Dear Colleagues,

on behalf of the ESNI Organizing Commitee I cordially invite you to participate in the 15th European School of Neuroimmunology (ESNI) course, which will be held on June 1st-4th, 2015 in Prague, Czech Republic. ESNI provides high level education in the rapidly developing field of neuroimmunology.

The programme of the 15th ESNI Course will be focused on many topics in contemporary neuroimmunology including the role of innate and adaptive immunity in broad spectrum of nervous system diseases, newest imaging and diagnostics tools, basic research and translational neuroimmunology. Active students participation during the ESNI course is encouraged especially in students´ presentation section by chairing, lectures and poster presentations. The aim of the scientific programme is to keep participants on the edge-level of neuroimmunology.

Prague belongs to one of the most popular destinations in the world with rich history, architecture, cultural life and gastronomy. It is the seat of the famous Charles University - the oldest university in the Central Europe. The venue Congress Centre of "Na Homolce Hospital" is fully equipped and will provide comfortable background for this exciting scientific course.

I am looking forward to welcome you in Prague.

Pavel Stourac
Local Organizer

BOARD MEMBERS

Altintas, Ayse | Turkey
Ben-Nun, Avraham | Israel
Comabella, Manuel | Spain
Comi, Giancarlo | Italy
Elovaara, Irina | Finland
Furlan, Roberto | Italy
Gilhus, Nils Erik | Norway
Graus, Francesc | Spain
Grigoriadis, Nikolaos | Greece
Hartung, Hans-Peter | Germany
Hintzen, Rogier | the Netherlands
Hohlfeld, Reinhard | Germany
Karabudak, Rana | Turkey
Karussis, Dimitrios | Israel
Lassmann, Hans | Austria
Leite, Maria Isabel | Portugal
Liblau, Roland | France
Linington, Christopher | UK
Miljkovic, Djordje | Serbia
Miller, Ariel | Israel
Nataf, Serge | France
Olsson, Tomas | Sweden
Owens, Trevor | Denmark
Polman, Chris | the Netherlands
Probert, Lesley | Greece
Sà, Maria José | Portugal
Schwartz, Michal | Israel
Scolding, Neil | UK
Selmaj, Krzysztof | Poland
Stinissen, Piet | Belgium
Stourac, Pavel | Czech Republic
Uccelli, Antonio | Italy
Vincent, Angela | UK
Woodroofe, Nicola | UK

COORDINATORS

Gianvito Martino
Institute of Experimental Neurology (INSpe)
Division of Neuroscience
San Raffaele Scientific Institute
Via Olgettina 58
20132 Milan - Italy
E-mail: martino.gianvito@hsr.it

Hugh Willison
Glasgow Biomedical Research Centre,
Oxford, UK
GBRC B330, 120 University Place
G12 8TA Glasgow - UK
E-mail: hugh.willison@glasgow.ac.uk

LOCAL ORGANIZING COMMITTEE

Pavel Stourac
MD, PhD
Department of Neurology
University Hospital Brno
Brno - Czech Republic

SCIENTIFIC COMMITTEE

Christopher Linington
PhD
University of Glasgow
Glasgow | UK

Trevor Owens
PhD
University of Southern Denmark
Odense | Denmark

Lesley Probert
PhD
Hellenic Pasteur Institute
Athens | Greece

Nicola Woodroofe
PhD
Sheffield Hallam University
Sheffield | UK

SPEAKERS & CHAIRS

Amor, Sandra BSc, PhD
VU Medical Center
Amsterdam | the Netherlands

Becher, Burkhard PhD
University of Zurich
Zurich | Switzerland

Bennett, Jeffrey MD, PhD
University of Colorado Denver
Denver, CO | United States

Dalmau, Josep MD
University of Barcelona
Barcelona | Spain

De Jager, Philip MD, PhD
Brigham and Women's Hospital
Boston, MA | United States

Furlan, Roberto MD, PhD
San Raffaele Hospital
Milan | Italy

Gomez-Nicola, Diego PhD
University of Southampton
Southampton | UK

Havrdova, Eva MD, PhD
Charles University in Prague
Prague | Czech Republic

Heneka, Michael MD
University of Bonn
Bonn | Germany

➔

Illés, Zsolt MD
Odense University Hospital
Odense | Denmark

Issazadeh-Navikas, Shohreh MD, PhD
University of Copenhagen
Copenhagen | Denmark

Kerschensteiner, Martin PhD
Medical Center of the University of Munich
Munich | Germany

Kirshnamoorthy, Gurumoorthy MD
Max Plank Institute of Neurobiology
Munich | Germany

Korn, Thomas MD
Technische Universitat Munchen
Munich | Germany

Lassmann, Hans MD
Medical University of Vienna
Vienna | Austria

Liblau, Roland MD, PhD
INSERM UMR1043 – CNRS UMR5282
Université Toulouse III
Toulouse | France

Linington, Christopher PhD
University of Glasgow
Glasgow | UK

Mahad, Don MD, PhD
University of Edinburgh
Edinburgh | UK

Martino, Gianvito MD
San Raffaele Hospital
Milan | Italy

Meinl, Edgar MD
Klinikum der Ludwig-Maximilians-Universitat
Munich | Germany

Owens, Trevor PhD
University of Southern Denmark
Odense | Denmark

Praksova, Petra MD, PhD
University Hospital Brno
Brno | Czech Republic

Probert, Lesley PhD
Hellenic Pasteur Institute
Athens | Greece

Salomon, Benoit DVM, PhD
UPMC Univ Paris 06
Paris | France

Stourac, Pavel MD, PhD
Masaryk University
Brno | Czech Republic

Tafti, Mehdi PhD
University of Lausanne
Lausanne | Switzerland

Uccelli, Antonio MD
University of Genoa
Genoa | Italy

Vezzani, Annamaria PhD
Mario Negri Institute
for Pharmacological Research
Milan | Italy

Ward, Roberta PhD
Imperial College London
London | UK

Willison, Hugh MBChB, PhD
Glasgow Biomedical Research Centre
Glasgow | UK

Woodroofe, Nicola PhD
Sheffield Hallam University
Sheffield | UK

Scientific Program

DAY 1 1ST JUNE 2015

MORNING SESSION | 9.00 - 12.30

INNATE IMMUNE SYSTEM AND GLIA

CHAIRS: GIANVITO MARTINO AND PAVEL STOURAC

	SPEAKERS AND TOPICS
09.00 – 09.45	**Trevor Owens, University of Southern Denmark, Odense | Denmark** Neuroinflammations
09.45 – 10.30	**Diego Gomez-Nicola, University of Southampton, Southampton | UK** CNS innate immune responses in chronic neurodegeneration
11.00 – 11.45	**Roberto Furlan, San Raffaele Hospital, Milan | Italy** Microglia microvescicles as a new biomarker in neuroinflammation and neurodegeneration
11.45 – 12.30	**Martin Kerschensteiner, Medical Center of the University of Munich, Munich | Germany** Immune mechanisms of axonal injury (advanced imaging techniques)

AFTERNOON SESSION | 13.30 - 17.00

INNATE IMMUNE SYSTEM AND GLIA

CHAIRS: ANNAMARIA VEZZANI AND HUGH WILLISON

	SPEAKERS AND TOPICS
13.30 – 14.15	**Lesley Probert, Hellenic Pasteur Institute, Athens | Greece** Animal models of neuroinflammatory diseases: strengths and weaknesses
14.15 – 15.00	**Michael Heneka, University of Bonn, Bonn | Germany** Neuroinflammation in AD
15.30 – 16.15	**Annamaria Vezzani, Mario Negri Institute for Pharmacological Research, Milan | Italy** Immune responses in the CNS in epilepsy
16.15 – 17.00	**Don Mahad, University of Edinburgh, Edinburgh | UK** Inflammation and accumulation of mitochondrial ceficits as a cause of neurodegeneration

DAY 2 2ND JUNE 2015

MORNING SESSION | 9.00 - 12.30

ADAPTIVE IMMUNE RESPONSES IN THE CNS- T CELLS

CHAIRS: ROBERTO FURLAN AND ANTONIO UCCELLI

	SPEAKERS AND TOPICS
09.00 – 09.45	**Burkhard Becher, University of Zurich, Zurich | Switzerland** Mechanisms of T cell pathogenesis
09.45 – 10.30	**Thomas Korn, Technische Universitat Munchen, Munich | Germany** Th17 and memory cells in CNS autoimmunity
11.00 – 11.45	**Roland Liblau, Université Toulouse III, Toulouse | France** CD8 T cell-mediated immunopathology in the CNS
11.45 – 12.30	**Shohreh Issazadeh-Navikas, University of Copenhagen, Copenhagen | Denmark** Neuronal regulation of neuroinflammation

AFTERNOON SESSION | 13.30 - 17.00

ADAPTIVE IMMUNE RESPONSES IN THE CNS- T CELLS

CHAIRS: DON MAHAD AND THOMAS KORN

	SPEAKERS AND TOPICS
13.30 – 14.15	**Philip De Jager, Brigham and Women's Hospital, Boston, MA | United States** Genetics and chromatin analyses, and beyond
14.15 – 15.00	**Pavel Stourac, University Hospital Brno, Brno | Czech Republic** Paraneoplastic syndromes- update focused on new and emerging syndromes
15.30 – 16.15	**Benoit Salomon, UPMC Univ Paris 06, UMR 7211, Paris | France** Treg in CNS autoimmunity and therapeutic perspectives
16.15 – 17.00	**Antonio Uccelli, University of Genoa, Genoa | Italy** Stem cells in immunomodulation and neuroprotection

DAY 3 3RD JUNE 2015

MORNING SESSION | 9.00 - 12.30
ADAPTIVE IMMUNE RESPONSES IN THE CNS- B CELLS

CHAIRS: ROBERTA WARD AND TREVOR OWENS

	SPEAKERS AND TOPICS
09.00 – 09.45	**Christopher Linington, University of Glasgow, Glasgow | UK** Neuroinflammation, emerging roles for B cells and antibody in CNS disorders
09.45 – 10.30	**Gianvito Martino, San Raffaele Hospital, Milan | Italy** Neurobiology of multiple sclerosis
11.00 – 11.45	**Hans Lassmann, Medical University of Vienna, Vienna | Austria** Immunopathology of B cell associated CNS disorders
11.45 – 12.30	**Roberta Ward, Imperial College London, London | UK** Neuroinflammation in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and Huntington's disease

AFTERNOON SESSION | 13.30 - 18.15
ADAPTIVE IMMUNE RESPONSES IN THE CNS- B CELLS

CHAIRS: LESLEY PROBERT AND HUGH WILLISON

	SPEAKERS AND TOPICS
13.30 – 14.15	**Jeffrey Bennett, University of Colorado Denver, Denver, CO | United States** Human Ab and CNS demyelination, new methods for analyzing antibody specificities
14.15 – 15.00	**Edgar Meinl, Klinikum der Ludwig-Maximilians-Universitat Munchen, Munich | Germany** B cell function and autoantibodies in MS
15.00 – 15.45	**Josep Dalmau, University of Barcelona, Barcelona | Spain** Pathogenic autoantibodies in human autoimmune encephalitis
15.45 – 16.30	**Sandra Amor, VU Medical Center, Amsterdam | the Netherlands** MS tissue and sample banks

DEBATE | 16.45 - 18.15
ON THE RELEVANCE OF MENINGEAL FOLLICLES IN MS

CHAIRS: SANDRA AMOR AND HANS LASSMANN

DAY 4 4TH JUNE 2015

MORNING SESSION | 9.00 - 12.30

DISEASE

CHAIRS: CHRISTOPHER LININGTON AND NICOLA WOODROOFE

	SPEAKERS AND TOPICS
09.00 – 09.45	**Mehdi Tafti, University of Lausanne, Lausanne | Switzerland** Narcolepsy
09.45 – 10.30	**Hugh Willison, Glasgow Biomedical Research Centre, Glasgow | UK** Autoimmune Neuropathies
11.00 – 11.45	**Eva Havrdova, Charles University in Prague, Prague | Czech Republic** Treatment of CNS demyelinating disorders
11.45 – 12.30	**Zsolt Illés, Odense University Hospital, Odense | Denmark** Immunological differences between NMO and MS / NMDA involvement

AFTERNOON SESSION | 13.30 - 15.30

STUDENT PRESENTATIONS AND CLOSING LECTURE

CHAIRS: NICOLA WOODROOFE AND PETRA PRAKSOVA

	SPEAKERS AND TOPICS
13.30 – 13.45	**Marc-André Lécuyer, Université de Montréal, Montreal | Canada** The novel role of ALCAM in neuroinflammation and BBB homeostasis
13.45 – 14.00	**Indre Dalgediene, Vilnius University, Vilnius | Lithuania** The immunogenicity of amyloid beta oligomers and their role in macrophage-mediated inflammation
14.00 – 14.15	**Laura Peferoen, VU Medical Center, Amsterdam | the Netherlands** Stressed oligodendrocytes trigger microglia activation a key event in early multiple sclerosis lesion formation
14.15 – 14.30	**Liza Lind, University of Gothenburg, Gothenburg | Sweden** Chemokine expression and migration of immune cells in the central nervous system in viral neuroinflammation, with focus on regulatory T cells
14.30 – 14.45	**Susanne Vainio, Turku University Hospital, Turku | Finland** TSPO-PET imaging reveals that anti-VLA-4 treatment leads to reduced microglial activation in focal rodent EAE
14.45 – 15.30	**Gurumoorthy Krishnamoorthy, Max Plank Institute of Neurobiology, Munich | Germany** Gut microbioma and autoimmunity –current status and perspectives

Abstract Index

Abstract Index

Treatment and prevention in neuroimmunological diseases

Glia in neuroimmunology

9 - REGULATORY ROLE OF CYTOSOLIC PHOSPHOLIPASE A2 ALPHA IN INDUCTION OF CD40 EXPRESSION IN MICROGLIA

Yafa Petpet Malada
Ben Gurion University of the Negev, Soroka Hospital / Ben Gurion University of the Negev, Beer Sheva, Israel

The role of neuroinflammation and activated microglia has been documented in the pathogenesis of neurodegenerative diseases. Since the activation of cytosolic phospholipase A2 alpha ($cPLA_2$) has been reported in inflammatory conditions, its role in activation of microglial cells was not entirely elucidated. The results of the present study show that activation of mouse BV-2 microglial cell-line by 50ng/ml LPS or 10ng/ml IFN gamma (IFNg) caused an immediate activation of $cPLA_2$, measured by its phosphorylated form, followed by a gradual elevation of its expression and activity during 24 h. Inhibition of $cPLA_2$ elevated expression and activity by the presence of specific antisense or specific inhibitor pyrrophenone prevented the release of nitric oxides and superoxides, consistent with our previous study and also prevented the induction of CD40 by either LPS or IFNg. However, inhibition of $cPLA_2$ induced by the anti-inflammatory cytokines IL4+IL10 (20 ng/ml) did not affect the significant expression of the M2-like neuroprotective microglia phenotype as detected by CD206, indicating that CD206 is not under $cPLA_2$ regulation. Since this is the first time to show that CD40 is under $cPLA_2$ regulation, the study focused on the location of $cPLA_2$ in the signal transduction pathways suggested for the induction of CD40 by either LPS or IFNg. Inhibition of either $cPLA_2$ or NADPH oxidase prevented the activation of redox-sensitive NFkB induced by either LPS or IFNg as evident by the phosphorylation of p65 subunit on Ser-536. While NADPH oxidase is regulated by $cPLA_2$, inhibition of NADPH oxidase, by DPI, did not inhibit the activation of $cPLA_2$ by both inducers, suggesting that $cPLA_2$ is upstream to the oxidase. Activation of STAT1 alpha (detected by its phosphorylation on Ser727 and Tyr701) that was shown to participate in the induction of CD40 by either of the inducers was not affected in the presence of antisense or pyrrophenone suggesting that STAT1 alpha is not under $cPLA_2$ regulation. Thus, our results show that $cPLA_2$ participates in CD40 upregulation induced by either LPS or IFNg via activation of NADPH oxidase and NFκB. Taken together, we suggest that the response of $cPLA_2$ to either LPS or IFNg is probably a key player in the in the induction of neurotoxic M1-like phenotype microglia, through the regulation of free radicals production by NADPH oxidase and iNOS and the elevated expression of CD40, that serves as an amplifier of the inflammatory response in the CNS.

32 - STRESSED OLIGODENDROCYTES TRIGGER MICROGLIA ACTIVATION A KEY EVENT IN EARLY MULTIPLE SCLEROSIS LESION FORMATION

Laura Peferoen [1] - Malika Bsibsi [2] - Zohra Layegh [1] - Hans van Noort [2] - Sandra Amor [1]
Pathology department, VU medical center, Amsterdam, Netherlands [1] - Delta Crystallon, Leiden, Netherlands [2]

Multiple sclerosis (MS) is characterised by lesions of inflammation and demyelination in the central nervous system. These lesions continually form and regress during disease and disease activity. Already

in the normal appearing white matter focal clusters of activated microglia are detected in the absence of myelin damage, leukocyte infiltration or blood-brain barrier breakdown. Yet little is known about what triggers microglia to form these so-called pre-active lesions.

In MS the microglia clusters are observed in close association with stressed oligodendrocytes that express the small heat shock protein HSPB5, up-regulated under stress conditions. We showed that microglia in preactive lesions express the key receptors for HSPB5, namely TLR1, TLR2 and CD14. To examine whether HSPB5 activate microglia in vitro, we stimulated primary human microglia with recombinant HSPB5. Transcript profiling of HSPB5-activated microglia revealed induction of immune regulatory factors, several chemokines and antiviral genes that are generally inducible by type I interferons.

By immunohistochemistry many of these molecular markers were detected in activated microglia in preactive lesions.

Taken together, these data indicate that microglia activation in preactive lesions is at least partly driven by HSPB5, expressed by oligodendrocytes.

To investigate whether, as well as activation, stressed oligodendrocytes attract microglia and thereby trigger preactive lesion formation, we performed a Boyden chamber chemotaxis assay. Primary human microglia were allowed to migrate over a 8µm pore size polyethylene terephthalate membrane towards supernatants collected from stressed MO3.13 cells, a human oligodendrocyte cell line. Our data show that microglia migrate significantly more towards stressed oligodendrocytes compared to unstimulated cells.

