragments in the epidermis. We show here that the accumulation of these filaggrin fragments is due to a defect in filaggrin degradation in the cornified layers of caspase-14-/- skin. Consequently, this lack of normal filaggrin degradation results in a significant reduction in the levels of natural moisturizing factors, such as urocanic acid and pyrollidone carboxylic acid, in the skin from caspase-14 deficient mice as compared to wild-type mice. In addition, we demonstrate that caspase-14 can directly cleave the filaggrin monomer. Taken together, our data identify caspase-14 as a crucial protease in filaggrin catabolism.

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PRECLINICAL EVALUATION OF UMBILICAL CORD MATRIX-DERIVED STEM CELLS AS REGENERATIVE THERAPY FOR MULTIPLE SCLEROSIS.

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Background and objective:

The use of stem cells (SC) is a promising novel approach for treatment of many degenerative diseases. Extraembryonal tissues such as umbilical cord are considered promising sources of mesenchymal-like stem cells (MSC) because of their abundance and less ethical issues. However, UCMS are not fully characterized yet, since large inconsistency among reports exists concerning SC isolation and expansion, marker expression, immunomodulatory properties and differentiation capacity. We aimed to isolate and characterize human umbilical cord matrix-derived stem cells (UCMS) and assess their clinical value for neurodegenerative disorders like multiple sclerosis (MS). We hypothesize that extraembryonic UCMS differ from classical MSC and show more multipotent characteristics.

Methods:

Primary cultures of UCMS were set-up from human post-partum umbilical cords using enzymatic and explant isolation. Growth kinetics, morphology and marker expression were evaluated for UCMS cultured in 4 different expansion media (standard low glucose DMEM versus CDM, KO-DMEM and KO-DMEM/F12). In this setting we sought to identify different SC populations in UCMS isolates, based on expression of specific SC related genes and markers e.g. mesenchymal markers (CD90, CD105), adhesion (CD49d, CD62L) & immunological molecules (HLA-DR, -G, CD80, CD86), chemokine receptors (CCR1,-2,-5,-7 and CXCR3,-4) and pluripotency markers (Oct4, Nanog). UCMS phenotype was determined using flow cytometry, PCR and immunocytochemistry. In addition, T-cell suppression was analyzed in co-cultures of (anti-CD3 activated) peripheral blood mononuclear cells (n=4) and increasing amounts of UCMS (n=6).

Results:

The differently isolated UCMS displayed similar marker expression patterns, yet enzymatically isolated UCMS showed a more heterogeneous population (broad granular-shaped) compared to explant-derived UCMS (spindle-shaped). UCMS grew best in KO-DMEM/F12, but no significant difference in marker expression was observed between the culture media. In general, UCMS expressed Oct4, Sox2 and Rex1 (until passage 2), HLA-G (mRNA), CXCR3 (15.3%±3.3) and CXCR4 (5.0%±1.9). Additionally, UCMS suppressed proliferation of activated T-cells, both in transwell and contact co-cultures, as well as after irradiation, suggesting involvement of (a) soluble mediator(s).

Conclusion:

In vitro propagated UCMS resemble classical MSC, are lowly immunogenic and show potent suppressive capacity towards activated T-cells. Overall, this preclinical evaluation is a first step to further evaluate UCMS potency for use in MS.

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A TRANSGENIC NON-INVASIVE CASPASE DETECTION SYSTEM REPORTING APOPTOSIS AND DIFFERENTIATION DURING NORMAL XENOPUS DEVELOPMENT.

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Apoptosis plays an essential role in animal development and homeostasis. Disorders of this process cause various pathologies, including autoimmune and neurodegenerative diseases. Caspases are central mediators of the apoptotic process, but there is growing evidence that these cysteine proteases also have apoptosis-independent functions, e.g. in the differentiation of lens fiber cells and erythrocytes. To get insight into the dynamics of caspase-activation in vivo, we have designed and evaluated novel non-invasive fluorescent reporter systems that register caspase activity in living Xenopus embryos. Because of their external development and transparency, Xenopus embryos are ideal for real time fluorescent analysis. During early development specific and highly dynamic patterns of caspase activity were detected (e.g. in the brain, spinal cord, eye, kidneys) in the transgenic reporter lines. Overlap with TUNEL-positive cells proves the ability of the reporters to detect apoptotic cells. To evaluate the developmental role of different caspases during early development, loss-of-function experiments were performed with specific morpholinos. Depletion of caspase 3 (C3) did not induce major changes in the fluorescent patterns of the C3-reporter line, pointing to functional redundancy, e.g. with caspase 7 (C7). Indeed, combined C3-C7 MO injection impaired reporter activation and was detrimental for the developing embryo suggesting an important role of these caspases during early development. Interestingly, the caspase 9 (C9)-reporter, but not the C3-reporter, was found to be activated in the ventral blood island (VBI), where the embryonic blood is formed. Moreover, targeted knock-down of C9 decreased T3 globin expression, a marker for primitive erythrocytes. Since no TUNEL-positivity is found in the VBI, these data suggest a novel, apoptosis-independent function for caspase-9 in early blood formation.

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MUTANT HSPB8 CAUSES MOTOR NEURON SPECIFIC NEURITE DEGENERATION.

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Missense mutations (K141N and K141E) in the =537,-crystallin domain of the small heat shock protein HSPB8 (HSP22) cause distal hereditary motor neuropathy (distal HMN) or Charcot-Marie-Tooth neuropathy type 2L (CMT2L). The mechanism through which mutant HSPB8 leads to a specific motor neuron disease phenotype is currently unknown. To address this question, we compared the effect of mutant HSPB8 in primary neuronal and glial cell cultures. In motor neurons, ex<x>pression of both HSPB8 K141N and K141E mutations clearly resulted in neurite degeneration, as manifested by a reduction in number of neurites per cell, as well as in a reduction in average length of the neurites. Furthermore, ex<x>pression of the K141E (and to a lesser extent, K141N) mutation also induced spheroids in the neurites. We did not detect any signs of apoptosis in motor neurons, showing that mutant HSPB8 resulted in neurite degeneration without inducing neuronal death. While overt in motor neurons, these phenotypes were only very mildly present in sensory neurons and completely absent in cortical neurons. Also glial cells did not show an altered phenotype upon ex<x>pression of mutant HSPB8. These findings show that despite the ubiquitous presence of HSPB8, only motor neurons appear to be affected by the K141N and K141E mutations which explains the predominant motor neuron phenotype in distal HMN and CMT2L.