In conclusion, although oligodendrocytes are thought to be "victims" in multiple sclerosis, our data suggests an pro-active role for oligodendrocytes in early multiple sclerosis lesion formation by attracting and activating microglia.

36 - CYTOKINES INDUCE RELEASE OF SHEDDING VESICLES IN A HUMAN MICROGLIA CELL LINE INDEPENDENTLY FROM THE PURINERGIC SIGNALING PATHWAYS

Federico Colombo [(1)] ***- Giacomo Casella*** [(1)]
Annamaria Finardi [(2)] ***- Roberto Furlan*** [(3)]
San Raffaele Scientific Institute, Università Vita-Salute San Raffaele, Milano, Italy [(1)] - San Raffaele Scientific Institute, Ospedale San Raffaele, Milano, Italy [(2)]
San Raffaele Scientifc Institute, Ospedale San Raffaele, Milano, Italy [(3)]

In recent years the field of cell-to-cell communication has been revitalized thanks to the increasing attention about the biology of the extracellular microvesicles (EMV), small lipid particles that can deliver active biomolecules among cells. The two types of EMVs currently isolated, exosomes and ectosomes, differ in dimension and site of origin: the former are nanoparticles of 20-100 nm originating in the lumen of late endosomes, whereas the latter are bigger (100-1000 nm) and bud directly from the plasma membrane. Their multiple roles have been recognized either in phisiology or in pathological conditions, ranging from tumor and coaugulation defects to neurodegenerative and inflammatory diseases. In multiple sclerosis patients a huge increase in the myeloid cells-derived ectosomes in the cerebrospinal fluid has been observed, with a high degree of correlation with the diasease severity.

Therefore we decided to use a human microglia cell line, namely CHME-5, as a model for studying some aspects of the ectosomes biology.

This cells are able to release detectable amounts of EMVs upon either ATP treatment or cytokine priming, which are conditions that well mimic the *in vivo* environment during inflammation. Vesicles were analyzed by transmission electron micoscopy or by western blot analysis of the raft-associated protein flotillin-1/reggie-2; interestingly the cyto-

kine-mediated ectosomes release seems to be independent from either the signaling components of the purinergic system (P2X7 receptor and pannexin-1) or the lipid metabolizing enzyme acid-sphingomyelinase, whose roles in the ATP-driven EMVs biogenesis are well recognized. Moreover the time course analysis of citokine-mediated EMVs release reveals an overlap with the phenotypic skeewing of myeloid cells according to the proposed M1/M2 model.

51 - THE MIF-CD74 SYSTEM IN HUMAN GLIOMAS

Pia Zeiner [1] ***- Corinna Preusse*** [2] ***- Anna-Eva Blank*** [1] ***Peter Baumgarten*** [1] ***- Hansjürgen Bratzke*** [3] ***Christian Senft*** [4] ***- Ria Winkelmann*** [5] ***- Karl H. Plate*** [1] ***- Jörg Wischhusen*** [6] ***- Werner Stenzel*** [2] ***Patrick Harter*** [1] ***- Michel Mittelbronn*** [1]
Edinger Institute, Institute of Neurology, Goethe University Frankfurt, Frankfurt am Main, Germany [1] Department of Neuropathology, Charité Berlin, Berlin, Germany [2] - Institute of Forensic Medicine, Goethe University Frankfurt, Frankfurt, Germany [3] Department of Experimental Neurosurgery, Goethe University Frankfurt, Frankfurt, Germany [4] Senckenberg Institute of Pathology, Goethe University Frankfurt, Frankfurt, Germany [5] - Interdisciplinary Center for Clinical Research, Department for Obstetrics and Gynecology, University of Würzburg, Würzburg, Germany [6]

CD74, the macrophage migration inhibitory factor (MIF) receptor, is up-regulated in numerous neoplasms, mainly in hematologic tumors, and currently investigated in clinical trials. The CD74 surface expression displays a rapid turnover with quick internalization and recycling after antibody binding, what makes the molecule an attractive target for antibody-based treatment strategies. Because CD74 has been further described as one of the most overexpressed molecules in human glioblastomas, we wanted to assess the potential relevance for anti-CD74 treatment by determining firstly the cellular source and clinico-pathologic relevance of CD74 expression in human gliomas by immunohistochemistry, immunofluorescence, immunoblotting and cell sorting analysis. Our results indicate a restriction of the MIF receptor to glioma-associated microglia/macrophages (GAMs) *in vivo*, while being absent in neoplastic cells, the latter strongly expressing its ligand. Most interestingly, a higher amount of CD74-positive GAMs was associated with beneficial patient survival constituting an independent prognostic parameter. Therefore, we addressed the characterization of the immunological polarization profile of GAMs fractionated from primary tumors via the well-established method of CD11b MACS isolation. Our analyses via quantitative polymerase chain reaction (qPCR) suggest a particular immune polarization phenotype of GAM with expression of classical M1-polarization markers in combination with particular M2 markers, that on functional level are associated with specific functions in the glioma microenvironment e.g. phagocytosis. With regard at the MIF-CD74 system, there is evidence for an association with an anti-tumoral M1 polarization, whereas other GAM subpopulations might display distinct immunological phenotypes. In conclusion, CD74 represents a positive prognostic marker most probably because of its association with an M1-polarized immune milieu in high-grade gliomas.

Neuroinflammation

5 - INFLUENCE OF AUTOIMMUNE INFLAMMATION ON REMYELINATION IN CUPRIZONE-INDUCED DEMYELINATION

Patrik Kunz - Angelika Escher - Wolfgang Brück
Stefan Nessler - Alonso Barrantes-Freer
Christine Stadelmann
Department of Neuropathology,
University Medical Center, Göttingen, Germany

Background
Remyelination frequently fails in chronic multiple sclerosis (MS) lesions, yet it is usually successful in early lesions and experimental models. An anti-myelin immune response may contribute to remyelination failure in MS. In this respect, overexpression of IFN-gamma was shown to diminish remyelination in the cuprizone model. On the other hand, adaptive and innate inflammation was shown to increase remyelination in long-standing experimental demyelinated lesions.

Question/Methods
In order to determine the *in vivo* effects of a myelin-specific T cell response on remyelination, we combined cuprizone-induced de- and remyelination with active immunization with myelin oligodendrocyte glycoprotein (MOG) 35-55 and assessed the effect on oligodendroglial lineage cells and remyelination using light and electron microscopy.

Results
Immunization led to a marked T cell infiltration of the corpus callosum, an increase in IFN-gamma production and blood brain barrier breakdown. However, the density of oligodendroglia lineage cells was similar in immunized and non-immunized mice with cuprizone induced demyelination. Also, in situ hybridization for PLP revealed similar densities of myelinating oligodendrocytes. Importantly, no differences in the proportion of remyelinated axons were observed using histochemistry and electron microscopy.

Conclusion
We conclude that the remyelination response after cuprizone-induced demyelination is robust and not modulated by an additional adaptive immune response.

10 - DISSECTING THE CELLULAR AND MOLECULAR REQUIREMENTS FOR TNF-MEDIATED NEUROPROTECTION AGAINST GLUTAMATE EXCITOTOXICITY

Irini Papazian - Lesley Probert
Laboratory of Molecular Genetics,
Hellenic Pasteur Institute, Athens, Greece

Tumor necrosis factor (TNF) is a master inflammatory cytokine that is up-regulated in neurodegenerative disorders including Alzheimer's disease, stroke, and multiple sclerosis (MS). However, while non-selective TNF inhibitors that block both transmembrane (tmTNF) and cleaved soluble (solTNF) forms of TNF are effective treatments for chronic human diseases in the periphery, such as rheumatoid arthritis, they provide no benefit to patients with MS; indeed, they exacerbated disease and can even induce demyelination. In addition, genome-wide association studies (GWAS) in large patient cohorts showed that TNFRSF1A, the gene that encodes TNFRI, is associated with MS and one vari-

ant with reduced function is implicated in disease pathogenesis. These clinical data provide the most convincing evidence that further to pro-inflammatory effects, TNF exerts essential beneficial functions in the CNS and ask for improved understanding of its molecular and cellular interactions under physiological and pathophysiological conditions so that it can be more safely targeted for the treatment of human diseases.

While the roles of TNFRII in direct neuroprotection under conditions that prevail in ischemia and in remyelination in an MS model are well accepted, the effects of TNFRI are much more complex and controversial. TNFRI induces pro-inflammatory effects that promote autoimmune demyelination, cell survival pathways that protect neurons and glia against various death stimuli and, under some circumstances, triggers cell apoptosis or necrosis. The differential roles of tmTNF and solTNF in the CNS are also incompletely understood. In this study we used a genetic approach to identify the essential cellular and molecular interactions that underlie TNF-mediated neuroprotection against glutamate excitotoxicity, a type of neuron death that prevails in many acute and chronic CNS disorders. By preparing co-cultures of murine astrocytes and cortical neurons with different combinations of cells isolated from wild type and TNF or TNFR deficient mice, we were able to confirm that both TNFRI and TNFRII are individually required on neurons for protection against NMDA-induced death and further reveal that astrocyte-derived tmTNF plays an essential role in inducing neuroprotection.

Our results identify a previously unknown neuroprotective role for astrocytes that is mediated through a cell contact mechanism, confirm that both TNFR relay neuroprotective signals in neurons and further support selective inhibition of the pro-inflammatory effects of solTNF, while preserving beneficial effects of tmTNF, TNFRI and TNFRII, as a promising therapeutic approach for a wide range of inflammatory CNS disorders.

13 - THE NOVEL ROLE OF ALCAM IN NEUROINFLAMMATION AND BBB HOMEOSTASIS

Marc-André Lécuyer - Lyne Bourbonnière
Sandra Larouche - Luis Alberto Pérez Quintero
Catherine Larochelle - Alexandre Prat
Centre de Recherche du CHUM, Université de Montréal, Montréal, Canada

The loss of blood-brain barrier (BBB) integrity is a hallmark of multiple sclerosis. It is associated with a disorganization of junctional molecules and an upregulation of cell adhesion molecules essential for immune cell transmigration. Identifying novel key players involved in this process is thus crucial for the development of MS therapies aimed at promoting BBB integrity and decreasing leukocytes trafficking into the central nervous system (CNS) during neuroinflammation. In this study, the specific role of the adhesion molecule ALCAM present on BBB endothelial cells (BBB-ECs) and its effects on leukocyte transmigration during the course of experimental autoimmune encephalomyelitis (EAE) was assessed. Using a modified adhesion assay under shear stress, we demonstrated a significant reduction in human monocytes and T lymphocytes adhesion to BBB-ECs following ALCAM blockade. The migration of both immune cell subsets across human BBB-ECs and meningeal endothelial cells was also significantly decreased. Furthermore, *in vivo* ALCAM blocking antibodies reduced leukocyte infiltration and EAE severity in WT C57Bl/6. Unexpectedly, ALCAM KO mice developed a more severe active EAE associated with a significant increase in perivascular infiltration of pro-inflammatory lymphocytes (Th1/Th17) and M1 monocytes/macrophages, as compared to WT controls. ALCAM KO mice also displayed more extensive CNS demyelination and astrogliosis. In order to explain these data, we performed passive EAE transfer in which ALCAM KO mice received WT MOG-reactivated splenocytes. These experiments suggested that the pathophysi-

ology observed in active EAE is linked to the absence of ALCAM on BBB-ECs. In addition, phenotypic characterization of un-immunized ALCAM KO mice revealed a reduced expression of BBB junctional proteins. Further analysis showed that ALCAM is indirectly associated with tight junction molecules of BBB-ECs, explaining the increased permeability of CNS blood vessels in ALCAM KO animals. Correlating with these data, primary culture of mouse brain BBB endothelial cells was shown to possess a lower TEER and an increase permeability coefficient assessed using a modified Boyden chamber assay. Collectively, our data provide evidence of the implication of ALCAM in leukocyte transmigration across human and mouse CNS endothelium and point to a biologically crucial function of ALCAM in maintaining BBB integrity in mouse.

14 - NEUROINFLAMMATORY CHANGES FOLLOWING SURGICAL DECOMPRESSION FOR CERVICAL SPONDYLOTIC MYELOPATHY AND THEIR IMPLICATIONS FOR THE DEVELOPMENT OF NOVEL PERIOPERATIVE TREATMENTS

Pia M. Vidal [(1)] · ***Spyridon K Karadimas*** [(2)]
Antigona Ulndreaj [(2)] - ***Stefania Forner*** [(3)]
Alex Laliberte [(2)] - ***Michael G Fehlings*** [(4)]
Toronto Western Research Institute and spinal program, Krembil Neuroscience Centre, Division of Genetics & Development, University Health Network, Toronto, Canada [(1)] - Toronto Western Research Institute and spinal program, Krembil Neuroscience Centre, Division of Genetics & Development, University Health Network and Institute of Medical Sciences, University of Toronto, Toronto, Canada [(2)] - Departmento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil [(3)]
Toronto Western Research Institute and spinal program, Krembil Neuroscience Centre, Division of Genetics & Development; Institute of Medical Sciences, University of Toronto; Dept of Surgery, Division of Neurosurgery and Spinal Program, Toronto, Canada [(4)]

Cervical spondylotic myelopathy (CSM) is the most common cause of spinal cord impairment in the world. It is caused by prolonged compression of the spinal cord from degenerative conditions, and is characterized by gait instability, bladder dysfunction, pain, weakness, as well as hand impairment. To date, treatment consists of surgical decompression in order to relieve the compression. Our previous studies in animal models of CSM have demonstrated that there is an ischemia perfusion injury (IRI) that follows surgical decompression.
The primary objective of this study was to characterise the nature of the post-decompression, IRI mediated inflammatory response and its role in the neurological recovery after surgical decompression.We performed a gradual compression of the spinal cord in C57B/L mice by inserting a piece of biomaterial underneath the C5-6 lamina. Afterwards, animals were surgically decompressed at 3 and 9 weeks after symptoms manifested, corresponding to moderate and severe compression, respectively. Non-decompressed animals served as a comparison. We evaluated pain response, gait deficits and hand dexterity using Von Frey, Catwalk and Capellini handling test respectively. ELISA and flow cytometry were used to characterise systemic and local changes in the immune system.Surgical decompression for CSM caused a local increase of cytokines and chemokines around the level of compression at 24 hours after surgery. In addition, there was a significant change in the subpopulations of circulating monocytes after decompressive surgery. Decompression in the less severely compressed "moderate" group led to a substantial improvement in hand dexterity function as well as a decrease in pain response compared to the "severe" group.
Our data suggest that surgical decompression triggers activation of the immune system both at the systemic and local levels by promoting the secre-

tion of cytokines and chemokines in the spinal cord as well as by changing the white blood cells composition. Furthermore our findings point to the fact that earlier decompression is associated with better outcomes.

15 - PREDICTORS OF OUTCOME AND CONVERSION TO MULTIPLE SCLEROSIS IN PATIENTS WITH A FIRST EPISODE OF INFLAMMATORY MYELOPATHY: A MULTICENTRIC RETROSPECTIVE STUDY

Matteo Gastaldi [1] ***- Laura Dilodovico*** [2]
Sabrina Ravaglia [3] ***- Elisabetta Zardini*** [4]
Roberto Bergamaschi [3] ***- Alfredo Romani*** [3]
Andrea Cortese [3] ***- Enrico Alfonsi*** [5]
Alessandro Lozza [5] ***- Giovanni Piccolo*** [3]
Anna Pichiecchio [6] ***- Stefano Bastianello*** [6]
Paola Borrelli [7] ***- Cristina Montomoli*** [7]
Paola Bini [3] ***- Valeria Mariani*** [8] ***- Paola Banfi*** [9]
Giorgio Bono [10] ***- Enrico Marchioni*** [11]
Mauro Ceroni [2] ***- Diego Franciotta*** [4]
Neurology department, University of Pavia/ University of Insubria, Pavia, Italy [1] - Neurology Department, University of Pavia, Pavia, Italy [2] - Neurology Department, National neurological Institute IRCCS Mondino, Pavia, Italy [3] - Laboratory of Neuroimmunology, National neurological Institute IRCCS Mondino, Pavia, Italy [4] - Neurophysiology department, National neurological Institute IRCCS Mondino, Pavia, [5] - Neuroradiology Department, National neurological Institute IRCCS C Mondino, Pavia, Italy [6] - Unit of Biostatistic and Clinical Epidemiology, University of Pavia, Pavia, Italy [7] Neurology Department, University of Pavia, Varese, Italy [8] - Neurology Department, Ospedale di Circolo/ Fondazione Macchi, Varese [9] - Ospedale di Circolo, University of Insubria, Varese, Italy [10] - Department of Neuro-oncology, National Neurological Institute IRCCS Mondino, Pavia, Italy [11]

Inflammatory myelopathies (IM) can occur as first episodes of CNS-restricted diseases, such as multiple sclerosis (MS), and neuromyelitis optica (NMO),[1] or of manifestations of the CNS involvement in systemic autoimmune vasculitis. Our aim was to identify predictors of disability, and of conversion to MS, in IM.

Methods

Clinical data on patients who presented in our hospitals with signs and symptoms of IM from 1998 to 2012 were collected retrospectively. Patients were classified as: a) MS according to the 2010 McDonald criteria,[2] b) post-infectious IM (PIM) if an infectious-like prodromal event was present in non-MS patients, c) idiopathic myelitis (IIM) if no clear etiology was found, and d) "Other disease" (OD) if any other cause for IM was known (e.g., IM associated with anti-aquaporin-4 antibodies). A bad outcome was defined as a median Rankin scale (mRS) score >2 at the end of follow-up [mean duration, 62 months (SD, 38)]. Some cases were classified as myeloradiculoneuritis (MRN) if they had abnormalities in nerve conduction studies (NCS), spinal root contrast enhancement on MRI, or both.

Results

We identified 159 patients: 48 PIM (30.2%), 54 IIM (34.0 %), 7 OD (4.4%), and 50 MS (31.4%). Predictors of conversion to MS were low cell counts and positive CSF oligoclonal bands (OCB) on CSF analysis, lesions fulfilling the Barkhof criteria on brain MRI, cervical lesions on spinal MRI.
Independent conditions associated with bad outcome were: a) MRN, b) PIM, c) complete transverse myelitis (CTM)[3], d) sphynteric and e) pyramidal symptoms, f) high mRS score at onset, g) severe blood-CSF barrier permeability damage, h) lumbosacral lesions, and i) spinal roots contrast enhancement. In a multivariate model, a bad outcome was more frequent when patients were older, and presented as CTM or MRN.

Conclusion

Our data confirm that, after first episodes of IM, the presence of brain lesions, CSF OCB, and cervical lesions predicts the conversion to MS[4]. As a novelty, MRN associated with bad outcome in both univariate and multivariate models. This finding has never been reported as a consistent prognostic factor in a large cohort of patients with first episode of IM. Moreover, in 20% of our MRN cases, the radicular inflammation was subclinical, thus suggesting that NCS and lumbosacral MRI should enter the IM diagnostic workup for a thorough prognostic assessment.

References

(1) De Sèze J, Stojkovich T et al. Acute Myelopathies: clinical, laboratory and outcome profiles in 79 cases. Brain 2001, 124:1509-1521.

(2) Polman CH, Reingold SC et al. Diagnostic Criteria for Multiple Sclerosis: 2010 revision of the McDonald criteria. Ann Neurol 2011;69:292–302.

(3) Scott FT. Nosology of Idiopathic acute transverse myelitis. Acta Neurol Scand 2007: 115: 371–376.

(4) Young J, Quinn S et al. Clinically isolated acute transverse myelitis: prognostic features and incidence. Multiple Sclerosis 2009;15(11):1295–1302.

17 - MESENCHYMAL STEM CELLS OVEREXPRESSING IL-13 DECREASE LESION SIZE AND DEMYELINATION AFTER SPINAL CORD INJURY

Dearbhaile Dooley [(1)] ***- Evi Lemmens*** [(1)]
Tim Vangansewinkel [(1)] ***- Stefanie Lemmens*** [(1)]
Chloé Hoornaert [(2)] ***- Debbie Le Blon*** [(2)]
Peter Ponsaerts [(2)] ***- Sven Hendrix*** [(1)]
Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium [(1)] - Laboratory of Experimental Hematology, Antwerp University, Antwerp, Belgium [(2)]

Background

Trauma to the CNS leads to a dramatic influx of immune cells from the periphery (e.g. T cells and macrophages) and activation of resident microglia and astrocytes. Mesenchymal stem cells (MSCs) have been reported to substantially improve functional outcome after spinal cord injury (SCI), mainly by immunomodulation via the secretion of chemokines and cytokines. The cytokine interleukin-13 (IL-13) is a key player as it induces neurite outgrowth in vitro and promotes functional recovery in a mouse model of SCI.

Aim

In this study, we investigated the therapeutic potential of grafting autologous MSCs overexpressing IL-13 (MSC/IL-13), in a mouse model of SCI.

Results

Transplantation of both MSCs and MSC/IL-13, significantly improved functional outcome following SCI, when compared to the control group (NaCl). Interestingly, no added effect of the local production of IL-13 by MSCs on locomotion was found, when compared to control MSCs. Both MSCs and MSC/IL-13 lead to a decrease in microglia/macrophage infiltration around the lesion site. IL-13-secreting MSCs lead to an significant increase in the number of $CD4^+$ T-cells within the spinal cord and resulted in a 50% decrease in lesion size and demyelination when compared to control MSCs.

Conclusion

IL-13 overexpression by MSCs significantly decreases lesion size and demyelination. This substantial improvement after SCI is accompanied by a significant increase in T cell numbers, suggesting a beneficial phenotype. Our data open the road for new therapies as we show for the first time that MSCs can be used as potent carriers of immunomodulatory factors, such as IL-13.

18 - STUDY OF THE IMPORTANCE OF ENDOPLASMIC RETICULUM STRESS AND AUTOPHAGY IN THE PATHOLOGY OF MULTIPLE SCLEROSIS

Sofie Voet - Conor Mc Guire - Geert van Loo
VIB, Inflammation Research Center, Ghent University, Ghent, Belgium

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS). The etiology of the disease is largely unknown, but is considered to be an autoimmune disease characterized by auto-reactive encephalitogenic T cells which home to the CNS. There they initiate an inflammatory reaction leading to axonal demyelination, loss of oligodendrocytes and neuronal damage. In addition, CNS resident cells, such as microglia and astrocytes produce inflammatory cytokines and chemokines which further contribute to MS disease development. Observations in human patient samples as well as experimental studies in mice suggest that endoplasmic reticulum (ER) stress may be involved in the pathology of MS. ER stress occurs upon the accumulation of unfolded or misfolded proteins in the ER. In order to restore ER homeostasis the unfolded protein response (UPR) is initiated, increasing the folding capacity of the ER. Also autophagy, a lysosomal degradation pathway important for the removal of protein aggregates, may be important in MS. However, more knowledge on the importance of ER stress and autophagy in the pathology of MS is needed in order to better understand the mechanisms behind the disease. Through *in vivo* targeting of crucial UPR signaling pathways and autophagy in mice, in combination with well-established mouse models of human MS, we aim to identify and assess the contribution of these pathways to the development of MS. Specific deletion of XBP1, a crucial transcription factor controlling UPR responses, in CNS progenitor cells protects mice from cuprizone-induced demyelination, a model used to study de- and remyelination in the absence of a peripheral immune response. This protection was not observed in conditions when XBP1 was deleted in oligodendrocytes alone, suggesting the involvement of ER stress in CNS cells other than oligodendrocytes. Current studies now focus on the involvement of IRE1alpha, the stress sensor upstream of XBP1, and Atg16L1, a crucial autophagy mediator, in CNS progenitor cells and their importance for MS.

19 - THE IMMUNOGENICITY OF AMYLOID BETA OLIGOMERS AND THEIR ROLE IN MACROPHAGE-MEDIATED INFLAMMATION

Indrė Dalgėdienė [(1)] - Asta Lučiūnaitė [(1)]
Rita Lasickienė [(1)] - Rima Budvytytė [(2)]
Ramunė Morkūnienė [(3)] - Vilmantė Borutaitė [(3)]
Aurelija Žvirblienė [(1)]
Institute of Biotechnology, Vilnius University, Vilnius, Lithuania [(1)] - Institute of Biochemistry, Vilnius University, Vilnius, Lithuania [(2)] - Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania [(3)]

Keywords
Antibodies, beta amyloid, neurotoxicity

The central molecule in the pathogenesis of Alzheimer's disease (AD) is believed to be a small-sized polypeptide – beta amyloid (Abeta) which has an ability to assemble spontaneously into oligomers. Various studies concerning therapeutic and prophylactic approaches for AD are based on the immunotherapy using antibodies (Ab) against Abeta which in some cases of clinical trials led to neuroinflammation. However, knowledge on the mechanisms of Abeta-induced immune responses is rather limited. Previous research on Abeta oligomeric antigens in complex with Ab (Abeta+Ab) showed that neurotoxic effects on primary neurons

are increased by Fc-dependent microglia activation. In the current study, using BALB/c mice we evaluated the dependence of immunogenicity of Abeta on the size of oligomeric particles and investigated by flow cytometry and ELISA how Abeta oligomers alone or Abeta+Ab influence macrophage phenotype in vitro.

Results

The analysis of mouse serum antibodies revealed that 1-2 nm Abeta oligomers are highly immunogenic. In contrast, larger Abeta oligomers and monomers induced a weak IgG response in immunized mice. Epitope mapping of both monoclonal and polyclonal Ab demonstrated that the main immunodominant region of the Abeta oligomers is located at its aminoterminus, between amino acids 1 and 19. The profiles of secreted cytokines and changes of cell surface marker levels in primary mouse spleen macrophages and J774A.1 cell line treated with either Abeta or Abeta+Ab suggest that macrophages are directed towards the M1 phenotype.

Conclusions

Small Abeta oligomers induce the strongest immune response in mice. The amino-terminus of Abeta oligomers represents an immunodominant epitope which indicates its surface localization. Abeta oligomeric antigens in complex with antibodies form inflammatory conditions in macrophage cultures. The results of the current research may be important for further development of Abeta-based vaccination and immunotherapy strategies.

Acknowledgments

This research was funded by a grant (No. LIG-04/2012) from the Research Council of Lithuania.

24 - ENCEPHALOPATHY ASSOCIATED SEPSIS: VITAMIN B6 IN THE RELEASE OF CYTOKINES AND DISRUPTION OF THE BLOOD BRAIN BARRIER

Lucinéia Danielski - Drielly Florentino - Andriele Vieira - Maryane Martins - Diego Nascimento Fabricia Petronilho

Laboratório de Fisiopatologia Clínica e Experimental FICEXP- Programa de Pós-Graduação em Ciências da Saúde- Universidade do Sul de Santa Catarina UNISUL – Tubarão, SC, Brasil

Objective

Encephalopathy Associated Sepsis (EAS) is defined as diffuse brain dysfunction caused by systemic inflammatory response in response to infection. Knowing that the pro inflammatory cytokines play an important role in the pathophysiology of EAS. Also, in recent years, has been extensively studied the role of regulation of the kynurenine pathway metabolism in diseases that affect the CNS and have in its pathophysiology involvement exacerbated inflammatory response. The aim of this study was to evaluate levels of pro-inflammatory cytokines and the permeability of the blood brain barrier (BBB) after adminstration of vitamin B6 in rats submitted to CLP.Male Wistar rats (250-350g) were subjected to CLP model, with sham control. Groups divided into sham + saline, sham + B6, CLP + saline and CLP + B6 (600 mg / kg, via s.c) n = 10. Twenty-four hours, were euthanized or subjected to evaluation of the BBB, hippocampus and prefrontal cortex removed for analysis of cytokines . Data analyzed by ANOVA with post hoc Tukey test and log-rank test with $P < 0.05$. There was a decrease in the levels of cytokines in the hippocampus. TNF-alpha in the CLP + B6 group compared with Sham + B6, and IL-1beta in the CLP + B6 group compared with CLP group. However there was a reduction in permeability of the BBB only in the prefrontal, CLP + B6 group compared with CLP. Even so still preliminary, demonstrated the potential of vitamin B6 in En-

cephalopathy associated with sepsis, in the hippocampus and prefrontal, important structures and for damage that may explain part of the observed symptoms in patients.
Keywords: sepsis, sepsis-associated encephalopathy , vitamin b6, kynurenine pathway.
Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Universidade do Sul de Santa Catarina (UNISUL)

25 - THE ROLE OF INLAMMASOMES FOR AMYLOID-BETA MICROGLIA PHAGOCYTOSIS IN ALZHEIMER'S DISEASE

Francesca La Rosa [(1)] - Marina Saresella [(1)]
Mario Clerici [(1)] - Michael Heneka [(2)]
Don C.Gnocchi Fondation ONLUS IRCCS, University of Milan, Milan, Italy [(1)] - Departiment of Neurology, University Hospital of Bonn, Bonn, Germany [(2)]

Neuroinflammation plays a key role in the modulation of the pathogenesis of neurodegenerative disorder such as Alzheimer's disease (AD). Microglia, the main immune effector of the brain, are able to migrate to sites of amyloid-beta (Ab) deposition to eliminate Ab phagocytosis upon activation by multiple receptors: toll like receptors and scavenger receptors.
The issue of whether microglia are able to eliminate pathological lesions such as neurofibrillary tangles or senile plaques from AD brain still remains the matter of controversy. Recent data suggest that the Nod Like Receptor3 (NLRP3), multiprotein inflammasome complexes, plays a role in AD, as its activation in the microglia by Ab triggers. Interleukin-1beta produced as a biologically inactive pro-form and requires caspase-1 for activation and secretion. Caspase-1 activity is controlled by inflammasomes. We investigate about the importance of inflammasomes complex in the Ab phagocytosis and its degradation. The preliminary results of phagocytosis assay and immunofluorescent experiment on Primary Microglia cells to lipopolysaccaride (LPS) an Ab exposure show that a previous treatment with LPS reduce Ab phagocytosis. Different results were obtained in Primary Microglia wild type, NLRP3 and ASC Knockout suggesting a real inflammasomes involvement in Alzheimer's pathology. Inflammasomes inactivation reduces the production of inflammatory cytokines prolonging the protective activity of microglia and Ab clereance, featuring a typical microglia phenotype of the early stage of AD.

26 - EFFECTS OF NURR1 REDUCTION ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN MICE

Francesca Montarolo - Simona Perga
Serena Martire - Antonio Bertolotto
Neurobiology Unit, Neurologia 2 CReSM & Neuroscience Institute Cavalieri Ottolenghi NICO, AOU San Luigi Gonzaga, Orbassano, Italy

The Nuclear receptor related 1 protein (Nurr1) is a transcription factor belonging to the subfamily of steroid nuclear hormone receptors. This factor plays an anti-inflammatory role by inhibiting the expression of inflammatory genes in microglia and astrocytes. Furthermore, Nurr1 is associated to Parkinson disease since it is involved in the development of midbrain dopamine neurons. In Multiple Sclerosis (MS), Nurr1 is down-regulated in peripheral blood mononuclear cells with respect to healthy controls and its gene expression level negatively correlates with the aggressiveness of the pathology and clinical parameters such as the relapse rate and the Expanded Disability Status Scale progression.
In order to better understand the consequences of Nurr1 reduction during inflammatory conditions such as MS, we evaluate the clinical and neuropathological course of induced myelin oligodendro-

cyte glycoprotein (MOG35-55) chronic EAE in heterozygous Nurr1 knockout mice (Nurr1-KO) since homozygous knockout mice died within 12 hours after birth.
The analysis revealed that the Nurr1-KO mice developed EAE symptoms as well as wild type, but Nurr1 deficiency was able to anticipate the disease onset. Neuropathological quantification revealed an increase in perivascular inflammatory infiltrates in Nurr1-KO mice compared to wild type mice, whereas the percentage of demyelination and axonal loss areas were not different.
These results suggest that Nurr1 deficit anticipates EAE onset increasing perivascular inflammatory infiltrates in spinal cord. Therefore, this study represents a good starting point for a possible future therapeutic implication of Nurr1 in reducing the inflammatory component of MS.

28 - DECREASED ANIMAL BEHAVIOR AND ALTERED CELLULAR PROFILE IN BRAINS OF IL-10-DEFICIENT MICE INFECTED WITH *PLASMODIUM CHABAUDI CHABAUDI (AS)*

Kyle Wilson [(1)] ***- Sonja Stutz*** [(2)] ***- Lorenzo Ochoa*** [(3)]
Kelly Dineley [(4)] ***- Petra Cravens*** [(5)] ***- Gracie Vargas*** [(6)]
Robin Stephens [(5)]
Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, United States [(1)] - Center for Addiction Research, University of Texas Medical Branch, Galveston, TX, United States [(2)] - Center for Biomedical Engineering, University of Texas Medical Branch, Galveston, TX, United States [(3)] - Department of Neurology, University of Texas Medical Branch, Galveston, TX, United States [(4)] Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, United States [(5)] Center for Biomedical Engineering, University of Texas Medical Branch, Galveston, TX, United States [(6)]

Cerebral malaria (CM) remains one of the most severe complications of *Plasmodium falciparum* infection, accounting for over 500,000 cases per year, mostly in young African children. This syndrome is thought to result from high levels of parasitemia and inflammation, and death has been linked to severe cerebral edema.
There is little evidence that specific strains of Plasmodium falciparum are linked to this syndrome and the causality of parasite adhesion in the brain is unclear; while host genetic factors like the cytokines IL-10 and TNF have been clearly linked to severe disease. *P. chabaudi chabaudi* (AS), a rodent malaria species, leads to uncomplicated disease in wild-type animals; however, IL-10 knock-out (KO) mice suffer lethal disease characterized by gross cerebral edema and hemorrhage, and increased levels of the pro-inflammatory cytokines IFN-gamma and TNF. In order to characterize this model more fully, we investigated the neurological consequences of this infection through animal behavior studies and phenotypic analysis of the immune cell populations involved in brain invasion during peak infection. We observed 100% mortality by day 9 post-infection, accompanied by micro-leakages throughout the cerebrum. We also showed that KO mice suffered significant declines in behavioral and physical capacities during the peak of infection compared with C57Bl/6J (WT) mice as measured by a general behavioral screen (SHIRPA), grip strength, and latency to tail flick. IL-10 KO animals showed a decline in neuro-muscular coordination and balance during the early recovery period, which was restored at later time points. When we looked at the cell populations in the perfused brain on day 7 post-infection by flow cytometry, we found a significant increase in inflammatory monocytes ($CD11b^{hi}$, $CD45^{+}$, $Ly6C^{+}$), as well as the activation state of resident microglia ($CD11b^{int}$, $CD45^{+}$, $MHC\text{-}II^{+}$) in IL-10 KO mice compared to WT mice. As we do not observe parasite sequestration in the brain of infected animals, these studies support the role of increased inflammation in the development of experimental cerebral malaria.

29 - IL27 PRIMED MYELOID DENDRITIC CELLS ARE TOLEROGENIC AND OVEREXPRESS THE ANTI-INFLAMMATORY MOLECULES PD-L2 AND IDO1.

Felipe von Glehn [1] - Gopal Murugaiyan [1]
Chantal Kuhn [1] - Marta Olah [1] - Keren Regev [1]
Radhika Raheja [1] - Maria Antonietta Mazzola [1]
Sushrut Jangi [1] - Anu Paul [1]
Leonilda dos Santos [2] - Howard Weiner [1]
Roopali Gandhi [1]
Harvard Medical School, Brigham and Women's Hospital, Boston, United States [1] - Neuroimmunology Unit, University of Campinas, Campinas, Brazil [2]

Background

The pathogenesis of multiple sclerosis (MS) is driven by central nervous system (CNS) invading encephalitogenic T cells. Dendritic cells (DCs) are considered the most efficient antigen-presenting cells that contribute significantly to regulate effector immunity and tolerance. CNS perivascular infiltrating myeloid DCs (mDCs) are important contributors to reactivation of auto reactive T-cells in MS and EAE. Interleukin 27 (IL-27) is an IL-12 family cytokine known to have broad inhibitory effect on both inflammatory T cells and DCs. However, the molecular mechanisms by which IL-27 mediates its suppressive effect on DCs and T cells is not well understood.Objective: To determine the molecular mechanisms involved in IL-27 driven tolerogenic effect in mDCs comparing RRMS patients to controls.