13: Johnen Nicolas
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SPATIO-TEMPORAL LOCALIZATION OF BETA III TUBULIN IN THE ORGAN OF CORTI AND THE SPIRAL GANGLIA BETWEEN THE EMBRYONIC DAY (E18) AND THE POST-NATAL DAY (P25) IN RAT.

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The mammalian auditory organ, the organ of Corti (OC), is composed of mechanosensory hair cells and nonsensory supporting cell types. Based on their morphology and physiology, at least two types of sensory cells can be identified in the OC: inner and outer hair cells. The structure of this organ is well reported in adult but its development is still little-known.

By using confocal microscopy, we studied the spatial-temporal distribution of beta tubulin III during the differentiation of the OC in rat from the embryonic day 18 (E18) to the postnatal day (P25).

The beta tubulin III is typical for neural cells in the OC. We observed that beta III tubulin is present in the extensions innerving the row of inner hair cells at E18. At E19, the extensions innerving the inner hair cells and the two first rows of outer hair cells were immunolabelled. From E21 to P25, all of hair cells were connected to the spiral ganglion. In the latter, the intensity of immunolabelling decreased between E18 to P25 and the labelling only concerned some cells.

These results reveal that beta III tubulin appears before birth in the nervous extensions connecting the sensory cells of the OC according to a modiolar-to-striolar gradient. In the spiral ganglia, the labelling progressively decreases during its development.

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EXPRESSION OF FUNCTIONAL HYPO-OSMOSENSITIVE TRP CHANNELS IN THE AIRWAY EPITHELIUM.

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The transient receptor potential (TRP) channel superfamily consists of at least 7 subfamilies that are widely expressed in virtually all mammalian cell types, mediating responses to a variety of physical and chemical stimuli. Especially during the last decade, extensive research has resulted in a gradual identification of the biological significance of many members of this heterogeneous family. Two TRP channels, TRPV4 and TRPM3, have been characterized as plasma membrane proteins that mediate Ca2+ entry in cells upon extracellular application of hypo-osmotic solutions. Expression of TRPV4 has been reported in human and mouse tracheal epithelial cells, and in human bronchial epithelial cell cultures. TRPM3 is expressed in several types of epithelial cells, but has not yet been localized to the airway epithelium. The present study aimed at investigating the expression and functionality of the osmosensitive TRPV4 and TRPM3 channels in the epithelium of mouse intrapulmonary airways, by combination of high resolution confocal live cell Ca2+ imaging and multilabel immunohistochemistry.

Immunocytochemical staining of lung cryosections revealed the expression for both TRPV4 and TRPM3 on the majority of mouse airway epithelial cells. Multilabeling experiments showed a strong co-localization between TRPV4 and TRPM3, and confirmed that both ciliated and Clara cells express these TRP channels, but also that NEB cells are negative. Confocal live cell imaging of mouse lung slices taught us that short term (30-60s) administration of hypo-osmotic solutions (200-150 mOsm/kg H2O) results in a reversible and reproducible graded [Ca2+]i rise in all Clara and ciliated cells.

The present study showed that all airway epithelial cells except for the endocrine cells composing NEBs abundantly express functional TRPV4 and TRPM3 channels that are activated by extracellular hypo-osmotic stimulation. Both TRP channels may allow the cells to adjust to changes in extracellular osmolarity and therefore potentially play a central role in airway epithelial homeostasis by modulating epithelial barrier function. The presented approach, using real time confocal live cell imaging in a whole lung slice model, opens interesting new perspectives for unraveling functional aspects of many cell and tissue types in control lungs and disease models.

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PULMONARY NEUROEPITHELIAL BODIES ARE SELECTIVELY ACTIVATED BY MILD HYPO-OSMOTIC STIMULI: A MOLECULAR LIVE CELL IMAGING STUDY.

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Neuroepithelial bodies (NEBs) are morphologically well-defined airway receptors composed of densely innervated groups of neuroendocrine cells that are shielded from the airway lumen by Clara-like cells, and together compose the so-called NEB microenvironment. Based on their extensive vagal sensory innervations, NEBs are suggested to be the morphological counterparts of at least a subpopulation of electrophysiologically characterized vagal mechanosensory airway receptors. So far, however, physiological evidence is lacking. In the present study, we aimed to find out whether NEB cells are sensitive to hypo-osmotic stimuli, which cause cell swelling and may represent an interesting approach to study mechanosensitivity in the NEB microenvironment.

Mouse lung vibratome slices (120 µm thick) were used for confocal live cell imaging of pulmonary NEBs, which were visualized by loading with the styryl pyridinium dye 4-Di-2-ASP. Ca2+ mediated cell activation was monitored by loading with the intracellular Ca2+ indicator Fluo-4. Short term (30s) exchange of the perfusion of the lung slices with a hypo-osmotic solution (230 mOsm/kg H2O instead of 290 mOsm/kg) resulted in a fast, reversible and reproducible Ca2+ rise in NEB cells, which was dependent of extracellular Ca2+. Similar to a control depolarizing stimulus with high K+, osmomechanical activation of NEB cells gives rise to a typical delayed activation of Clara-like cells that is known to be mediated by the release of ATP from activated NEB cells. All other airway epithelial cells, which in contrast to NEBs were demonstrated to abundantly express the well-accepted hypo-osmosensitive TRP channels TRPV4 and TRPM3, appeared to show a Ca2+ rise only at osmolarities lower than 200 mOsm/kg H2O, suggesting a different signaling mechanism than found in NEB cells. The hypo-osmotic activation of NEB cells may well be caused by mechanical deformation of the plasma membrane and associated mechanosensitive ion channels in the plasma membrane. Since ATP is released by activated NEB cells, and a subpopulation of the myelinated vagal afferents with terminals between NEB cells expresses P2X2/3-ATP receptors, the present results offer a possible pathway for NEB cells to transduce osmo-mechanical signals to the central nervous system. The first physiological evidence is provided for direct activation of NEBs by mild acute hypo-osmotic stress that potentially mimics mechanical stimulation.

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OLIGODENDROCYTE-SPECIFIC FADD DELETION PROTECTS MICE FROM AUTOIMMUNE-MEDIATED DEMYELINATION.

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Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model for multiple sclerosis (MS), the most common inflammatory demyelinating disease of the central nervous system (CNS). The disease is characterized by activated auto-reactive myelin-specific lymphocytes that home to the CNS where they initiate a vicious cycle of inflammation and tissue damage. In both MS and EAE, myelin and the myelin-producing cells, the oligodendrocytes (ODCs), are targets of the autoimmune attack, and their loss is directly associated with neuronal dysfunction and damage leading to the clinical manifestations of the disease. Since ODC destruction is central in MS pathology, considerable efforts have been made to clarify the molecular mechanisms behind this process.

A number of studies have suggested that ODCs undergo caspase-dependent apoptosis upon death receptor (DR) stimulation in the context of EAE. DRs are capable of inducing an apoptotic cell death program, involving the recruitment of procaspase-8 to the receptor complex leading to caspase-8 and downstream effector caspase activation. Crucial in this process is the receptor adaptor protein Fas-associated death domain (FADD), which bridges the receptor with procapsase-8. Germline deletion of FADD in mice results in embryonic lethality as a result of cardiac failure and abdominal hemorrhage. Fibroblasts from these mice are completely resistant to TNFR-1-, Fas- and death receptor 3 (DR3)-mediated apoptosis, demonstrating its crucial role.

In this study we sought to address the role of FADD-dependent ODC apoptosis in the pathogenesis of EAE. Therefore, we generated mice lacking FADD specifically in ODCs (FADDODC-KO). ODCs isolated from these mice were tested for their sensitivity to DR-induced apoptosis in vitro, and the response of FADDODC-KO mice was analyzed in an EAE model.

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LIVER X RECEPTORS REGULATE CHOLESTEROL HOMEOSTASIS IN OLIGODENDROCYTES.

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Liver X receptors (LXRs) are ligand activated nuclear receptors that play an important role in the control of cellular and whole-body cholesterol homeostasis. Both LXR isoforms (LXR-alpha and LXR-beta) are expressed in the central nervous system (CNS) and are involved in the regulation of brain cholesterol metabolism. However, their presence in oligodendrocytes (OLGs) and their function in OLG cholesterol homeostasis remain largely unknown. High cholesterol levels in OLGs are essential for myelin membrane growth during maturation of the CNS. In order to gain insight into cholesterol homeostasis in OLGs, expression levels of the LXRs and their target genes were investigated in neonatal or mature rat OLG cultures using quantitative real time PCR. The LXRs, as well as the LXR target genes (ApoE, ABCA1, ABCG1, ABCG4 and LDLR) were detected in both primary OLG cell types. Treatment of primary neonatal rat OLGs with the LXR agonist T0901317 induces the expression of several established LXR target genes, including ApoE, ABCA1, ABCG1, ABCG4 and LXR-alpha itself. Furthermore, treatment of the OLGs with T0901317 resulted in an enhanced cholesterol efflux in the presence of Apolipoprotein AI or high density lipoprotein particles. LXR activation also induces morphological changes. These data show that LXRs and their target genes are present in OLGs and regulate their cholesterol homeostasis.

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IL-4 AND IL-13 EXERT OPPOSING EFFECTS ON FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY.

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Increasing evidence suggest that inflammatory processes after spinal cord injury (SCI) may cause detrimental as well as beneficial effects. Previous studies indicate that T cells may influence axon regeneration via secreted anti-inflammatory cytokines such as interleukin-4 (IL-4) and IL-13. The anti-inflammatory cytokines IL-4 and IL-13 have multiple functions in common and share the IL-4 receptor alpha-chain (IL-4Ralpha) which is thought to be responsible for most of the functional characteristics. In the current study we have investigated the therapeutic potential of recombinant IL-4 and IL-13 administered locally in gelfoam beads above the lesion at selected time points after SCI. Single administration of recombinant IL-4 immediately and 1 day after the injury significantly improved locomotor restoration as assessed by the open-field Basso Mouse Scale (BMS). Histological analysis revealed that recombinant IL-4 significantly stimulates axon regrowth in the lesioned spinal cord in vivo. Surprisingly, immediate local administration of recombinant IL-13 worsens clinical outcome after SCI. In summary, our results indicate that acute treatment with recombinant IL-4 significantly promotes functional recovery after SCI, while IL-13 exerts opposing effects and thus impairs clinical outcome after SCI in vivo although both cytokines share the IL-4Ralpha.

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COMPARATIVE ANALYSIS OF CELLULAR GRAFT BEHAVIOR IN VIVO REVEALS CELL TYPE-ASSOCIATED AND INFLAMMATION-INDUCED DIFFERENCES IN SURVIVAL, MIGRATION AND IMMUNOGENICITY.

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Although the neuro-protective and -regenerative effects of cell transplantation in the central nervous system (CNS) have been demonstrated, most pre-clinical cell therapy studies report mainly on clinical observations, while currently little is known regarding the actual in vivo fate of grafted cell populations. In this study, we transplanted neural stem cells (NSC), bone marrow mononuclear cells (BMMNC), dendritic cells (DC), mouse embryonic fibroblasts (MEF) and splecocytes (SPLEEN) into the CNS of healthy C57BL/6 mice and into the demyelinated CNS of C57BL/6 mice that received a 4-week Cuprizone-supplemented diet. For each experimental group, extensive histological analysis was performed at week 2 post-grafting in order to: (i) quantify cell graft migration, survival, and toxicity, and (ii) determine and characterize endogenous neuro-immune responses against the different cell types grafted. While MEF, NSC and SPLEEN grafts did not significantly migrate from the injections site in both healthy and demyelinated CNS, the significant migration observed for BMMNC and DC grafts was associated with substantial toxicity to both healthy and demyelinated CNS tissue. In addition, none of the grafted cell populations were able to contribute to the natural remyelination process occurring following arrest of Cuprizone-supplemented diet. Moreover, no endogenous remyelination was observed at the site of cell injection in demyelinated CNS, while MEF, BMMNC, DC and SPLEEN grafts, but not NSC grafts, induced substantial damage to the myelin structure of the graft site in healthy CNS. Furthermore, we investigated whether and how the CNS innate immune system interacted with the different cell types grafted. NSC, BMMNC and SPLEEN grafts became highly infiltrated with microglia and astrocytes, both in healthy and in demyelinated CNS. MSC grafts however, became highly infiltrated by microglia, but encapsulated by astrocytes. DC grafts in contrast, due to their extreme toxicity, were mainly surrounded by an endogenous astrocytic scar tissue. In summary, despite many previously published observations that cell grafting in the CNS can contribute to tissue regeneration, we warrant that further research should be undertaken to understand - and eventually control - cell graft induced tissue damage and activation of the CNS innate immune system.