Methods

MDCs were purified from PBMCs, isolated from MS or controls, by FACS sorting. DCs were activated with LPS or LPS+IL-27 for 24 hrs. Total RNA was analyzed using hybridization assays containing 500 target immune genes and 752 microRNAs assays. FACS and confocal microscopy were used to measure expression of PDL2 and IDO1. Purified T-cells were co-cultured with activated mDCs. T cell proliferation was measured by thymidine incorporation assays and the cytokines in the supernatants were measured using LUMINEX assay.

Results

We found that the mDCs treated with IL-27 induced anti-inflammatory T cells that were less proliferative and secreted decreased pro-inflammatory cytokines, like IL17F, IL23 and TNF-alpha, but increased anti-inflammatory cytokine IL-10. We found that the regulatory phenotype of T cells induced by IL-27-primed mDCs was associated with an altered expression of immunoregulatory genes involved in NF-κβ, IDO1 and PD-L2/PD-1 signaling pathways in DCs. In addition, we found that IL-27 induced mDCs expressed altered microRNA signatures that could be linked to their tolerogenic phenotype. Furthermore, our preliminary data also suggests that this IL-27 induced immunoregulatory pathway in DCs could be deregulated in patients with MS.

Conclusion

IL-27 induced an anti-inflammatory gene signature, phenotype and function in mDCs from healthy controls, an effect that was reduced when using mDCs from RRMS patients. Our data suggests that an increase of PD-L2 and IDO1 expression in mDCs could be responsible for the IL-27 driven tolerogenic effect.

34 - CAN SOLUBLE L-SELECTIN (CD62L) BE USED FOR ASSESSING THE RISK OF PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY?

Pabitra Basnyat [1] - Sanna Hagman [1] - Marcin Kolasa [1] - Keijo Koivisto [2] - Auli Verkkoniemi-Ahola [3] - Laura Airas [4] - Irina Elovaara [5]
Neuroimmunology Unit, medical school, University of Tampere, Tampere, Finland [1] - Department of Neurology, Seinäjoki Central Hospital, Seinäjoki, Finland [2] - Department of Clinical Neurosciences,

Helsinki University Central Hospital, Helsinki, Finland [3] Department of Clinical Neurosciences, Turku University Hospital, Turku, Finland [4] - Department of Neurology, Tampere University Hospital, Tampere, Finland [5]

Background

Natalizumab is a humanized monoclonal antibody for the treatment of relapsing-remitting multiple sclerosis (RRMS). It is an alpha-4 integrin (CD49d) antagonist that prevents the migration of peripheral leukocytes across the blood-brain barrier (BBB). Despite its efficacy, long-term treatment with this drug is associated with the complication of developing progressive multifocal leukoencephalopathy (PML), a JC virus (JCV)-mediated disorder of the CNS. JC virus seropositivity has been established as one of the risk factors for developing PML in natalizumab-treated MS patients. L-selectin-expressing CD4+T cells have been proposed as a biomarker for individual PML risk assessment.

Objective

L-selectin is rapidly shed from the cell surface of activated T cells to blood where it remains as a functionally active soluble form. Therefore, in this study, our aim was to examine whether the levels of soluble L-selectin (sL-selectin) in sera of RRMS patients treated with natalizumab can predict the risk of PML.

Methods

This study included 99 subjects of whom, 44 RRMS patients were treated with natalizumab, 30 RRMS patients with IFN-beta and 25 subjects were healthy controls. The levels of sL-selectin in sera were measured by ELISA and the anti-JC Virus (JCV) antibody index was determined by the second-generation ELISA (STRATIFY JCV™ DxSelect™) assay. The association of sL-selectin with anti-JCV antibody index in natalizumab and IFN-beta- treated RRMS patients was assessed.

Results

The significant correlation was found between the levels of sL-selectin and anti-JCV-antibody indices in sera in the natalizumab-treated patients (r=0.402; p=0.007; n=44), but not in those treated with IFN-beta. This correlation became even stronger in JCV-seropositive patients treated with natalizumab longer than 18 months therapy (r=0.529; p=0.043; n=15).

Conclusion

Our data suggests that the measurement of sL-selectin should be evaluated further as a potential biomarker for predicting the risk of developing PML.

35 - MICROGLIAL MICROVESCICLES AS THERAPEUTIC VECTOR FOR NERUOINFLAMMATION

Giacomo Casella [1] - Federico Colombo [1]
Annamaria Finardi [2] - Roberto Furlan [2]

Ospedale San Raffaele, Università Vita e Salute San Raffaele, Milan, Italy [1] - Ospedale San Raffaele, Ospedale San Raffaele, Milan, Italy [2]

Extracellular vesicles (EVs) are membrane-bound particles formed from inside a cell or directly from its membrane, and released to the extracellular space that carry information whose function is cell-to-cell communication without direct contact. EVs can be divided by their biogenesis, cell origin and morphologic characteristics, in to three classes: exosomes, microvescicles (MVs) and apoptotic blebs All most of cells release EVs; emerging evidence started to support the notion that EVs are a universal mechanism of communication. MVs may represent a very promising strategy to gain pathogenic information, identify therapeutic targets, and select specific biomarkers for neurological disorders. The idea of using MVs as therapeutic delivery vehicles has recently emerged. MVs may be act as “physiological

cargo" and with their low immunogenicity can be engineered to carry target molecules mRNA, siRNA and drugs, as potential therapy delivery systems. Recent evidence suggest that activated microglia uses EVs to communicate to neighbouring microglia and modulate their phenotype and function. We propose here to exploit microglia-derived EVs as drug delivery tool for neuroinflammation and neurodegeneration, trough production of microglial MVs able to cross the blood brain barrier (BBB) and deliver therapeutic molecules to the central nervous system CNS. We have produced a stably engineer murine microglia cell lines, by antibiotic selection G418, to express interleukin 4 and Rabies viral glycoprotein (RVG). Our interest is direct to use IL-4, because it can shift microglia to a protective phenotype called M2. Our group shown in the past, through gene therapy that directs CNS delivery of IL-4 is extremely efficient in inhibiting the animal model of Multiple Sclerosis, Experimental Autoimmune Encephalomyelitis EAE and the RVG has a high affinity to achetil-coline receptor, in particular the isoform Ð7 that is expressed on the surface of neurons, astrocyte, microglia and other cells. It is already demonstrated the capacity of RVG to target the CNS. We optimized the microglial MVs production using ATP at 1 µM and PMA, the collection by different step of centrifugation. We evaluated if the MVs can transfer their content in vitro, using the farnesylate GFP; another approach that we are testing is the CREr2 model. MVs IL-4+, have showed the ability to promote the polarization of the microglia in vitro, by the expression of a typical anti-inflammatory gene like YM1 and induce a slight reduction of pro-inflammatory gene iNOS.

37 - THE ROLE OF MICROGLIA AND INFLAMMATION IN AN ANIMAL MODEL OF ALS

Chiara Rossi - Annamaria Finardi - Andrea Bergamaschi - Roberto Furlan - Angelo Quattrini Giancarlo Comi - Gianvito Martino - Luca Muzio
Ospedale San Raffaele, Division of Neuroscience, Milano, Italy

Background

Microglial (MG) cells activation and CNS-inflammation are characterizing Sod1^{G93A} mice - i.e. the animal model of Amyotrophic Lateral Sclerosis (ALS) disease. The central role of MG cells in mediating part of the damage occurring into the CNS of these transgenic mice has been highlighted by several pioneering works. Indeed, the MG exact role and how neuroinflammatory episodes might influence early steps of the disease progression and motor neurons (MN) degeneration are aspects that need further investigation.

Methods

Here we provide electrophysiological recordings of neuronal networks from WT and Sod1^{G93A} mice receiving a pro-inflammatory cocktail of cytokines, that is mimicking the inflammatory environment occurring *in vivo*. We next characterized MG cells in the spinal cord of Sod1^{G93A} mice, trying to establish a functional correlation between MG activation and neuronal damage. Finally, we attempted to polarize MG cells in Sod1^{G93A} mice toward the M2 paradigm by using a gene therapy approach based on the administration of lentiviruses encoding IL4.

Results

The administration of pro-inflammatory cytokines on neuronal networks did not perturbed the total number of spikes recorded in both genotypes and similarly the burst organization was unchanged -

i.e. the length of bursts, percentages of channels displaying bursting activity and the intra-burst spikes frequencies -. All in all, these results suggest that the general firing activity of ALS neurons is not perturbed by this selected inflammatory cytokines. Finally we demonstrated that the *in vivo* over expression of IL4 in $SOD1^{G93A}$ mice reduces the number of MG cells and modulates microglia-related inflammatory genes. Surprisingly, the reduction of microgliosis does not affects mice survival and the progression of the pathology suggesting that this gene therapy cannot drive the complete switch of microglia to the protective M2 phenotype.

38 - CHEMOKINE EXPRESSION AND MIGRATION OF IMMUNE CELLS IN THE CENTRAL NERVOUS SYSTEM IN VIRAL NEUROINFLAMMATION, WITH FOCUS ON REGULATORY T CELLS

Liza Lind [(1)] ***- Charlotta Movitz*** [(2)] ***- Joakim Ek*** [(3)]
Carina Mallard [(3)] ***- Marie Studahl*** [(4)]
Kristina Eriksson [(1)]
Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden [(1)] Sahlgrenska Cancer Center, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden [(2)] - Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden [(3)] - Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden [(4)]

The Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are double-stranded DNA viruses with ~50% DNA homology that initially infects mucosal tissue and later establish infection in sensory neurons. Despite their genetic similarity, the clinical manifestations caused by these two viruses are different. HSV-1 mainly cause cold sores and establish infection in trigeminal ganglia, while HSV-2 give rise to genital blisters and resides in sacral ganglia. In rare cases both virus causes neuroinflammation, encephalitis (HSV-1) or meningitis (HSV-2). Migration of cells is controlled by expression of chemotactic cytokines – chemokines. We have assessed chemokines in cerebrospinal fluid (CSF) from patients with active HSV-1 encephalitis or HSV-2 meningitis and observed a strikingly different chemokine pattern in these diseases, with significantly more chemokines being produced during meningitis. Preliminary data suggests that these findings can be translated to mice. We are therefore characterizing immune cell trafficking over the blood-brain-barrier in HSV-2 induced meningitis, with focus on regulatory T cells. In mice we have observed large numbers of CD4+ T cells and a moderate quantity of NK cells in the central nervous system during HSV-2 meningitis. However, the frequency of regulatory T and CD8+ T cells is greatly suppressed. Chemokine levels in human CSF confirms that recruitment of proinflammatory cells expressing CXCR3 (Th1 and NK cells) is highly prioritized in HSV-2 meningitis, with high levels of ligands CXCL10 (IP-10) and CXCL9 (MIG). However, CD8+ T cells are also known to express CXCR3 and the limited recruitment of CD8+ T-cells into the CNS, despite high viral titers, warrants further investigation. In conclusion, we show a highly restricted recruitment of cells into the CNS during viral meningitis despite high levels of chemokines in the CSF.

39 - A PILOT STUDY OF ADENOSINE 2A-RECEPTORS IN AN ACUTE MODEL OF NEUROINFLAMMATION USING 11C-TMSX

Joni Merisaari [1] - Susanne Vainio [1] - Semi Helin [2]
Alex Dickens [3] - Merja Haaparanta-Solin [1]
Olof Solin [2] - Juha Rinne [4] - Laura Airas [5]
Turku PET Centre, Medicity/Preclinical imaging, University of Turku, Turku, Finland [1] Radiopharmaceutical Chemistry Laboratory, University of Turku, Turku, Finland [2] - John Hopkins School of Medicine, John Hopkins University, Baltimore, United States [3] - Turku PET Centre, Turku University Hospital, Turku, Finland [4] - Division of Clinical Neurology, Turku University Hospital, Turku, Finland [5]

Introduction

Neuroinflammation is a key factor in many neurological diseases, such as multiple sclerosis, Parkinson's disease and Alzheimer's disease. The role of adenosine 2_A-receptor ($A_{2A}R$) in neuroinflammation is still somewhat unknown. Nevertheless, it has been established that $A^{2A}R$ has a role in the activation of microglial cells in neuroinflammation. This study aims to visualize the A2AR expression in the central nervous system during an acute model of neuroinflammation. ^{11}C-TMSX radiotracer was used to visualize $A_{2A}R$ activity with positron emission tomography (PET) and *ex vivo* autoradiography (ARG). An acute model of neuroinflammation was induced by intrastriatal injection of lipopolysaccharide (LPS). Immunohistochemistry was used to confirm the inflammation by staining the microglia.

Methods

Lewis rats (n = 8) were injected with LPS (10 µg) or saline (1 µL) into the left striatum. The animals were imaged *in vivo* at 16 - 24 h after the injection using ^{11}C-TMSX (n = 4) and *ex vivo* ARG was performed (n = 4) to confirm the results. Immunohistochemistry was conducted with three different stainings. Anti-OX-42 and anti-IBA-1 were used to visualize microglial cells and luxol fast blue for myelin degeneration. Biodistribution of the tracer was further investigated with naïve Sprague-Dawley rats (n = 5).

Results

In vivo imaging of the acute model of neuroinflammation revealed a nearly significant increase (n = 2 + 2, p = 0.07) in ^{11}C-TMSX-binding when compared to the vehicle treated animals. Furthermore, autoradiography showed an increase of (n = 2 + 2, p = 0.09) ^{11}C-TMSX binding in the LPS treated animals when compared to the vehicle treated animals. To supplement literature of ^{11}C-TMSX biodistribution studies an increase in ^{11}C-TMSX-binding in the adrenals was observed.

Conclusion

The results indicate that the acute model of neuroinflammation in rats increases the expression of $A_{2A}R$ when compared to the vehicle treated animals. Study shows that ^{11}C-TMSX can be used to detect $A_{2A}R$ activity in rodents. A greater number of animals would strengthen the significance of the results. This work was funded by the European Community´s Seventh Framework Programme (FP7/2007–2013) under grant agreement no. HEALTH-F2-2011-278850 (INMiND).

43 - DNA METHYLATION CHANGES IN NEURONS FROM MULTIPLE SCLEROSIS PATIENTS

Lara Kular - Tatiana Kramarova
David Gomez-Cabrero - Milena Z Adzemovic
Jesper Tegnér - Lou Brundin - Maja Jagodic
Karolinska Institute, Karolinska University/Hospital, Stockholm, Sweden

Background

Multiple Sclerosis (MS) is a chronic inflammatory

disease characterized by autoimmune destruction of myelin and subsequent loss of neurons in the brain and spinal cord. It is the second most common cause of neurological disability in young adults. Current treatments are only effective during relapsing-remitting stage of disease, while there are no treatments for secondary progressive stage which is characterized by persistent neuronal loss. Since neurodegeneration persists at later stages without prominent inflammatory activity, we speculate that neurons acquire sustained changes under neuroinflammatory conditions that make them more prone to dysfunction and eventually to degeneration. In this regard, we aim to characterize DNA methylation changes in neurons from affected and healthy brains.

Results

The material comprises snap frozen brain tissue blocks collected within 24h post-mortem from MS patients and controls (Multiple Sclerosis and Parkinson's Tissue Bank, Imperial College London) that were characterized according to the type of MS lesion and anatomical location of the tissue block. We optimized method for sorting neuronal nuclei from frozen brain tissue and performed preliminary genome-wide DNA methylation analysis on white matter neurons from MS patients and controls (n=12), using Infinium HumanMethylation450 BeadChip. Bioinformatics and statistical analysis identified differentially methylated positions (DMPs) between MS and controls. Pathways analysis on autosomal DMPs allowed us to select interesting candidates and pathways affected in MS neurons (e.g JAK/STAT signaling and oxidative stress/metabolic pathways). We are currently validating this promising data in an independent set of samples. The functional consequences of DNA methylation changes will be further investigated in vitro and *in vivo* using rodent models of MS.

Conclusion

Our data suggest that neurons, which are the targets of autoimmune attack in MS, acquire permanent changes that can be detected (postmortem) on the level of DNA methylation. Our preliminary findings implicate pathways involved in oxidative stress and detoxification as putative key processes that could contribute to impaired neuronal activity and inability to repair after immune insult. Thus, this study may open promising insights into pathogenesis and treatment of MS as well as other neurodegenerative diseases.

46 - FIBROBLAST GROWTH FACTOR 9 AND ITS DOWNSTREAM REGULATORS IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

Daniel McElroy [(1)] ***- Katja Thuemmler*** [(1)] ***- Maren Lindner*** [(1)] ***- Cornelia Schuh*** [(2)] ***- Hans Lassman*** [(2)] ***Christopher Linington*** [(1)]

Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom [(1)]

Department of Neuroimmunology, University of Vienna, Vienna, Austria [(2)]

Fibroblast growth factor 9 (FGF9) plays important roles in coordinating the proliferation, differentiation, migration and survival of neurons and glia in the developing nervous system, however it is now becoming apparent that this pleiotropic growth factor may also play an important role in the pathogenesis of multiple sclerosis (MS). Our data demonstrate FGF9 is upregulated in active MS lesions where it appears to be a response to inflammatory demyelination. In vitro studies demonstrate FGF9 is a powerful inhibitor of (re)myelination, but this is not mediated by a direct effect of FGF9 on cells of the oligodendrocyte lineage, but in response to soluble products secreted by astrocytes in response to FGF9 treatment. Transcriptional profiling of myelinating culture cells treated with FGF9 demonstrates it induces complex downstream changes in gene expression that result in a pro-inflammatory

signalling environment in which (re)myelination fails. These effects are associated with marked increases in expression of the negative feedback inhibitors Spry2, Spry4, Dusp5 and Dusp6. In order to investigate the pathophysiological significance of these in vitro observations we explored the expression of these feedback inhibitors in MS lesions using immunohistochemistry. This confirmed FGF9 expression is upregulated in oligodendrocytes, and to a lesser extent in astrocytes, in both active acute lesions and at the active rims of chronic active lesions and that this is associated with increased expression of Sprouty2 and Sprouty4 by astrocytes. In contrast Sprouty2/4 expression was minimal in normal appearing white matter adjacent to these lesions and in white matter from healthy controls. These findings provide further evidence that FGF signalling in astrocytes plays a role in the development of MS lesions. Elucidating the function of Spry2/4 during lesion development will help determine how the astrocytic response in MS may be manipulated to promote remyelination; a strategy predicted to restore function and prevent further axonal loss in MS.