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AUTOANTIBODY PROFILING IN EARLY MULTIPLE SCLEROSIS.

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Background and objective:

Oligoclonal band antibodies in cerebrospinal fluid (CSF) are a characteristic of Multiple Sclerosis (MS). However, the targets of these antibodies remain elusive. In addition, it is difficult to establish an early diagnosis of MS. The objective of this study is to identify a panel of MS-associated antigenic targets suitable as biomarkers.

Methods:

To identify markers for early disease, autoantibody profiling was performed with a phage display technique, called serological antigen selection (SAS), on pooled CSF from Clinically Isolated Syndrome (CIS) patients (n=4), who developed definite MS, early Relapsing Remitting (RR) MS (n=6) and Primary Progressive (PP) MS (n=6) patients. This procedure is based on expression of a cDNA library fused to filamentous phage protein VI and interactions between exposed proteins and Immunoglobulin G (IgG) antibodies. Two cDNA display libraries have been constructed: a normalized cDNA library derived from active chronic MS plaques and a cDNA library made from healthy normal white matter.

Results:

The results of the SAS procedures were characterized with PCR and restriction digestion analysis. Subsequently, enriched putative antigenic targets displayed on phage were identified with sequence analysis. This led to the identification of 25 CSF candidate antigens for CIS, 18 for RRMS and 15 for PPMS. Some of the enriched clones identified were identical, although derived from separate SAS procedures with the two cDNA libraries, indicating a specific antibody response. Several other clones express proteins involved in related cell processes. Additionally, clones that have been identified show homology with MS-related proteins. However, no antibody reactivity against these targets has been described so far.

Conclusions:

We have identified several candidate markers for MS and to confirm their specific immunoreactivity, phage ELISA will be performed on CSF and serum of individual patients used in the SAS procedure, additional MS patients and controls with other (neurological) diseases. Eventually, more studies will be performed on MS specific antigenic targets to study their biological relevance and biomarker potential.

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THE MOUSE PULMONARY NEUROEPITHELIAL BODY (NEB) MICROENVIRONMENT HARBORS A GABAERGIC SIGNALING MECHANISM.

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Gamma-aminobutyric acid (GABA) serves a multitude of known physiological functions. Although best studied as the main inhibitory neurotransmitter in the central nervous system (CNS) of vertebrates, GABA also fulfills roles outside the CNS as a signaling molecule during embryonic development and in adult regeneration. The synthesis of GABA from glutamic acid is a decarboxylation reaction that is catalyzed by the enzyme glutamic acid decarboxylase (GAD), that in mammals exists in two isoforms, GAD65 and GAD67.

The airway epithelium harbors morphologically well characterized complex organizations of densely innervated groups of neuroendocrine cells, the neuroepithelial bodies (NEBs). The latter are invariably shielded from the airway lumen by so-called Clara-like cells, which reveal stem cell properties and together with the nerve terminals and endocrine cells are referred to as the NEB microenvironment. Recently, the existence of a GABAergic system has been proposed in NEBs in monkey lungs. However, for the moment very little is known about the possible involvement of GABAergic signaling pathways in the lungs in health and disease. The presented study intended to further elaborate the idea using mouse models that offer more extensive possibilities for fundamental experimental research. Interest was focused on the possibilities of the models for confocal live cell imaging of the NEB microenvironment.

Immunocytochemistry on cryostat sections of prenatal (gestational day 17-20), three-week-old and adult mice, revealed that in mouse lungs GAD was expressed in highly selective cell groups of the airway epithelium. Double staining with calcitonin gene-related peptide (CGRP), a marker for NEBs in several species, identified all GAD-expressing cells as NEB cells. Multilabel immunostaining with markers for NEB-associated nerve fiber populations and Clara cells clearly demonstrated that in mouse lungs GAD is present in NEB cells only. We further studied the expression of GAD67-green fluorescent protein (GFP) in lungs of GAD67-GFP knock-in mice, and found that all CGRP-ir NEBs expressed GAD67-GFP, and vice versa.

Although the functional significance of GABA synthesis in NEBs remains to be determined, the identification of GFP-fluorescent NEBs in the GAD67-GFP mouse model will certainly boost future functional NEB studies using our recently established ex vivo lung slice model for confocal molecular live cell imaging of NEBs.

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A FETAL LUNG SLICE MODEL FOR MOLECULAR LIVE CELL IMAGING OF THE PULMONARY NEUROEPITHELIAL BODY MICROENVIRONMENT.

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The pulmonary neuroepithelial body (NEB) microenvironment is composed of extensively innervated groups of neuroendocrine cells that are shielded from the airway lumen and the surrounding epithelium by Clara-like cells. The prominent presence of differentiated NEBs from early embryonic development on, suggests that NEBs may contribute to airway epithelial growth and maturation. Moreover, the relatively highest density of NEBs during the perinatal period reflects their potential importance for the transition from fetal to neonatal life. For confocal molecular live cell imaging of physiological reactions in NEBs and surrounding epithelial cells in their 'natural environment', we recently established an ex vivo lung vibratome slice model in postnatal mouse lungs (postnatal days 1-21 and adult). The main goal of the present study was to further extend the possibilities of this model to the study of fetal lungs (gestational days 17-20).

Lung slices were prepared of in situ agarose-filled fetal mouse lungs. After incubation with the fluorescent stryryl pyridinium dye 4-Di-2-ASP, NEBs could be identified as clusters of small, rounded fluorescent epithelial cells, and were typically surrounded by a continuous layer of larger, rounded and virtually non-fluorescent Clara-like cells. Ciliated cells appeared polygonal and strongly fluorescent. Using Fluo-4, changes in the intracellular free calcium concentration ([Ca2+]i) were monitored in NEBs and surrounding airway epithelial cells. Similar to observations in neonatal lung slices, application (5s) of 50mM extracellular high K+, an established control stimulus for postnatal NEBs, also evoked a fast, reversible and reproducible [Ca2+]i increase in fetal NEB cells. Clara-like cells displayed a delayed rise in [Ca2+]i, suggestive of an indirect NEB-mediated activation. Stimulation of Fluo-4 loaded lung slices with 20 µM ATP (10s) caused a rise in Fluo-4 fluorescence in Clara and Clara-like cells, to a lesser extent in ciliated cells, and in contrast to observations in postnatal lungs, also in fetal NEB cells. Immunocytochemical staining on fixed lung slices for calcitonin gene-related peptide, a marker for NEB cells, revealed pulmonary NEBs in all slices of prenatal mice, enabling the use of fetal lung slices to link physiology to neurochemistry. A population of NEB-associated vagal sensory nerve terminals, expressing P2X3 ATP receptors, could also be detected.

In conclusion, the presented fetal mouse lung slice model for molecular live cell imaging offers excellent possibilities to further unravel the significance of NEBs during the prenatal and perinatal period.

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ONCOSTATIN M REGULATES THE DEVELOPMENT OF INFLAMMATORY CNS LESIONS.

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The neuropoietic cytokine oncostatin M (OSM) is undetectable in healthy CNS, but it is expressed in multiple sclerosis (MS) lesions. Whether or how OSM affects CNS lesion development remains unknown. OSM limits neuronal death induced by excitotoxicity, but in non-neuronal disease models such as arthritis, it is associated with inflammation and tissue damage. This study was designed to elucidate the role of OSM in the development of MS lesions. We induced expression of OSM in the CNS of healthy mice by means of lentiviral vectors. OSM expression disrupted the blood-brain barrier and induced a local inflammatory response, characterized by an upregulation of adhesion molecules, MHCII expression and an infiltration of T-cells and macrophages/microglia in the otherwise healthy CNS. This indicates that OSM is a potent inducer of inflammatory CNS lesions. However, the incidence of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), was significantly reduced in mice with CNS-targeted OSM expression. While we did find a local inflammatory response at the site of OSM expression, infiltration of typical EAE-related immune cells in the spinal cord was significantly reduced. In contrast to CNS-targeted OSM, systemic administration of the cytokine did not affect development of autoimmune CNS lesions. Our study reveals a dual role for OSM in MS pathogenesis. While its local expression is sufficient to induce several aspects characteristic of CNS lesion development, it inhibits the development of autoimmune CNS lesions.

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SAFETY AND T CELL MODULATING EFFECTS OF HIGH DOSE VITAMIN D3 SUPPLEMENTATION IN RELAPSING REMITTING MULTIPLE SCLEROSIS.

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Background:

A poor vitamin D status has been associated with a high disease activity of multiple sclerosis (MS). In vitro, vitamin D has anti-inflammatory effects on T cells. Recently, we described associations between in vivo vitamin D status and peripheral T cell characteristics in relapsing remitting MS (RRMS) patients. In the present study, we studied the effects of high dose vitamin D3 supplementation on safety and T cell related outcome measures.

Methods:

Fifteen RRMS patients on Beta Interferon treatment were supplemented with 20 000 IU/d vitamin D3 for 12 weeks. Vitamin D and calcium metabolism were carefully monitored, and T cell characteristics were studied. Regulatory T cell (Treg) function was assessed in a proliferation suppression assay. T cell phenotype and cytokine profile were analyzed by flowcytometry.

Results:

All patients finished the protocol without side-effects, hypercalcaemia, or hypercalciuria. The median vitamin D status increased from 50 nmol/L (31–175) at week 0 to 380 nmol/L (151–535) at week 12 ($P<0.001$). During the study, 1 patient experienced an exacerbation of MS and was censored from the T cell analysis. The proportions of (naïve and memory) CD4+ Tregs remained unaffected. Although Treg suppressive function improved in 9 subjects, this effect was not significant in the total cohort ($P=0.143$). A decrease of the ratio between IFN-g+ and IL-4+ CD4+ T cells was observed ($P=0.035$). Additionally, the ratio between IL-17+ and IL-10+ CD4+ T cells was decreased ($P=0.003$).

Conclusion:

Twelve week supplementation of high dose vitamin D3 in RRMS patients was well tolerated and did not induce decompensation of calcium metabolism. The skewing towards an anti-inflammatory cytokine profile supports the evidence on vitamin D as an immune-modulator, and may be used as outcome measure for upcoming randomized placebo-controlled trials.

This study is registered at www.clinicaltrials.gov as NCT00940719.

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SPAG16 ISOFORM 2: THE ROLE OF A NOVEL AUTOANTIGEN IN MULTIPLE SCLEROSIS.

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Background and goals:

In a previous study aimed at the high-throughput profiling of the autoantibodies present within multiple sclerosis (MS) cerebrospinal fluid (CSF), we identified a novel MS CSF autoantibody target 'sperm associated antigen 16 isoform 2' (SPAG16-2). As tissue expression, protein function and disease-contributing role of SPAG16-2 are unknown, further investigation into this candidate MS autoantigen was warranted. In this study, we aimed to further characterize SPAG16-2 as a novel candidate MS autoantigen.