47 - NON-LYTIC AUTOANTIBODY MEDIATED INJURY INDUCES CHEMOKINE EXPRESSION IN MYELINATING CULTURES

Tiia Semenoff - Katie Chapple - Katja Thümmler Julia Edgar - Christopher Linington
Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom

Multiple sclerosis (MS) is characterised by sustained intrathecal immunoglobulin synthesis within the central nervous system (CNS) that is established in ~90% of patients at diagnosis. It persists throughout the course of the disease and manifests itself as discrete oligoclonal bands of IgG in cerebrospinal fluid (CSF), but its pathophysiological significance remains obscure. The specificity profile of this antibody repertoire is heterogeneous and complex, and varies between patients; however, this intrathecal response may recognise any of a wide variety of microbial antigens, as well as myelin, axonal and neuronal autoantigens.

A significant proportion of the antibodies are directed against myelin-derived lipids, in particular galactosyl ceramide and sulphatide. These surface lipids have shown to provide targets for antibody-dependent, complement-mediated demyelination in a variety of experimental settings. However, does this occur in MS, in particular in the progressive forms of the disease in which the blood brain barrier is intact and complement is unable to access the CNS? To address this, we explored if glycolipid-specific antibodies played other, more subtle roles in disease pathogenesis than mediating complement-dependent lysis. The possible effects were investigated in myelinating cultures derived from embryonic rat spinal cord; a model system that allows exploration of sulphatide reactive IgM mAb O4 effects in the absence of exogenous complement and effector cells. After 24 hours, in the absence of complement, mAb O4 was unable to induce demyelination. However, over a prolonged period it inhibited ongoing myelination, which was associated with microglial proliferation and activation. Gene microarray analysis indicated multiple transcriptional changes, including induction of multiple chemokines, suggesting "immune" activation in the complement-independent cultures. This transient increase in mRNA transcripts resulted in sustained protein synthesis and secretion of biologically active products as demonstrated by culture supernatant analysis.

The glycolipid specific antibody therefore mediates a complex non-lytic response associated with induction of chemokines implicated in recruitment of T cells (CCL5, CCL20, CXCL11), B cells (CXCL13, 10 and 9) and monocytes/macrophages (CCL2, CCL7) into the CNS; a response predicted to exacerbate disease activity in MS.

48 - MANUAL AND AUTOMATED METHODS FOR ASSESSING MITOCHONDRIAL TRANSPORT WITHIN NEURONS

Elena Bros [1] - Anja Hauser [2] - Raluca Niesner [2]
Friedemann Paul [3] - Carmen Infante-Duarte [1]
Institute for Medical Immunology, Charité University Medicine, Berlin, Germany [1] - DRFZ, Deutsches Rheuma Forschungszentrum, Berlin, Germany [2]
NeuroCure Clinical Research Center, Charité University Medicine, Berlin, Germany [3]

Mitochondrial damage appears to contribute to the pathogenesis of neuroinflammatory disorders of the central and the peripheral nervous system. Mitochondria are crucial to cell survival, not only by producing ATP, but also by maintaining ion homeostasis and regulating apoptosis. Within the axons, they are delivered to, and remain in areas where metabolic demand is highest. The health of neurons depends critically on the continuous traffic, distribution and function of their mitochondria. We have previously shown that alterations of mitochondrial motility following an oxidative insult precede axon degeneration, and might therefore be an early indicator of neuropathology. Thus, understanding mitochondrial dynamics in health and pathology might be crucial to identify key pathogenic events in neuroinflammation. However, there is so far no consensus on what methods are most adequate for analyzing mitochondrial behavior. Here, we tracked mitochondria in central and peripheral axons with both manual and various automated tracking tools. We showed that the correlation between tracking strategies was very poor. Compared with manual tracking, automated tools dramatically underestimated track length, mitochondrial displacement and movement duration, with reductions ranging from 45 to 77 % of the values obtained manually. On the contrary, they generally overestimated mitochondrial velocity. Further, automated tools generated a significant number of tracks that did not correspond to real mitochondrial movements, thus misrepresenting mitochondrial behavior. Despite these discrepancies, we further showed that automated tools successfully detected reductions of mitochondrial transport after incubating the axons with an oxidant agent. Thus, although automated tracking methods do not appear adequate for absolute quantification of mitochondrial dynamics, they might be suitable for assessing relative transport differences between experimental groups.

49 - BRAIN MAGNETIC RISONANCE SPECTROSCOPY IN CHILDREN WITH SYDENHAM'S CHOREA AND TOURETTE SYNDROME

Maria Giuseppina Petruzzelli [1] - Franca Di Cuonzo [2]
Maddalena Toto [1] - Laura Cortese [1]
Raffaella Pantaleo [1] Giuseppina Zagaria [1]
Lucia Margari [1]
Child Neuropsychiatry Unit, Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari Aldo Moro, Bari, Italy [1]
Neuroradiology Unit, Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari Aldo Moro, Bari, Italy [2

Background
Different autoimmune mechanism may be involved in the pathogenesis of childhood onset movment disorders, as Sydhenam's Chorea and Tourette Sindrome. The mesurement of brain metabolites with magnetic risonance spectroscopy (MRS) provides, to date, a unique prespective on the brain bases of neuropsychiatric disorders.

Objective
the aims of this study were to investigate metabolite changes in basal ganglia of children with Sydhenam's Chorea and Tourette Sindrome, and to compare each other.

Methods

15 children with Sydhenam's Chorea and 30 children with Tourette Sindrome were included into the study. All patients underwent a detailed neuropsychiatric evaluations. Brain magnetic risonance imaging (MRI) and multi voxel magnetic risonance spectroscopy (MRS) in basal ganglia was performed in all subjects. N-acetyl aspartate(NAA)/creatine (Cr) and cholin/Cr ratios were calculated.

Results

The study of MRS spectra showed an abnormal pick at 3.9 ppm, suggesting the presence of sugar and amino acids, in 7 patients (46,7%) with Sydhenam's Chorea but in no one of Tourette Syndrome, with a significant diffrence between the two groups (p =0.000). Moreover in Sydhenam's Chorea we found a slight decreased NAA/Cr in 1 patient and a decreased of NAA in 3 patients, suggesting a neuronal dysfunction.

Conclusions

Our findings suggest as an abnormal presence of sugar and amino acids in Sydhenam's Chorea may be related to antibodies against basal ganglia, and highlight the importance of MRS methods to study brain metabolic markers involved in the pathogenesis of disease.

54 - THE CHOROID PLEXUS AS LEUKOCYTE ENTRY GATE DURING SYSTEMIC INFLAMMATION

Delphine Demeestere
Roosmarijn Vandenbroucke - Claude Libert
Flemish Institute for Biotechnology (Inflammation Research Center), University of Ghent, Ghent, Belgium

The systemic inflammatory response syndrome (SIRS) is associated with systemic inflammation and organ dysfunction. SIRS is a prevalent disease with a high mortality rate and when encephalopathy occurs, mortality is even higher. Current treatments are mainly supportive, which urges the need for new therapeutics. Recently, we observed disruption of the blood-cerebrospinal fluid barrier (BCSFB), formed by the choroid plexus (CP), early after induction of SIRS. This was accompanied by increased cytokine levels in the CSF and activation of astrocytes in the brain parenchyma. It is believed that the BCSFB is an important site of immune cell trafficking from the periphery into the brain. Macrophages, dendritic cells and T cells are the resident immune cells at the CP; however the infiltration of leukocytes during systemic inflammation at the CP is not thoroughly described. Indeed, early after induction of systemic inflammation, we observed increased expression of several adhesion molecules (VCAM, ICAM) involved in cell-cell interaction. Additionally, analysis of the CSF revealed an increase of white blood cells (WBCs) after inflammatory stimulus. Flow cytometry data showed changes in the number of macrophages and T cells at the CP accompanied by an influx of neutrophils. However, no infiltration in the brain parenchyma was observed. In conclusion, we have evidence that systemic inflammation induces changes at the BCSFB, which eventually results into WBC trafficking across the BCSFB. Consequently, preventing BCSFB changes and WBC trafficking could have therapeutic potential for the treatment of SIRS.

55 - TARGETING MICROGLIAL ADAM17 TO IMPROVE FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

Daniela Sommer - Myriam Gou-Fabregas
Pia M Vidal - Dearbhaile Dooley - Evi Lemmens
Sven Hendrix
Biomedical Research Institute, Hasselt University, Hasselt, Belgium

Traumatic spinal cord injury (SCI) is characterized by a strong post-traumatic inflammation and microglia/macrophage activation leading to secondary injury damage and limited functional recovery. Inflammation and scar tissue formation are major barriers that dampen neuronal regeneration after central nervous system injury. Therefore, a better understanding of the processes involved in the secondary injury may provide new targets to improve neuroregeneration and functional outcome of SCI patients.

Microglia are the primary immune effector cells in the central nervous system (CNS) and are apart from macrophages the most effective modulators after CNS damage. Similarly to macrophages, microglia can be polarized into various directions including the M1 phenotype (producing pro-inflammatory cytokines such as TNFalpha and IL-1beta) and the M2 phenotype (generating a pro-regenerative favorable milieu). Therefore, it is tempting to speculate that a reduction of M1 and/or an increase in M2 phenotype activation from microglia and macrophage cells might be beneficial for neuroregeneration and functional recovery after SCI.

Previous results from our group indicated that "A disintegrin and metalloproteinase 17" (ADAM17), which induces the release of soluble TNFalpha, substantially influences SCI outcome as well as oligodendrocyte and microglia survival. To elucidate the involved pathological mechanisms, we performed further experiments using the ADAM17 hypomorphic mouse model ($ADAM17^{ex/ex}$). Preliminary data indicate that $ADAM17^{ex/ex}$ mice show a better functional recovery after SCI compared to wild type controls based on the 9-point Basso Mouse Scale analysis. Moreover, $ADAM17^{ex/ex}$ primary microglia cultures show impaired capacity to produce soluble TNFalpha even after LPS stimulation.

T and B cells autoimmunity

4 - CELL-INTRINSIC ESTROGEN RECEPTOR α ACTIVATION IN CD4+ T CELLS CONTROLS TH1/TH17 DIFFERENTIATION IN TRANS AND PROTECTS FROM CNS AUTOIMMUNITY

Laure Garnier - Sophie Laffont - Nelly Rouquié Alexandra Gouazé - Jean-Charles Guéry
INSERM CPTP U1043, Université Paul Sabatier, Toulouse, France

Estrogens influence many physiological processes in mammals. In autoimmune diseases, estrogens can display either beneficial or deleterious effects. Clinical remissions in patients with multiple sclerosis (MS) during pregnancy have suggested that sex hormones, particularly estrogens, could modulate CNS-autoimmunity and inflammation. Indeed, 17ß-estradiol (E2) treatment has been shown to inhibit the development of experimental autoimmune encephalomyelitis (EAE), the animal model of (MS). Two molecular targets essentially mediate E2 actions: estrogen receptor alpha (ERα) and beta (ERβ). We and others have shown that the protective effect of E2 on EAE was strictly dependent on ERα. However, the underlying mechanisms responsible for the anti-inflammatory effect of E2 in EAE protection are still ill defined. Using tissue-specific ERÐKO mouse models, we demonstrated that ERα-signaling in T lymphocytes, but not myeloid cells (monocytes/macrophages or dendritic cells), was required for sustained EAE protection by E2 (Lélu et al., J. Immunol. 2011 187:2386). Using adoptive transfer model of MOG-specific 2D2 CD4 T cells, we now show that ERα-signaling in endogenous host $CD4^+$ T lymphocytes rather than responding 2D2 $CD4^+$ T cells orchestrates the inhibition of MOG-specific Th1/Th17 cell priming. Co-administration of naive 2D2 $ER\alpha^{-/-}$ T cells with E2-responsive $ER\alpha^{+/+}$, but not $ER\alpha^{-/-}$, CD4 T cells strongly delayed EAE development in $Rag2^{-/-}$ $ER\alpha^{-/-}$ hosts. Thus, these results identify a suppressive activity of E2-primed $CD4^+$ T cells, by which they can restrict differentiation of Th17 cells in *trans* and protect from CNS autoimmunity.

6 - IMMUNE RESPONSE TO EPSTEIN-BARR VIRUS IN MULTIPLE SCLEROSIS

Gisella Guerrera (1) - Eleni Anastasiadou (2)
Eleonora Piras (1) - Giovanna Borsellino (1)
Rosella Mechelli (3) - Viviana Annibali (3)
Giovanni Ristori (3) - Francesca Aloisi (4)
Pankaj Trivedi (2) - Marco Salvetti (3)
Luca Battistini (1) - Daniela F. Angelini (1)
Neuroimmunology Unit, Fondazione Santa Lucia-I.R.C.C.S., Roma, Italy (1) - Department of Experimental Medicine and Pathology, University of Rome "La Sapienza", Roma, Italy (2) - Centre for Experimental Neurological Therapies, S. Andrea Hospital-site, Department of Neuroscience, Mental Health and Sensory Organs (NESMOS), Fac, Sapienza University of Rome, Roma, Italy (3) - Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Roma, Italy (4)

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), where cells of the immune system cause damage to myelin, the coating of the axons of the neurons. The causes include genetic predisposition, immune dysregulation and enviromental factors including viruses. Epstein-Barr virus (EBV), an ubiquitous γ-herpesvirus, has been strongly associated with MS, but the mechanism by which EBV might contribute to MS

is still unclear.
Our hypothesis is that, in susceptible individuals, defects in the control of EBV facilitate the establishment of viral infection and of continuous cycles of inflammation in the CNS, due to the recruitment and activation of inflammatory cells in the brain. Moreover, through B lymphocytes, which are latently infected with EBV, may produce autoantibodies, and may present self antigens to autoreactive T lymphocytes. Finally, virus reactivation may cause cellular lysis with subsequent spread of self antigens and inflammation.
To study the immune response to EBV, we characterized the $CD8^+$ T cells response specific for EBV using pentamers, fluorescently labelled reagents composed of HLA class I molecules, loaded with several epitopes, and which bind to the respective antigen-specific TCR. In our study we used the immunodominant epitopes of proteins expressed during the lytic cycle of the virus, BMLF-1 and BZLF-1, and to two viral proteins expressed during the latent cycle of EBV, EBNA-3 and LMP-2. In our previous study we found a lower prevalence of $CD8^+$ T cell responses to EBV in MS patients with inactive disease, and a higher frequency of $CD8^+$ T cells specific for EBV lytic antigens during the active disease. In contrast, the $CD8^+$ T cell response to cytomegalovirus (CMV), another herpesvirus selected as a control, did not differ between HD and MS patients, irrespective of the disease phase. The data suggests a strong correlation between the active phase of the disease and the reactivation of the virus in the CNS.
We also used autologous B-EBV transformed lymphoblastoid cell lines generated spontaneously or by in vitro infection from PBLs of MS patients and healthy controls, and we characterized them for the expression of costimulatory molecules, which are important for the cross talk between B and T lymphocytes.
We found that spontaneous LCL derived from MS patients express high levels of costimulatory molecules, and this may contribute to auto-reactive immune responses by presenting self antigens. Also, we find that these two cell lines elicit different responses in T cells when used in co-culture experiments, suggesting that infection with different strains of the EBV virus my lead to different outcomes, and that indeed healthy donors positive for EBV infection may harbor different strains of EBV compared to MS patients.
Our data underlines the role of dysregulation of the EBV-specific response in MS.

7 - T HELPER 9 CELLS INDUCED BY PLASMACYTOID DENDRITIC CELLS REGULATE INTERLEUKIN-17 IN MULTIPLE SCLEROSIS.

Gabriella Ruocco [(1)] ***- Silvia Rossi*** [(2)] ***- Caterina Motta*** [(2)] ***- Giulia Macchiarulo*** [(2)] ***- Francesca Barbieri*** [(2)] ***- Marco De Bardi*** [(1)] ***- Giovanna Borsellino*** [(1)] ***- Annamaria Finardi*** [(3)] ***- Maria Grazia Grasso*** [(4)] ***- Serena Ruggieri*** [(5)] ***- Valeria Studer*** [(2)] ***- Claudio Gasperini*** [(5)] ***- Roberto Furlan*** [(3)] ***- Diego Centonze*** [(6)] ***- Luca Battistini*** [(1)] ***- Elisabetta Volpe*** [(1)]
Neuroimmunology Unit, Santa Lucia Foundation, Rome, Italy [(1)] - Department of Medicina dei Sistemi, University Tor Vergata, Rome, Italy [(2)] Neuroimmunology Unit, Institute of Experimental Neurology - Division of Neuroscience - San Raffaele Institute, Milan, Italy [(3)] - Multiple Sclerosis Centre, Santa Lucia Foundation, Rome, Italy [(4)] - Department of Neuroscience Lancisi, San Camillo Hospital, Rome, Italy [(5)] - Neuroimmunology and Synaptic Plasticity Unit, Santa Lucia Foundation, Rome, Italy [(6)]

Multiple sclerosis (MS) is an autoimmune disease characterized by persistent inflammation generated by cytokines mainly produced by T helper (Th)1 and Th17 cells, such as IFN-α and IL-17. The role of other Th cytokines in MS is still unclear.
We identified a novel immunoregulatory role for interleukin-9 (IL-9) in MS. IL-9 is a cytokine produced by a subset of CD4+ T lymphocytes called Th9 cells, and it is known to be involved in allergic diseases

and helminthic infections affecting mast cells and eosinophils.