Methods and results:

To analyze the presence and biomarker potential of anti-SPAG16-2 antibodies in MS serum, a recombinant protein ELISA was used for immunoreactivity screening in sera from MS patients (n=69) and controls (healthy controls, n=43, inflammatory and non-inflammatory neurological diseases, n=47). Elevated serum antibodies against SPAG16-2 were detected in 13% (9/69) of MS patients with 100% specificity (0/90 controls) for the disease. In addition, to investigate the biological relevance of SPAG16-2 in MS, the tissue expression of the antigen in MS and control brain was analyzed by immunohistochemical approaches. We demonstrated increased expression of SPAG16-2 in the center of MS brain lesions (n=2), while no detectable staining was seen within the normal appearing white matter of MS and control brain tissue (n=2).

Moreover, passive antibody-transfer experiments with anti-SPAG16-2 monoclonal antibodies in MOG-peptide induced experimental autoimmune encephalomyelitis (EAE), the animal model for MS, indicated a disease-exacerbating effect of these antibodies. Mice receiving anti-SPAG16-2 antibodies (n=5) had a higher disease burden indicated by significantly higher disease scores compared to animals injected with isotype control antibodies (n=4) ($P<0.01$). Spinal cords from mice injected with anti-SPAG16-2 antibodies were characterized by increased macrophage infiltration without any obvious effect on demyelination. We are currently further investigating how anti-SPAG16-2 antibodies exert their pathogenic effects.

In addition, we are currently comparing T cell reactivity against SPAG16-2 between MS patients and controls to provide further evidence of a distorted autoimmune response against the antigen.

Conclusions:

In conclusion, the findings in this study indicate that SPAG16 isoform 2 constitutes a novel candidate MS autoantigen. Future experiments are aimed at the elucidation of the function of this protein and the role of autoantibody reactivity against it in MS.

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MICROGLIA IN THE EMBRYONIC NEOCORTEX – MATERNAL INFLAMMATION AFFECTS EMBRYONIC MICROGLIA.

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Infection during pregnancy can lead to maternal inflammation. Several studies have suggested that maternal inflammation increases the risk on neuropsychiatric disorders, like autism, in the offspring. The cause of autism remains unknown. It is thought to be a complex interaction of different factors. Vargas et al. demonstrated the presence of an active neuroinflammatory process in the brains of autistic patients, with marked microglial cell activation. Microglia colonize the central nervous system early in embryonic development, at the moment that neuronal migration to the cortical plate is peaking and neuronal differentiation and synaptogenesis are underway. By their production of growth factors, it has been suggested that microglia can influence axonal growth and synaptogenesis.

The aim of this study is to determine the localization, activation stages and migration routes of the microglia present in the embryonic murine neocortex, in healthy embryos and embryos subjected to maternal inflammation. Pregnant mice were injected with PBS (control) or polyinosinic–polycytidylic acid (inflammation) on E11,5 and the transgenic CX3CR1 +/eGFP embryos were isolated at the various ages (E12,5 – E16,5). Coronal sections were stained for a set of microglial activation markers (Iba-1, CD11b, CD68 and MHC class II) and nestin was used to visualize radial glial cells.

In the control group, as expected, the cell density and number of microglial ramifications increase as the embryo ages. This increase in cell density is also present in the inflammation group, however compared to the control it is more pronounced. ba<x>sed on the expression levels of CD68 and CD11b, the microglial cells of the embryos subjected to maternal inflammation show a higher activation profile as those from the control group. Microglial cells are also present in the lateral ventricle and at the pial surface. It has been shown that microglia enter the nervous parenchyma from these sites. The orientation of the protruding ramifications of microglia present in the parenchyma suggests that the cells migrate along radial glial fibers to reach their final position. Confocal images confirm contact between both cell types.

27: Thewissen Kristof
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MULTIPLE SCEROSIS IS ASSOCIATED WITH INCREASED NUMBERS OF CIRCULATING DENDRITIC CELLS.

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Background + Objective:

Dendritic cells (DC) belong to the innate immunity and are widely known as professional antigen-presenting cells. Due to their specialized antigen-presenting capacity an important link is provided to the adaptive immune system where they regulate the balance between immunity and tolerance. Recent studies have shown that DC can control autoreactive T cells and even induce regulatory T cells. Moreover there is evidence for a bidirectional interaction between regulatory T cells and DC. We and others previously demonstrated that CD4+CD25+ regulatory T cells are less functional in patients with multiple sclerosis (MS), but the cause of this remains still unknown. We hypothesize that a disturbance in DC-Treg axis can ultimately lead to the induction or perpetuation of an autoimmune disease like MS. To test this, an ex vivo analysis was performed on DC and Tregs in peripheral blood of MS patients and healthy controls.

Methods:

An ex vivo analysis of different subsets of DC was carried out on the peripheral blood of MS patients and healthy controls with flow cytometry. Dendritic cell subsets were investigated for their phenotype (CD62L and CD86) and frequency in a group of MS patients (n=46) and age- and gender-matched healthy controls (n=37).

Results:

The frequency of plasmacytoid (BDCA2+BDCA1-, $p<0.01$) and myeloid DC (BDCA1+BDCA2-, $p<0.05$) is increased in the blood of MS patients as compared to healthy controls. This increase is even more pronounced in SP/PP MS patients ($p<0.05$). Furthermore, plasmacytoid DC of MS patients do not express the costimulatory molecule CD86 ($p<0.001$) whereas myeloid DC show a reduced expression of the cell adhesion molecule CD62L ($p<0.001$).

Conclusion:

The increased frequency of DC subsets in MS patients suggests an important role of DC in the induction or perpetuation of the immunological imbalance present in MS patients. Furthermore the absence of CD86, which preferentially induces Th2 responses, on plasmacytoid DC proposes a skewing toward Th1. Myeloid DC on the other hand are possibly retained in the periphery due to a lower expression of CD62L which controls migration to secondary lymphoid organs.

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LIVER X RECEPTORS IN CENTRAL NERVOUS SYSTEM INFLAMMATION.

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Background:

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating disease of the central nervous system (CNS) in which macrophages play a pivotal role. Initially, macrophages where thought to be only detrimental in MS, as they phagocytose myelin and secrete toxic mediators. However, recent evidence suggests that macrophages can also have anti-inflammatory effects and have the capacity to induce repair. Nonetheless, underlying mechanisms inducing a protective phenotype in macrophages remain to be clarified. Liver X receptors (LXRs) are ligand dependent transcription factors that regulate the expression of genes involved in cholesterol metabolism. In addition, LXRs have been described to repress the expression of certain inflammatory genes in macrophages. Since myelin contains cholesterol, which is the natural ligand for LXRs, these receptors may play a role in the induction of a protective phenotype in macrophages. We hypothesize that LXRs are activated after myelin phagocytosis and induce a protective, anti-inflammatory phenotype in macrophages. The goal of this study is to unravel the role of LXRs in the macrophage response after myelin phagocytosis.

Methods:

The activation of LXR response genes, after myelin phagocytosis, was studied in rat peritoneal macrophages by real-time PCR. Next, macrophage production of inflammatory mediators after myelin phagocytosis was investigated with NO-assays and DHR-assays.

Results:

LXR response genes are upregulated in macrophages after 48h incubation with both T09, a LXR agonist, and myelin. Furthermore, NO produced by macrophages significantly decreases after 24h incubation with both T09 and myelin. Finally, incubation of macrophages with both myelin and T09 significantly increases reactive oxygen species release.

Conclusions:

These results indicate that LXRs are activated after myelin phagocytosis and multiple pathways, probably including LXR signalling, are responsible for the myelin-induced protective phenotype in macrophages. Thus, during demyelination, macrophages with a protective phenotype may be induced that limit lesion progression. Targeting LXRs in an early stage of lesion-formation may prevent loss of axonal conduction and improve disease outcome in MS.

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PRESENCE AND NEUROCHEMICAL CODING OF INTRINSIC VIP/PACAP-POSITIVE ENTERIC NEURONS IN THE ADULT ZEBRAFISH.

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In a previous study, detecting the co-expression of neurochemical markers, five different neuronal classes were identified in the zebrafish enteric nervous system (ENS): a serotonergic, a nitrergic non-cholinergic, two cholinergic non-nitrergic subpopulations, and one subpopulation expressing both choline acetyltransferase (ChAT) and neuronal nitric oxide synthase (nNOS). Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP) did not yield immunoreactivity (IR) in neuronal cell bodies, only in nerve fibers. At present, we attempted using an in vitro colchicine pretreatment to detect VIP and PACAP in neuronal cell bodies to determine peptidergic subpopulations in the ENS of the adult zebrafish. Colchicine, previously used in mammals to improve immunostaining of neuronal cell bodies, is known to cause derangement of cellular microtubules, which leads to inhibition of axonal transport and accumulation of axonally transported substances in the neuronal cell body.

To enhance the IR for neuropeptides within the neuronal somata, whole mount preparations of the intestines of adult animals were, prior to fixation, maintained in organotypic culture in sterile Dulbecco's modified Eagle medium supplemented with 10 mg/ml antibiotic-antimycotic, 50 µg/ml gentamycin, 2.5 µg/ml amphotericin B, 10% fetal bovine serum, 1µM nifedipine and 88 µM colchicine, at 28°C for 16 hours. The proportional distribution of VIP and PACAP in the ENS was studied using multiple immunofluorescent staining methods. An antibody directed against human neuron-specific HuC/HuD proteins was used as a pan-neuronal marker. Furthermore, co-expression of VIP and PACAP with other neurochemical markers was examined.

Treatment of colchicine enhanced IR for VIP and PACAP as such that IR was not only observed in nerve fibers, i.e., varicosities, but also in neuronal cell bodies. A complete colocalization for VIP and PACAP was observed. VIP/PACAP-expressing neurons accounted for ±20% of the total neuronal population. Coexpression of VIP/PACAP with calretinin (CR) or calbindin (CB) was detected in few neuronal cell bodies (<5% of the total neuron population). ±70% of the VIP/PACAP-positive neurons showed nNOS-IR (14% of the total neuron population) and ±20% showed serotonin(5HT)-IR (5% of the total neuron population). ChAT-IR was not observed after pretreatment with colchicine.

These results complement and extend previous data regarding neurochemical properties of the zebrafish ENS. The neurons expressing VIP and PACAP make up 20% of the total neuronal population, from which 20% are serotonergic and 70% are nitrergic. Further immunostainings are necessary to reveal which of the previous identified subpopulations coexpress VIP and PACAP.

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DETECTION OF PHOSPHORYLATED BRAINSTEM PROTEINS THAT ARE DIFFERENTIALLY EXPRESSED OVER THE DISEASE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE).

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Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system in which axons and myelin are damaged. The underlying molecular processes remain poorly understood, but are crucial in the search for new therapeutic options. Protein phosphorylation, important for protein function, may be involved in the pathology of MS and experimental autoimmune encephalomyelitis (EAE), an animal model of MS. The identification of a panel of differentially expressed phosphorylated proteins along the disease course could provide valuable insights into global disease processes of EAE and MS.

Protein extracts from 'blood-free' brain stems of control and EAE Lewis rats were separated by 2D-gel electrophoresis. To detect phosphorylated proteins, gels were stained with a fluorescent dye, Pro-Q diamond phosphoprotein gel stain (Invitrogen). A fluorescent total protein staining was used as quality control and thus normalization of the phosphoprotein signals. Moreover total protein staining enables matching of different gels. Quantitative data were obtained by comparison to a previous two-dimensional difference in-gel electrophoresis (2D-DIGE) study at different disease stages (control, onset, top and recovery, three biological replicates each). All identified differentially expressed (ANOVA X04,0.05) proteins from this 2D-DIGE study (95) were checked for possible phosphorylation.

Both sensitivity and specificity of the Pro-Q diamond phosphoprotein gel stain were analyzed using different concentrations of the peppermintstick phosphoprotein standard (Invitrogen). Some background staining was detected, making it difficult to distinguish between differences in phosphorylation load. The presence of phosphorylated, and differentially expressed proteins is currently being confirmed by immunohistochemistry (IHC) and 1D or 2D western blotting (WB). These techniques allow us to determine the presence of the phosphorylated protein, but also the expression pattern if multiple time points are included. The global overview of phosphorylation during disease invites new studies to unravel the complicated molecular biological processes in the pathology of MS.