In this study we analysed the content of IL-9 in the cerebrospinal fluid (CSF) of relapsing-remitting (RR)-MS patients and we found that IL-9 levels are inversely correlated with indexes of inflammatory activity, neurodegeneration, and disability progression of MS disease.

We also analysed the role of IL-9 in vitro and we found that IL-9 activates STAT1 and STAT5 phosphorylation in Th17 cells and reduces the production of IL-17 and IRF-4 expression by Th17-polarized cells. We then analysed the levels of IL-9 and IL-17 in the CSF of RR-MS patients and we found that high levels of IL-9 are associated with the absence of IL-17, indicating that the inhibition of IL-17 by IL-9 might occur also *in vivo*.

In order to identify the cellular source of IL-9, we purified cells from CSF of RR-MS patients, we analysed the frequency of different cell types, and we measured the expression of IL-9. We found that most cells in the CSF are CD4 T cells, and that they express IL-9 after T cell specific stimulation, thus indicating that IL-9 in CSF could originate from Th9 cells.

Moreover, we investigated the mechanisms inducing Th9 induction in MS, and we found that cytokines and costimulatory molecules of plasmacytoid dendritic cells (pDCs) mediate Th9 polarization from naïve CD4 T cells.

In conclusion, these results suggest an important immunoregulatory role of the Th9 response, initiated by pDCs, which attenuate the exaggerated Th17 inflammatory response in MS.

11 - STUDY OF THE NATURAL IMMUNE TOLERANCE TO MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG) AND NEUROFILAMENT MEDIUM (NF-M)

Pierre-Paul Axisa [1] ***- Liliana E. Lucca*** [1] ***- Pierre Rufas*** [1] ***- Bruno Kyewski*** [2] ***- Jens Derbinski*** [2] ***Lennart T. Mars*** [1] ***- Roland S. Liblau*** [1]

Centre physiopathologie Toulouse Purpan, Université Toulouse 3, Toulouse, France [1]

Division of Developmental Immunobiology, Tumor Immunology Program, German Cancer Research Center, Heidelberg, Germany [2]

We established that MOG_{35-55}/NF-M_{15-35} bi-specific T cells exist in the CD4 T cell compartment of all C57BL/6 mice, and that these cells are functionally competent and capable of driving MOG_{35-55}-induced EAE. T cells recognizing two self-antigens would be expected to be more likely to be tolerized, and it is thus surprising that these cells arise in the periphery at such frequency. We assessed the immunogenicity of MOG_{35-55} in mice deficient for MOG and NF-M alone or in combination, or in wild-type mice. Polyclonal CD4 T cells from $MOG^{-/-}$ and $MOG^{-/-}$NF-$M^{-/-}$ mice display enhanced responses compared to wild type and NF-$M^{-/-}$ mice. This increase is correlated to a higher frequency and encephalitogenicity of autoreactive T cells. To understand how the bi-specific T cell repertoire escapes central tolerance, we initially assessed the thymic expression of the two neural antigens. Previous studies have demonstrated that MOG is expressed in the thymus. By RT-PCR, we showed that NF-M transcripts are also detectable in medullary thymic epithelial cells. We observed that expression of MOG, but not NF-M, partially tolerizes MOG_{35-55}-specific CD4 T cells, including bi-specific cells. As a consequence, we investigated why NF-M does not impart tolerance to the bi-specific CD4 T cells despite being expressed in the thymus. We established that the affinity of

NF-M_{15-35} to I-A^b is lower as compared to MOG_{35-55} and that the antigen presentation of NF-M_{15-35} is unstable over time, probably due to the lack of a stable I-A^b anchoring residue. In order to further investigate this hypothesis, we used an NF-M_{15-35} mutated peptide that exhibits higher affinity with I-A^b. NF-M_{15-35} mutant is immunogenic and allows activation of both a MOG_{35-55} and NF-M_{15-35} specific repertoire. These results suggest that for NF-M^{15-35} the threshold for tolerance induction in the thymus is not met because of poor antigenic presentation, resulting from formation of unstable pMHC complexes. Conversely, MOG expression, despite at low levels, suffices to partially tolerize the MOG_{35-55}-specific T cell compartment of C57BL/6, including the bi-specific CD4 T cell population. Our project documents how CNS autoantigens fail to tolerize the CD4 T cell repertoire thus allowing the development of an autoimmune disease.

21 - HISTOPATHOLOGY AND CLINICAL COURSE OF MOG-ANTIBODY ASSOCIATED ENCEPHALOMYELITIS

Melania Spadaro [(1)] ***- Lisa Ann Gerdes*** [(1)]
Marie Cathrin Mayer [(1)] ***- Birgit Ertl-Wagner*** [(2)]
Sarah Laurent [(1)] ***- Markus Krumbholz*** [(1)]
Constanze Breithaupt [(3)] ***- Tobias Högen*** [(4)]
Andreas Straube [(4)] ***- Armin Giese*** [(5)] ***- Reinhard Hohlfeld*** [(1)] ***- Hans Lassmann*** [(6)] ***- Edgar Meinl*** [(1)]
Tania Kümpfel [(1)]
Institute of Clinical Neuroimmunology, Medical Campus Grosshadern, Ludwig Maximilian University, Munich, Germany [(1)] - Department of Radiology, Medical Campus Grosshadern, Ludwig Maximilian University, Munich, Germany [(2)] - Institue of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Halle, Germany [(3)] - Department of Neurology, Medical Campus Grosshadern, Ludwig Maximilian University, Munich, Germany [(4)] Department of Neuropathology, Medical Campus Grosshadern, Ludwig Maximilian University, Munich, Germany [(5)] - Department of Neuroimmunology, Center for Brain Research, Vienna, Austria [(6)]

Autoantibodies against conformationally intact myelin oligodendrocyte glycoprotein (MOG) occur in a proportion of patients with different inflammatory demyelinating diseases of the central nervous system, such as childhood multiple sclerosis (MS) and acute disseminated encephalomyelitis (ADEM), and in patients with anti-aquaporin-4 (AQP4)-negative neuromyelitis optica spectrum disorders (NMOSD). Recent studies indicated that MOG antibodies are also present in some patients with optic neuritis and in very few patients with demyelinating syndromes associated with anti-NMDA-receptor abs. The aim of our study was to determine the presence of MOG-specific antibodies in adults with different demyelinating diseases and to analyse the target epitopes.
We established for this purpose a sensitive cell-based assay with transient transfection of MOG and a panel of mutated variants. Here we present histological, MRI, and clinical features of an adult patient with relapsing encephalomyelitis and antibodies against MOG. Furthermore, we report molecular details of the recognized epitopes. A brain biopsy revealed multiple sclerosis (MS)-type II pathology. Some features overlapped with both MS and NMOSD, whereas others were distinct from both MS and NMOSD. This case contributes a new, so far missing link in the emerging spectrum of MOG-antibody-associated encephalomyelitis.

22 - METHYLOME CHARACTERIZATION OF CD4+ T CELLS IN MULTIPLE SCLEROSIS - ESTABLISHING A ROLE FOR MIR-21 IN AUTOIMMUNE DISEASE

Sabrina Ruhrmann [1] - Eliane Piket [1] - Petra Bergman [1] - Lara Kular [1] - Julio Cesar Cetrulo Lorenzi [1] - Shahin Aeinehband [1] - Roham Parsa [1] David Gomez-Cabrero [2] - Jesper Tegnér [2] Fredrik Piehl [1] - Maja Jagodic [1]
Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden [1] - Department of Medicine, The Unit of Computational Medicine, Karolinska Institute, Stockholm, Sweden [2]

Evidence for methylation changes in CD4+ T cells and brain tissue of multiple sclerosis (MS) patients and healthy controls (HC) strongly suggest a role for epigenetics in disease pathogenesis. We here sought to identify methylation changes in one of the key players in MS disease, $CD4^+$ T cells, between MS patients and HC. The $CD4^+$ T cells were sorted using a MoFlow sorter from peripheral blood mononuclear cells (PBMCs) isolated from MS cases and HC. DNA extracted from the $CD4^+$ T cells, was subjected to genome-wide DNA methylation quantification using Illumina Infinium Human Methylation 450K Bead chip. The top scoring changes in DNA methylation between groups were confirmed using bisulfite pyrosequencing and miRNA expression was detected by TaqMan microRNA-assay. Preliminary analyses of the genome-wide methylation data revealed higher methylation rate at all CpG positions of the miR-21 gene in MS patients compared to controls. In line with this finding there was a lower miR-21 expression in these patients as methylation is generally associated with gene silencing. We further investigated the involvement of miR-21 in miR-21 $^{-/-}$ and wild type mice in an animal model of MS, experimental autoimmune encephalomyelitis (EAE). miR-21$^{-/-}$ mice were protected against EAE as compared to littermate controls. Taken together these findings support the notion that epigenetic regulation of miR-21 expression affects autoimmune neuroinflammation. To mechanistically dissect the impact of miR-21 on EAE, the inflammatory response will be characterized in miR-21$^{-/-}$ animals and littermate controls during the course of EAE.

30 - THE EFFECT OF IL27 SIGNALING ON MIRNA EXPRESSION IN MYELOID DENDRITIC CELLS IN MULTIPLE SCLEROSIS

Radhika Raheja [1] - Felipe von Glehn [1] - Gopal Murugayan [1] - Chantal Kuhn [1] - Marta Olah [1] Keren Regev [1] - Maria Mazzola [1] - Sushrut Jangi [1] Anu Paul [1] - Leonilda dos Santos [2] Howard Weiner [1] - Roopali Gandhi [1]
Brigham and Women's Hospital, Harvard Medical School, Boston, United States [1] - Neuroimmunology Unit , University of Campinas, University of Campinas, Campinas, Brazil [2]

Background
Dendritic cells contribute to the pathogenicity of multiple sclerosis (MS) by promoting the entry, activation and differentiation of pathogenic T cells into the central nervous system (CNS). Interleukin 27 (IL-27) signaling in DC's, on the other hand, reduces the differentiation of pro-inflammatory Th1 and Th17 T cells and the development of CNS autoimmunity in an EAE mouse model. We identified differences in microRNA (miRNA) expression in IL27 primed mDCs from HCs and RRMS patients. The role of miRNAs in mDCs and how they might contribute to the IL-27 mediated tolerogenicity of mDCs in MS is not understood.

Objective
To determine potential miRNA candidates in mDCs from HCs and RRMS patients and how its function is affected by IL-27 signaling.

Methods

We purified mDCs from HCs (n=6) and RRMS patients (n=6) by flow cytometry. The mDCs were either activated with LPS only or LPS with IL-27 (IL-27 primed mDCs). Total RNA was isolated and analyzed using PCR-based microRNA array. The findings were validated by Taqman RT- PCR. Activated mDCs were cocultured with purified T cells to detect the expression of cytokines in the supernatant by LUMINEX.

Results

In our MicroRNA array data from HCs, we detected 7 miRNAs that were significantly downregulated and 3 miRNAs that were upregulated in IL-27 primed mDCs compared to those treated with LPS only. When we validated these findings by Taqman RT-PCR, only miR27a was significantly decreased in IL-27 primed mDCs compared to mDCs treated with LPS alone ($p<0.05$). On the other hand, 4 miRNAs were significantly upregulated and 6 downregulated in Il-27 primed mDCs from RRMS patients compared to mDCs treated with LPS only. Interestingly, however, miR27a was not significantly reduced in IL-27 primed mDCs from RRMS patients by microarray analysis or Taqman RT-PCR ($p=0.126$). Further, we detected an increased expression of the anti-inflammatory cytokine IL-10 in the supernatant from IL-27 primed mDCs colcultured with T cells from HCs.

Conclusions

IL-27 mediated decrease in miR27a expression in mDCS from HCs might generate an anti-inflammatory response and consequently limit the differentiation of Th1 and Th17 cells. This effect might be deregulated in RRMS patients. Further studies will enable us to assess the significance of miR27a expression in mDCs and how it might contribute to disease pathogenesis.

31 - IL-10-PRODUCING REGULATORY B CELLS (B10 CELLS) IN NEUROMYELITIS OPTICA SPECTRUM DISORDER

Hye-Jin Cho [1] - Eun Bin Cho [1] - Jin Myoung Seok [1]
Byoung Joon Kim [1] - Eun-Suk Kang [2]
Ju-Hong Min [1]
Samsung Medical Center, Sungkyunkwan University, Department of Neurology [1]
Samsung Medical Center, Sungkyunkwan University, Department of Laboratory Medicine and Genetics [2]

Background

Whereas the main pathogenesis of multiple sclerosis (MS) is considered to be mediated by T-helper (Th) 1 cell, the discovery of aquaporin 4-antibody (AQP4-ab) in neuromyelitis optica (NMO) emphasizes the involvement of humoral immune system. However, the pathogenic cascade in MS and NMO remains to be determined. We investigated the balance of the proinflammatory lymphocytes and suppressive lymphocytes in NMO spectrum disorder (NMOSD).

Methods

Twenty-three seropositive NMOSD patients (30 samples; N=8, during attack; N=22, during remission), 11 MS patients (14 samples; N=5, during attack; N=9, during remission), and 13 healthy controls (14 samples) were enrolled. All NMOSD and MS patients have received one or more disease modifying drugs or immunosuppressive drugs. Among lymphocytes, the skewness of B cell, regulatory B (Breg) cell, IL-10 secreting Breg (B10) cell, T cell, Th cell (Th1, Th2, and Th17), cytotoxic T (Tc) cell, and regulatory T (Treg) cell were measured by flow cytometry. Significance was evaluated by Kruskal-Wallis test or ANOVA as appropriate for the data distribution and spearman correlation coefficients were used to evaluate potential relations between specific lymphocyte subsets and AQP4-ab intensity using cell-based indirect immunofluorescence assay.

Results
The proportion of B cells was higher in the MS group, compared to the NMOSD and control groups ($p = 0.003$ and $p = 0.025$, respectively) and the proportion of B10 was significantly higher in both NMOSD and MS groups, compared to the control group ($p < 0.001$). In NMOSD, the proportion of B cells during attack was significantly higher than that during remission ($p < 0.001$). In addition, only the proportion of B10 cells showed a significant positive correlation with AQP4-ab intensity (rho = 0.40, $p = 0.02$).

Conclusion
Our results suggest that in NMOSD patients, the increase of B cells may indicate of the relapse, a highly active status, although the overall proportion of B cells was lower than in MS patients. Moreover, the positive correlation between the proportion of B10 and AQP4-ab intensity supports that B10 may contribute to suppress the disease activity in NMOSD. Further functional studies could help to elucidate the immunological role of B cell and B10 in NMOSD.

40 - BTLA-EXPRESSING B REGULATORY CELLS IN MULTIPLE SCLEROSIS

Federica Piancone - Francesca La Rosa - Ivana Marventano - Laura Mendozzi - Domenico Caputo Marco Rovaris - Marina Saresella - Mario Clerici
Don C. Gnocchi Foundation, IRCCS Santa Maria Nascente, Milan, Italy

Background
Despite the popularity of the B cell/autoantibody-involvement in autoimmune disease the contributions and the role of B cells in human autoimmune diseases remained unclear until recently. Alterations in immune regulation could result in the breakdown of immune tolerance; a phenomenon associated with the development of Multiple Sclerosis (MS). B lymphocytes are key players in the pathogenesis of MS. Recent studies have confirmed an important role for these lymphocytes in the negative regulation of immunity. Within this scenario, BTLA, a novel inhibitory receptor that ligates B7 family members proteins, was identified. Similar to CTLA-4 and PD-1, BTLA is expressed on activated B and T cells alone. Binding of BTLA to B7-H4 (B7S1, B7x), its putative ligand, inhibits TCR-initiated cytokine production and cell cycle progression, suggesting a role for BTLA in peripheral tolerance. Recently a novel subset of regulatory B lymphocytes (Breg), which regulate immune responses through the production of the anti-inflammatory cytokine interleukin-10 (IL-10), was identified.

Objectives
To define the possible role of B cells in the pathogenesis of MS.

Methods
We analysed by flow-cytometry the surface expression of BTLA, as well as IL-10 and TGFbeta production, in un-stimulated or in myelin oligodendrocyte glycoprotein (MOG)- stimulated CD19+ B lymphocytes of MS patients with a clinical and MRI diagnosis of either relapsing-remitting (RRMS), primary progressive (PPMS), secondary progressive (SPMS) or benign MS (BEMS); results were compared to those obtained in age and sex matched healthy controls (HC).

Results
Whereas no differences were seen in un-stimulated cells, MOG-stimulated, BTLA-expressing CD19+ cells were augmented in HC compared to PPMS ($p<0,001$) and to RRMS ($p=0,015$), in BEMS compared to PPMS ($p<0,01$)) and in SPMS compared to PPMS ($p<0,05$); TGFbeta-expressing and IL-10 producing B lymphocytes were significantly reduced in PPMS patients compared to all the other groups of patients ($p<0,05$) and HC ($p=0,005$); BTLA express-

ing and IL-10 producing CD19+ B cells were augmented in HC compared to all MS groups.

Conclusions

Data herein indicate that a quantitative and qualitative impairment of Breg accompanies the breakdown of immune tolerance associated with MS. Results herein indicating a significant reduction of BTLA-expressing and IL-10 producing CD19+ cells offer support to the concept that this pathway plays a pivotal role in this disease.

Grant by Italian Ministry of Health : Ricerca Corrente 2013-14.

41 - MYELIN-REACTIVE ANTIBODIES TRIGGER T CELL-MEDIATED CNS AUTOIMMUNITY BY OPSONISATION OF AUTO-ANTIGEN

Silke Kinzel [(1)] ***- Klaus Lehmann-Horn*** [(2)] ***- Claude C. Bernard*** [(3)] ***- Patrice H. Lalive*** [(4)] ***- Christine Stadelmann-Nessler*** [(1)] ***- Wolfgang Brück*** [(1)] ***Martin S. Weber*** [(1)]

Institute of Neuropathology, University Medical Centre Goettingen, Göttingen, Germany [(1)] - Department of Neurology, Technische Universität München, Munich, Germany [(2)] - Monash Immunology and Stem Cell Laboratories, Monash University, Clayton, Australia [(3)] Department of Clinical Neurosciences, University Hospital of Geneva, Geneva, Switzerland [(4)]

Objective

To dissect the relative pathogenic relevance of myelin-specific antibodies (ab) from myelin-specific B cells in the multiple sclerosis model experimental autoimmune encephalomyelitis (EAE).