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INTERFERON ALPHA SUPPRESSES ALPHAHERPESVIRUS IMMEDIATE EARLY PROTEIN LEVELS IN SENSORY NEURONS, LEADING TO THE ESTABLISHMENT OF A LATENT INFECTION.

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Alphaherpesviruses are a subfamily of the herpesviruses containing closely related human and animal pathogens, including human herpes simplex virus (HSV-1) and porcine pseudorabies virus (PRV).

Cycles of latency and reactivation are a very important characteristic of these alphaherpesviruses, for example leading to recurrent episodes of cold sores and genital lesions in HSV. Neurons of the trigeminal ganglion (TG) are the predominant site of latency in both HSV-1 and PRV. Establishment of latency is generally thought to be the result of a delicate balance between virus, neuron, and unidentified immune effectors. Using an in vitro 2-chamber model based on porcine TG cultures, we have identified interferon alpha (IFN) as an immune effector that is capable to drive both HSV-1 and PRV, in a latent state in vitro.

The IFN-induced establishment of in vitro latency was found to correlate with suppression of the immediate early (IE) protein ICP4 in HSV-1 and its homologue IE180 in PRV. IFN-mediated IE suppression was more efficient and rapid in HSV-1 than in PRV, correlating with a more efficient establishment of in vitro latency using HSV-1 versus PRV. To further investigate the mechanism of IFN-mediated IE suppression and the differences in efficiency in IFN-mediated IE suppression between HSV-1 and PRV, we made use of rat dorsal root ganglion neuronal cells (50B11) (Chen et al., 2007) because of the technical limitations associated with primary TG cultures.

At the protein level, at 4h post inoculation (hpi), for HSV-1, ICP4 protein expression was strongly reduced in IFN-treated samples (75% reduction) while for PRV, IFN treatment only slightly affected IE180 protein levels (15% reduction). At 8hpi and 12hpi the IE protein levels were significantly suppressed for both viruses. Using qRT-PCR, mRNA levels of either HSV-1 ICP4 or PRV IE180 at 4hpi were found not to be significantly different in IFN-treated samples versus control samples, whereas a strong reduction was observed at 8hpi and 12hpi (76.5 to 96%).

To investigate the lack of IE translation inhibition during PRV infection, we analyzed IFN-mediated phosphorylation and thereby inactivation of the translation initiation factor eIF2alpha. As expected, treatment of cells with IFN and subsequent infection with HSV-1 resulted in a strong increase in phosphorylation of eIF2alpha. However, this increase was entirely absent in PRV-infected cells, showing that PRV circumvents IFN-mediated translation inhibition interfering with phosphorylation of eIF2alpha.

In summary, IFN-mediated suppression of viral IE proteins may be a key step in establishment of alphaherpesvirus latency. IFN acts at two stages to suppress IE protein levels: first at the translational level and later at the transcriptional level. However, PRV (but not HSV-1) is able to avoid IFN-mediated translational control of IE levels by phosphorylation of the translation initiation factor eIF2alpha.

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CHARACTERIZATION OF THE AUTO-ANTIBODY REPERTOIRE IN MYASTHENIA GRAVIS BY IMMORTALIZING THYMIC B CELLS.

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Introduction:

Immortalization of thymic B cells by Epstein Barr Virus (EBV) transformation provides a useful tool to characterize the auto-antibody repertoire in AChR-positive myasthenia gravis patients.

Methods:

Mature B lymphocytes were isolated from the thymi of 3 MG and 3 control patients and immortalized by EBV transformation. To overcome the low efficiency of EBV-ba<x>sed immortalization, the polyclonal B cell activator CpG 2006 was added to the B cells.

Results:

The immortalized B cell clones produce monoclonal antibodies mainly belonging to the IgM subclass. Screening of the antibodies by immunohistochemistry on muscle tissue showed immunoreactivity to striated muscle proteins in about 25% of MG and 5% of control B cell lines. However, analysis by ELISA showed that these monoclonal antibodies were not directed against muscle proteins myosin, actin, alpha-actinin, nor titin which have been previously described to be expressed in MG thymi. 5 out of 250 B cell clones of MG thymus co-precipitated with the AChR as detected by radioimmunoassay, suggesting that the thymus does not contain an enriched population of mature B cells with AChR specificity. However, one B cell clone was found to produce pathogenic anti-AChR antibodies which internalized 50% of AChRs at the surface of TE671 cells. Finally, the coding genes of the IgM and IgG heavy chains were sequenced. The distribution of the VH families in immortalized MG B cells was comparable to healthy controls. Parsimony trees created from heavy chain B cell clones from MG thymi showed a high homology between the different clones suggesting clonal expansion from a B cell ancestor.

Conclusion:

Characterizing monoclonal antibodies produced by MG thymocytes will provide further information in autoimmune processes of MG.

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ACUTE NEURODEGENERATION TRIGGERS AN ALTERNATIVE MACROPHAGE RESPONSE.

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Neurodegeneration triggers a strong immune response, which leads to the secretion of different cytokines and immune mediators. Traditionally this activation has been considered to negatively contribute to the disease progress. However, in recent years a protective role for the immune system in neurodegeneration has been shown as well.

Since most studies remain descriptive, we decided to focus on the underlying mechanisms that could determine the balance between protection and degeneration. To approach this we are studying a model of acute neurodegeneration, i.e. axotomy of the peripheral nerve, in which the immune system has been shown to have a protective role.

We observed that acute neurodegeneration led to a transient immune response, in which cytokines returned to basal levels at 72 hours upon injury. This was associated with the induction of several negative regulators of the innate immune system, which could be crucial for limiting inflammation. Although several cytokines were induced, typical markers for classical macrophage activation, like iNOS and interferon-gamma, were completely absent. On the other hand markers of alternative macrophage activation, like arginase 1 and chitinase 3-like-3, were clearly upregulated. High levels of IL-10 and the absence of IL-12 pointed towards the presence of a regulatory macrophage phenotype. All these processes seemed to rely on IL-13, but not IL-4, since the latter was not detectable in our model.

In conclusion these data suggest that acute neurodegeneration triggers an environment favoring the recruitment of alternatively activated macrophages. We are currently following up on how this alternative environment is established and if it is involved in determining the balance between neuroprotection and neurodegeneration.