Background

B cells and B cell-derived ab may both play a pathogenic role in CNS autoimmune disease. Mice in which B cells recognize myelin oligodendrocyte glycoprotein (MOG), plasma cells secrete high titres of MOG-specific ab and which further contain myelin-reactive T cells (Thx2D2 mice) spontaneously develop EAE. We utilized Thx2D2 mice in combination with B cell-depleting anti (a)-CD20 ab and adoptive transfer regiments to dissect the relative pathogenic contribution of myelin-reactive B cells from myelin-reactive ab in initiation and progression of EAE.

Methods

Thx2D2 mice were injected with a-CD20 weekly starting at the age of 4 weeks. Serum from Th mice immunized with rMOG 1-117 (rMOG) or myelin-reactive 8.18C5 ab was transferred i.v. into naïve 2D2 mice. Serum from WT mice immunized with MOG p35-55, or an IgG1 isotype ab (iso ab) served as controls. *In vivo* proliferation of T cells was determined by BrdU assay. In-vitro, bone-marrow derived macrophages (BMDM) were co-cultured with CFSE-labelled T cells in the presence of 8.18C5, an iso ab or Fc-cleaved 8.18C5 ($F(ab)_2$). T cell proliferation was determined by flow cytometry.

Results

In Thx2D2 mice, a-CD20 treatment did not interfere with development of encephalitogenic T cells or incidence/severity of spontaneous EAE. While all peripheral compartments were depleted of B cells, a-CD20 did not affect constitutive secretion of a-MOG ab. Serum from Th mice containing high titres of a-MOG ab triggered spontaneous EAE when transferred into naïve 2D2 recipients. Further dissecting the role of pathogenic ab, transfer of purified 8.18C5 ab to 2D2 recipients led to *in vivo* proliferation of T cells and similarly triggered spontaneous EAE. In in-vitro studies, 8.18C5, but not its F(ab)2 fragment enhanced phagocytosis of rMOG by BMDM and led to accentuated proliferation of T cells in co-culture assays at low antigen concentrations of rMOG.

Conclusion

Our data indicate that besides promoting CNS demyelination, a-MOG ab enhance myelin-recognition of antigen-presenting cells by opsonization resulting in accentuated activation of myelin-reactive T cells. This novel process may be of particular relevance for initiation of CNS autoimmune disease when a limitingly low concentration of self-antigen is initially recognized.

42 - GENE EXPRESSION PROFILING OF RESTING AND ACTIVATED CD4+ T CELLS IN PATIENTS WITH MULTIPLE SCLEROSIS

Sandra Hellberg [1] *- Daniel Eklund* [1]
Huan Zhang [2] *- Colm Nestor* [2] *- Magnus Vrethem* [3]
Maria Jenmalm [1] *- Mikael Benson* [2]
Mika Gustafsson [4] *- Jan Ernerudh* [5]
Dept of Clinical and Experimental Medicine, Unit of Autoimmunity and Immune regulation, Linköping University, Linköping, Sweden [1] - Dept of Clinical and Experimental Medicine, Center for Individualised Medicine, Linköping University, Linköping, Sweden [2] Dept of Neurology and Dept of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden [3] - Dept of Physics, Chemistry and Biology, Bioinformatics, Linköping University, Linköping, Sweden [4] - Dept of Clinical and Experimental Medicine and Dept of Clinical Immunology and Transfusion Medicine, Linköping University, Linköping, Sweden [5]

A key event in the chronic inflammatory disease multiple sclerosis (MS) is the entry of peripherally activated CD4+ T cells into the central nervous system. An understanding of the regulatory mechanisms that cause these cells to become auto-reactive and disease-promoting are of great importance for future treatments. Previous attempts to identify disease-specific changes in gene expression in resting immune cells have been inconclusive and have not revealed pathways that have been used as drug targets or implementation of biomarkers. In contrast to previous studies, we here investigate gene expression not only in resting but also in activated CD4+ T cells from MS patients to determine if the gene expression profile of activated cells or changes in the process of activation reveals the same or a different pattern of aberrations as compared to investigating resting cells only.

Blood was obtained from 14 treatment-free patients without preceding clinical relapse and a diagnosis of relapsing-remitting definite MS, and from 14 healthy age-matched controls. CD4+ T cells were cultured unstimulated (resting) or stimulated with anti-CD3 and anti-CD28 antibodies (activated) for 24 h. Flow cytometry was used to determine level of activation and the proportion of naïve and memory subsets. Agilent whole genome microarray was used for global gene expression.

All cells responded with low-moderate increase in CD69 expression following stimulation and there was no difference in response between MS patients and controls (mean 12.2%±5.1 (SD) and 12.5%±4.4, respectively). Gene expression analysis identified few genes that were differentially expressed between patients and control.

As expected, a low degree of overlap was observed with previously published studies performed on a mixture of immune cells. Preliminary results show that a supervised classification method was able to separate patients and controls in both resting and activated cells. The CD4+ T cells were further analyzed with both gene set enrichment analysis and Ingenuity Pathway Analysis, which showed significant enrichment for more than 60 gene sets including both immune-related gene sets as well as more general gene sets related to cell fate and differentiation. Furthermore, genes which constituted an MS-specific response to stimulation were highly associated to immune-related pathways. We are currently analyzing the data set using a modular approach.

45 - FOLLOWING ANTI-CD20 TREATMENT, REPLETION AND IMMUNE-COMPETENCE DEPENDS ON PERIPHERAL STIMULATION OF REAPPEARING B CELLS

Linda Feldmann - Wolfgang Brück
Martin S. Weber
Department of Neuropathology, University Medical Center Göttingen, Göttingen, Germany

Clinical studies indicate that anti-CD20 B cell depletion may be an effective therapy for multiple sclerosis (MS) and neuromyelitis optica (NMO). To date, no long-term treatment regimen exists, while emerging data in NMO suggest that clinical activity may return before circulating B cells can be detected in blood. In order to investigate whether this may refer to an earlier reoccurrence of immune competent B cells in secondary lymphoid organs, we monitored compartment-specific repletion with B cells in formerly anti-CD20-treated mice.
C57BL/6 mice received 3 weekly subcutaneous injections with 0.2 mg murine anti-CD20 or isotype antibody (ab). Mice then remained naive or were immunized with T cell determinant MOG peptide 35-55 or MOG protein 1-117, which is recognizable by B cells. Mice were monitored for reappearance of B cells and evaluated phenotypically for markers of activation and differentiation as well as functionally for their capacity to act as antigen-presenting cells (APC) for CFSE labelled MOG-specific T cells.
Following anti-CD20 treatment, B cell depletion was virtually complete in blood and lymph node, while around 5% and 20% of B cells remained detectable in the spleen and bone marrow, respectively. In naïve and in MOG p35-55 immunized mice, a model which does not involve B cells, B cell repletion started in spleen and bone marrow around week 7, while repletion of lymph node and blood was substantially delayed. In these two settings, reappearing B cells remained phenotypically naïve and acted as weak APC. In contrast, in MOG protein EAE, a model in which B cells get activated, contribute as APC and differentiate into ab-secreting plasma cells, B cells repleted earlier and more simultaneously within the immune relevant compartments. Functionally, in MOG protein, but not in MOG peptide induced EAE reappearing B cells fastly differentiated and acted as potent APC.
Upon cessation of anti-CD20 treatment, B cells repopulate in immune-relevant organs before they can be detected in the blood. The phenotype and function of reappearing B cells depend on the presence of peripheral B cell stimulation; reappearing B cells remain naive in the absence of B cell antigen-recognition, while B cells rapidly gain pathogenic function when B cell-stimulating antigen is present at the time B cells reappear. In perspective, these findings indicate that MS and NMO may require distinct treatment schedules.

53 - INCREASED FREQUENCIES OF PERIPHERAL BLOOD TH17 AND TC17 CELLS IN MULTIPLE SCLEROSIS PATIENTS

Andreia Monteiro [1] - Catarina Cruto [2] - Pedro Rosado [2] - Mafalda Fonseca [3] - Luiza Rosado [2] Tiago Carvalheiro [4] - Artur Paiva [4]
Faculdade Ciências da Saúde, Centro Hospitalar Cova da Beira / Universidade da Beira Interior, Covilhã, Portugal [1] - Serviço Neurologia, Centro Hospitalar Cova da Beira, Covilhã, Portugal [2] - Faculdade Ciências da Saúde, Universidade da Beira Interior, Covilhã, Portugal [3] Laboratório citometria de fluxo, Instituto Português do Sangue e da Transplantação, Coimbra, Portugal [4]

The pathophysiology of multiple sclerosis (MS) is heterogeneous and complex. It was initially suggested that Th1 cells were the main subset responsible for the MS pathogenesis, although Th17subset has been shown to be also involved in this process. Considerable evidence exist that points toward an

important pathogenic and/or regulatory role for cytotoxic CD8 T cells(Tc) in MS. Tc cells also acquire different effector and/or memory phenotype, that may contribute to the initiation of CNS autoimmunity by supporting Th17 cell pathogenicity. In this context, the aim of the present work was to quantify and functional characterize Th(c)1 and Th(c)17 cell populations in the peripheral blood from patients diagnosed with MS, according to the MacDonald criteria2010. 38 patients, subdivided in 2 subgroups, according to the phase of the disease, stable (n=30, 26female/4 male, mean age 41±15) or relapse (n=8, 5female/3male, mean age 44±11) and a control group (n=20, 16female/4male, mean age 50±9) were evaluated in this study. Th(c)1 and Th(c)17 characterization was done by intracellular cytokine staining after in vitro stimulation with PMA/ionomycin, in the presence of Brefeldin A during 4 hours. The expression of cell surface markers and intracellular production of IL-17, TNFα, IFNγ and IL-2 were assessed by flow cytometry (FACSCanto; BD), and the obtained results were analyzed using Infinicyt software (Cytognos). Statistical evaluation of data were analysed using the non-parametric Mann-Whitney U test. We observed a significant increase in the frequency of Th17cells in stable MS group when compared with relapse or control groups. Moreover, an increased expression of TNFÐ was observed in those cells. The frequency of Tc17cells, and among them, those that concomitant produced IL-17 and IFN-γ were also increased in stable MS, as well as TNFα expression. A same pattern was observed in relapse MS when compared with control group. Concerning the frequency of Th1 and Tc1 cells no statistically significant differences were observed between the studied groups. In summary, it seems that Th17 and Tc17cells could play an important role in the pathophysiology of MS, since increased frequencies of those cells were observed in MS patients. We also may speculate that the decreased frequencies of those cells observed in relapse patients could reflect a specific migration from blood to CNS. We believe that a more comprehensive knowledge of the plasticity of these cells in MS is highly pertinent for future therapeutic interventions.

Treatment and prevention in neuroimmunological diseases

8 - THE ROLE OF CYTOSOLIC PHOSPHOLIPASE A2 ALPHA IN THE DEVELOPMENT OF AMYOTROPHIC LATERAL SCLEROSIS

Yulia Solomonov
Ben-Gurion University of the Negev, Ben-Gurion University of the Negev, Beer Sheva, Israel

Amyotrophic Lateral Sclerosis (ALS) is fatal neurodegenerative disease characterized by selective death of motor neurons in the cortex, brainstem and spinal cord. Recent studies provide evidence that expression and activation of Cytosolic Phospholipase A2 alpha ($cPLA_2$) increased in spinal cord of patients with sporadic and familial ALS, and spinal cord of G93A SOD1 transgenic mice. Our recent studies showed the role of $cPLA_2$ rat primary microglial activation and in rat primary neuronal death induced by Amyloid beta 1-42. The role of the present study was to determine whether $cPLA_2$ has a role in the development of ALS. The elevation of $cPLA_2$ protein expression in the spinal cord was first detected at 6 weeks old hmSOD1 mice before the development of the sign of the disease and remained elevated during the whole lifespan of the mice. Microglia activation (detected by CD40 and Iba1) appeared at week 15 preceding the reduction in the size and number on neurons (detected by NeuN). Infusion of specific antisense oligonucleotide against $cPLA_2$ (AS) to brain lateral ventricle (using ALZET Osmotic Pump) inhibited the elevated expression of $cPLA_2$ in the brain and spinal cord of G93A SOD1 transgenic mice in comparison to the corresponding sense infusion. 10ug/day AS given to mice at week 15 (at early symptomatic stage of disease) for 6 weeks delayed the motor neuron dysfunction (detected by Rotarod). To determine the molecular processes that are affected by $cPLA_2$ during the development of the disease symptoms and to characterize the effect of prevention of $cPLA_2$ upregulation on the different processes taking place at the transition to the rapid phase of the disease, mice were brain infused with AS or with sense at week 15, and the experiments were terminated around week 19 (in a stage that the two groups exhibited a significant difference in the symptoms of the disease) and analyzed in comparison to non-treated ALS and WT mice. $cPLA_2$ protein expression was much lower in spinal cord and brain stem of AS treated mice in comparison to ALS mice or sense treated mice. The AS treatment prevented the reduction in the number of the neurons (detected by NeuN) and inhibited astrocyte (detected by GFAP) and microglia activation (detected by Iba1or by CD40) compared with ALS mice and sense treated mice. Thus, the $cPLA_2$ may offer an efficient target for treatment of ALS.

12 - PRE-CLINICAL EVALUATION OF A SELECTIVE INHIBITOR OF SOLUBLE TNF FOR TREATMENT OF CHRONIC NEUROINFLAMMATORY DISEASES

Maria Karamita [(1)] ***- Christopher J Barnum*** [(2)]
Ray J Tesi [(3)] ***- David Szymkowski*** [(4)]
Malu Tansey [(2)] ***- Lesley Probert*** [(1)]
Laboratory of Molecular Genetics, Hellenic Pasteur Institute, Athens, Greece [(1)] - School of Medicine, Emory University, Atlanda, United States [(2)] - FPRTbio, FPRTbio, Washington, United States [(3)] - Xencor, Xencor, Monrovia, United States [(4)]

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system

(CNS) that shows autoimmune features. In most patients it starts as a relapsing-remitting disease but with age evolves into a chronic progressive disease. While treatment of relapsing-remitting MS has improved dramatically over the last decade, the therapeutic options for progressive MS are still very limited. Tumor necrosis factor (TNF) mediates chronic inflammatory pathologies including those affecting the CNS, but non-selective TNF inhibitors exacerbate MS and TNF receptor SF1A is associated with MS, indicating beneficial effects of TNF in CNS pathology. In this study, we compared the effects of soluble (sol) and transmembrane TNF (tm) by selectively and non-selectively inhibiting TNF in the cuprizone (CPZ) model of chronic MS. In this model inflammation is compartmentalized behind an intact blood brain barrier (BBB) and disease is monitored by activation and proliferation of microglia, astrocytes and oligodendrocyte precursor cells (OPC), and focal progressive demyelination and neurodegeneration. Withdrawal of CPZ from the diet allows almost complete remyelination. In this study, mice were fed with CPZ-supplemented diet and from the third week were treated with XPro1595, a dominant-negative analogue of human TNF that selectively blocks solTNF, or etanercept which blocks both tmTNF and solTNF. Mice were sacrificed and brains were processed for neuropathological analysis and measurement of human TNF levels. Interestingly, both naïve and CPZ mice that were treated with XPro1595, showed detectable levels of dominant-negative human TNF in the brain indicating that it can access the CNS. At an early time point XPro1595-treated mice showed equal levels of demyelination, neurodegeneration and microglial activation and proliferation, as well as similar levels of OPC, as control and etanercept-treated mice. However, at later time points, where neuropathology was further increased in control and etanercept-treated mice, XPro1595-treated mice showed reduced pathology, and this was accompanied by increased OPC. Our results show that in the this model of MS, selective inhibition of solTNF ameliorates neuropathology and enhances myelin repair, at least partly by increasing the number of OPC. These therapeutic effects of XPro1595, coupled with its ability to cross the BBB, may have important implications for the treatment of chronic CNS inflammatory pathologies.

16 - ANTI-MA2 ANTIBODY ENCEPHALITIS PRESENTING AS PSYCHOSIS

Suk-Won Ahn - Jae-Han Bae - Hae-Bong Jeong
Neurology, Chung-Ang Univertsity Hospital, Seoul, Republic of Korea

Background

Anti-Ma2 antibody encephalitis is a rare autoimmune disease and a paraneoplastic syndrome related with systemic cancers. The literature includes a few case reports in which anti-Ma2 antibody encephalitis involved the brainstem and limbic system, and these patients showed neuropsychiatric manifestations including neuro-ophthalmologic abnormalities, dizziness, headache, ataxia, seizure, and drowsiness. In this report, we describe a patient with anti-Ma2 antibody encephalitis and a hidden malignancy who initially presented with acute cognitive impairment and psychosis.

Case

A 64-year-old male presented to our hospital with a 2-month history of psychiatric manifestations including memory impairment, decreased speech, depressed mood, anxiety, disorganized language, aggressiveness, and excessive sleepiness. Brain magnetic resonance imaging (MRI) showed an infiltrative mass lesion in the right medial temporal lobe suggestive of a brain tumor. A follow-up brain MRI revealed further aggravation of the right medial temporal lobe lesion and new lesions involving the left temporal lobe. Chest CT and 18F-fluorodeoxyglucose positron-emission tomography (PET)

showed an ovoid nodule in the right apex with multiple lymph nodes in the hilum and mediastinum indicating lung cancer. In addition, anti-Ma2 antibodies were detected. Based on these findings, the patient was diagnosed with anti-Ma2 antibody encephalitis and lung cancer and underwent a right upper lung lobectomy.

Conclusion

This unusual case course serves to remind clinicians that patients who present a first-episode of rapidly progressing cognitive impairment and psychosis of an uncertain origin should undergo brain MRI, and the possibility of anti-Ma2 antibody encephalitis and hidden malignancies should be considered.

20 - DMF TREATMENT IN A B-CELL DEPENDENT EAE MODEL

Sarah Traffehn [1] – *Imke Metz* [1]
Wolfgang Brück [1] – *Martin S. Weber* [1, 2]
Department of Neuropathology, University Medical Center, Georg August University, Göttingen, Germany [1]
Department of Neurology, University Medical Center, Georg August University, Göttingen, Germany [2]

B cells are increasingly recognized as key players in the pathogenesis and progression of Multiple Sclerosis (MS). While earlier concepts focused on the pathogenic contribution of plasma cell-secreted antibodies possibly enhancing CNS demyelination, emerging evidence suggest that cellular B cell function, such as presentation of antigen and provision of pro-inflammatory cytokines may be equally important. Recently approved dimethylfumarate (DMF) substantially reduces MRI activity and relapse frequency in MS patients. While clinical benefit is associated with a decline of peripheral lymphocytes, the exact and possibly pleiotropic mechanism of action of DMF is currently evolving. We investigated whether DMF treatment modulates pathogenic B cell function in experimental autoimmune encephalomyelitis (EAE), an animal model of MS.

Methods

EAE was induced in female C57BL/6 mice by immunization with recombinant mouse myelin oligodendrocyte glycoprotein (rMOG) 1-117, a model in which B cells contribute in a pathogenic manner. 15 mg/kg body weight of DMF or methylcellulose (control) was fed twice a day by oral gavage. Treatment started either at day 0 post immunization (prevention) or when mice developed an EAE score ≥ 2. EAE onset and severity was scored daily. In representative mice, B cells were evaluated for expression of MHC-II, CD80, CD86, CD69, CD25, GL-7 and CD95 by FACS.

Results

Preventive DMF treatment did not significantly ameliorate the clinical disease course and was associated with an incline of anti-MOG ab serum levels. This result could be associated with the observation that preventive treated mice developed an overall less severe EAE compared to mice that were treated only after EAE onset.
Therapeutic DMF treatment significantly ameliorated disease severity in chronic EAE. This clinical effect was associated with substantial alterations in the expression of costimulatory molecules while expression of activation markers were largely unaffected.

Conclusion

Our data indicate that preventive treatment with DMF could be less effective as it may influences onset and severity of EAE. In contrast, therapeutic DMF treatment of established B cell-dependent EAE could significantly improve the disease course. This clinical benefit of DMF in CNS autoimmune disease is associated with pleiotropic immunological effects. Our data indicate that selective interference with B cell antigen presentation to T cells may represent a novel mechanism of action of DMF.

23 - THE NEUROTROPHIC FACTOR NEURTURIN PATHWAY IN AIRWAY IMMUNE CELLS

Marion Mauffray - Olivia Domingues
Jacques Zimmer - Tatiana Michel
Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg, Luxembourg

Neurturin (NTN) was previously described as a potent molecule for protecting against neuronal degeneration and this neurotrophic factor has also an anti-inflammatory function as it is able to reduce the LPS-induced expression of proinflammatory cytokines by microglia.
Recent studies suggest that neurotrophic factors can also be produced by immune cells and then regulate immune functions and participate in the pathogenesis of many features and symptoms of asthma.
We focus on the relationship between neurogenic and immune airway inflammation using models of acute and chronic allergic airway inflammation induced by the allergens HDM and ovalbumin. Our study of neurturin (NTN)-/- mice, in a context of airway inflammation, demonstrates an essential role of NTN in the control of epithelial and immune cells like DC and CD4+ T cells through the down-regulation of the Th2 responses. Furthermore, NTN-/- mice have significantly increased indicators of airway remodeling compared to WT mice.
Administration of NTN before challenge with the allergen ovalbumin partially rescued the phenotype of the NTN-/- mice. We demonstrated also that NTN has the capacity to decrease the level of IL-6 *in vitro* and thus plays a direct role as anti-inflammatory molecule (Michel et al., J Immunol, 2011, Mauffray et al., J Immunol, 2015). Finally, the pathway activated by NTN might go through the regulation of the transcription factor ATF3. Our work points out that NTN is not only involved in neuronal pathways but also in the immune system.

27 - CLINICAL CHARACTERISTICS AND TREATMENT RESPONSE OF PERIPHERAL NEUROPATHY WITH EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS (CHURG-STRAUSS SYNDROME): A SINGLE TERTIARY CENTER EXPERIENCE

Hye-Jin Cho [1] - Sehyo Yune [2] - Jin Myoung Seok [1]
Eun Bin Cho [1] - Ju-Hong Min [1] - Yeon Lim Seo [3]
Byung-Jae Lee [2] - Byoung Joon Kim [1]
Dong-Chull Choi [2]
Department of Neurology, Samsung medical center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic Of [1] - Department of Medicine, Samsung medical center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic Of [2] - Department of Pathology, Samsung medical center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea [3]

Background
Churg-Strauss syndrome (CSS) is a rare systemic small-vessel vasculitis accompanied by asthma, eosinophilia, and eosinophilic inflammation of various tissues including peripheral nerve. This study was done to describe the clinical course and their long-term outcomes of peripheral neuropathy in CSS.

Methods
Seventy-one physician-diagnosed CSS patients were identified at Samsung medical center between January 1995 and April 2014. About 86% of patients (n=61) were followed for more than 1 year. All patients with peripheral neuropathy received early combination treatment with corticosteroid and intravenous cyclophosphamide pulse therapy for 6 to 15 cycles. Demographic data, clinical features, laboratory and pathologic findings, treatments and outcomes were reviewed.

Results
Among the 61 patients, 46 (75%) had peripheral

neuropathy as initial manifestation of CSS. The mean follow-up duration of the neuropathic patients was 6.4 (1.2-18.8) years. The mean neurological functional disability scale before and after combination treatment with corticosteroid and cyclophosphamide was 2.43±0.86 and 0.54±0.95 ($p<0.001$), respectively. The peripheral neuropathy relapsed in only one patient after treatment.

Conclusion

The long-term clinical outcome of peripheral neuropathy in CSS patients with early corticosteroid and cyclophosphamide combination therapy was favorable with very low relapse rate.

33 - TSPO-PET IMAGING REVEALS THAT ANTI-VLA-4 TREATMENT LEADS TO REDUCED MICROGLIAL ACTIVATION IN FOCAL RODENT EAE

Susanne Vainio [(1)] ***- Alex Dickens*** [(2)] ***- Jouni Tuisku*** [(3)] ***Olli Eskola*** [(4)] ***- Merja Haapranta-Solin*** [(1)] ***Olof Solin*** [(4)] ***Daniel Anthony*** [(5)] ***- Juha Rinne*** [(3)] ***Laura Airas*** [(6)]

Turku PET Centre, Preclinical Imaging Laboratory, Turku University Hospital, Turku, Finland [(1)] - Johns Hopkins School of Medicine, Johns Hopkins, Baltimore, United States [(2)] - Turku PET Centre, Turku University Hospital, Turku, Finland [(3)] - Turku PET Centre, Radiopharmaceutical Chemistry laboratory, Turku University Hospital, Turku, Finland [(4)] - Department of Pharmacology, University of Oxford, Oxford, United Kingdom [(5)] - Division of Clinical Neurosciences, Turku University Hospital, Turku, Finland [(6)]

Background

Anti-VLA-4 is one of the most effective treatments for multiple sclerosis (MS) (Hutchinson et al., 2009). The anti-VLA-4 monoclonal antibody (mAb) binds to the α4-chain of $\alpha 4\beta 1$-integrins, also called very late activating antigen4 (VLA-4), which are expressed on all leukocytes excluding neutrophils. It inhibits leukocyte binding to vascular cell adhesion molecules (VCAM-1) and fibronectin and, thus, inhibits lymphocyte infiltration into tissues (Vosoughi and Freedman, 2010).The aim of this study was to evaluate the effect of anti-VLA-4 treatment on microglial activation in type 1 focal EAE rodent model of MS using PET imaging and [^{18}F]-GE-180, which is a radioligand that binds to the 18 kDa translocator protein expressed on activated microglia.

Methods

Focal EAE was induced by a stereotaxic injection of BCG into the striatum, and by subsequent peripheral activation of the lesion using complete Freund's adjuvant. Subcutaneous treatment with anti-VLA-4 mAb was initiated 30 days after the immunisation, and continued for 14 days in 4 rats. Control rats (n=4) were treated with an isotype matched non-binding control antibody. The animals were imaged *in vivo* using [18F]-GE-180 and positron emission tomography (PET) in the beginning and after 14 days of the treatment to evaluate the effect of the drug on lesion development. The results were confirmed by immunohistochemistry by staining the microglia with anti-Iba-1 mAb.

Results

In vivo PET imaging demonstrated a significant beneficial effect of the treatment, which was detected by reduced binding of the radioligand [^{18}F]-GE-180, and interpreted as a significant reduction in microglial activation following treatment. The lesion-to-contra ratio binding was reduced after 14 days of treatment when compared to the control group ($p = 0{,}036$). Analysis of histology confirmed the reduction of microglial activity in the treated animals compared to the control animals.

Conclusions

Our study demonstrates that anti-VLA-4 treatment reduces the inflammatory reaction and microglial activation in the focal EAE model. Importantly, this can be measured using *in vivo* PET imaging with novel second generation TSPO radioligands.

44 - NEURO-INFLAMMATORY AND ANXIETY PROFILES ARE REDUCED FOLLOWING INGESTION OF A SECOND GENERATION PREBIOTIC B-GOS: APPLICATION TO NEUROPSYCHIATRIC DISORDERS

Helene M Savignac [1] - Yvonne Couch [2]
David M Bannerman [3] - George Tzortzis [4]
Daniel C Anthony [2] - Philip WJ Burnet [5]
Clasado Research Services Ltd, University of Oxford, Reading - Oxford, United Kingdom [1] - Department of Pharmacology, University of Oxford, Oxford, United Kingdom [2] - Department of Experimental Psychology, University of Oxford, Oxford, United Kingdom [3]
Clasado Research Services Ltd, Reading University Campus, Reading, United Kingdom [4] - Department of Psychiatry, University of Oxford, Oxford, United Kingdom [5]

Introduction

Inflammation is increasingly linked to neuropsychiatric disorders, such as schizophrenia, anxiety, depression, multiple sclerosis, Alzheimer's and Parkinson's diseases [1, 2, 3]. These disorders are also increasingly related to disturbances of the intestinal microbiota and the brain-gut-microbiota axis [4, 5, 6], to which the immune system is a part of. Notably, the gut microbiota is currently thought to condition the proper development of the immune system and to help shaping brain functions, as evidenced by germ-free mice which display underdeveloped brain and immune system [7]. Conversely, modulation of the enteric microbiota notably via certain probiotic treatments has recently yielded positive results against some psychiatric disorders [8]. The second generation prebiotic galacto-oligosaccharide mixture (B-GOS), shown to modulate gut bifidobacteria, immune functions and gut health [9], was also recently demonstrated to modulate brain expression of key molecules involved in neuropsychiatric disorders and to decrease anxiety and cortisol levels in humans [10, 11]. Thus, we investigated here the effects of B-GOS on neuro-immuno processes and its potential in reducing anxiety induced by inflammation.

Material and Methods

B-GOS was administered to adult male CD1 mice via their drinking water for 3 weeks; control mice received normal water. After this, in the morning, mice received an acute intra-peritoneal injection of the inflammation-inducer lipopolysaccharides (LPS) or saline. After resting in their home cage for 6 hours, the locomotor activity of the animals was assessed as a cue of sickness behaviour, during 2 hours and this was followed 1 hour later by marble burying testing. The following morning, 24 hours post-LPS or saline injection, animals were tested in the light-dark box for inflammation-induced anxiety behaviour. As B-GOS modulates inflammatory cytokines and N-methyl-D-aspartate receptor (NMDAR) expression in the brain, as a clue of neuroinflammation, plasma cytokines levels and brain cytokines and NMDAR subunits were measured.

Results

B-GOS restored LPS-induced drop in locomotor activity to the levels of control animals (i.e. B-GOS-saline and water- saline mice). However, this effect was not observed in the marble burying test as B-GOS-LPS mice displayed the same reduction in burying behaviour as water-LPS mice, compared to both saline control groups. Importantly, B-GOS abolished the increased anxiety observed in water-LPS mice in the light/dark box. In the brain, B-GOS also reduced the LPS-induced increase in pro-inflammatory cytokines, to levels similar to those of both saline control groups. Regarding NMDAR subunits, the expression of NR2B subunit only was increased by LPS and this effect was attenuated by BGOS ingestion.

Discussion

B-GOS reduced sickness behaviour as assessed by locomotor activity measures, but not by the marble burying test and reduced post-inflammation anxi-

ety. These changes were paralleled by a decrease in specific cytokines and NR2B subunits, suggesting that NMDAR and specifically NR2B are key mediators in brain inflammation. Noteworthy, it is not possible to rule out that the data obtained in the light-dark box may be related to locomotor activity. However, these data add to the recent findings that B-GOS can alter brain functions and reduce anxiety in humans and shows, in addition to its effects on gut and immune health, that B-GOS can modulate many components of the brain-gut-microbiota axis. This has strong implications for all neuropsychiatric disorders that are related to immune dysfunctions. Further studies are now warranted to identify better both the mechanisms behind B-GOS effects and the full spectrum of its potential applications for neuropsychiatric disorders.

(1) Takeda S et al., 2014. Systemic inflammation, blood-brain barrier vulnerability and cognitive/ non-cognitive symptoms in Alzheimer disease: relevance to pathogenesis and therapy. Front Aging Neurosci. 29;6:171.
(2) Al-Amin MM and Reza HM, 2014. Neuroinflammation: Contemporary anti-inflammatory treatment approaches. Neurosciences (Riyadh). 19(2):87-92.
(3) Amor S et al., 2014. Inflammation in neurodegenerative diseases--an update. Immunology. 142(2):151-66.
(4) Scheperjans F et al., Mar 2015. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord. 30(3):350-8. doi: 10.1002/mds.26069.
(5) Hornig M, 2013. The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness. Curr Opin Rheumatol. 25(4):488-795
(6) Fetissov SO and Déchelotte P, 2011. The new link between gut-brain axis and neuropsychiatric disorders. Curr Opin Clin Nutr Metab Care. 14(5):477-82
(7) Borre YE et al., 2014. The impact of microbiota on brain and behavior: mechanisms & therapeutic potential. Adv Exp Med Biol. 817:373-403.
(8) Dinan TG et al., 2013. Psychobiotics: a novel class of psychotropic. Biol Psychiatry. 15;74(10):720-6.
(9) Tzortzis G, 2009. Development and functional properties of Bimuno®: a seond-generation prebiotic mixture. Food Science and echnology Bulletin: Functional Foods. 6 (7) 81-89.
(10) Savignac HM et al., 2013. Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. Neurochem Int. 63(8):756-64.
(11) Schmidt K et al., 2014. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology (Berl). DOI 10.1007/s00213-014-3810-0.

50 - EFFECCTOR MEMORY CD4+ T CELLS ARE ASSOCIATED WITH COGNITIVE PERFORMANCE IN A SENIOR POPULATION

Cláudia Serre-Miranda - Susana Roque Nadine Santos - Carlos Portugal-Nunes Patrício Costa - Joana Palha - Nuno Sousa - Margarida Correia-Neves
Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

Introduction

Longevity is increasing worldwide in part as a result of great improvements of public health and health care. Although with marked inter-individual differences, ageing is associated with a gradual decline in cognitive functions. Thus, the identification of factors that might delay cognition decline, promoting a healthy aging, is an increasingly relevant challenge. In addition to the cognitive alterations, the immune system also progressively changes with age. Recent data on the interplay between cognition and the immune system led to the cur-

rent vision that the brain rather then being an immune privileged organ, enjoys the privilege of being regulated by the immune system. The immune system has been shown to play modulatory functions in several brain functions, namely cognition.

Objective
Immunosenescence and cognitive decline are common markers of the aging process. Taking into consideration the heterogeneity observed in cognitive decline in aging and the now recognized link between lymphocytes and cognition, we herein explored the association between alterations in lymphocytic populations and cognitive performance parameters.

Methods
In a cohort of cognitively healthy adults (n=114), previously characterized by diverse neurocognitive/psychological performance patterns, detailed peripheral blood immunophenotyping of both the innate and adaptive immune systems was performed by flow cytometry.

Results
Better cognitive performance was associated with lower numbers of effector memory CD4+ T cells and higher numbers of naive CD8+ T cells and B cells. Furthermore, effector memory CD4+ T cells were found to be predictors of general and executive function and memory, even when factors known to influence cognitive performance in older individuals (e.g., age, gender, education and mood) were taken into account.

Conclusions
This is the first study in humans associating specific phenotypes of the immune system with distinct cognitive performance in healthy aging.

52 - CAN NATALIZUMAB TREATMENT IMPAIR NK CELLS DEFENSE AGAINST MELANOMA?

Ilaria Gandoglia [1] - Alice Laroni [1] - Federico Ivaldi [1] - Eric Armentani [1] - Paolo Carrega [2] Guido Ferlazzo [3] - Gianluigi Mancardi [1] Nicole Kerlero de Rosbo [1] - Antonio Uccelli [1]
Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genoa, Italy [1] Laboratory of Clinical and Experimental Immunology, Giannina Gaslini Institute, Genoa, Italy [2] - Laboratory of Immunology and Biotherapy, Department of Human Pathology, University of Messina, Messina, Italy [3]

Objectives
A possible association between Natalizumab (NTZ), an anti- CD49d antibody approved as treatment of multiple sclerosis (MS), and higher risk of melanoma is debated. As NK cells (NK), which contribute to controlling melanoma development, express CD49d, our objective was to assess if NK-mediated killing of melanoma cells and melanoma-driven NK migration are influenced upon NTZ exposure.

Methods
PBMC were extracted by density Ficoll. Polyclonal NK cells (bulk NK) were expanded from sorted NK cultured on irradiated feeder cells in presence of IL-2 and PHA. Expression of CD49d and its receptor CD106 by freshly isolated and bulk NK was assessed by FACS. Primary melanoma cell lines were established from biopsy samples. Degranulation of NK in presence of melanoma cells was measured by FACS with anti-CD107a antibody. Migration of NK towards melanoma cells was quantified in a overnight cell-permeable transwell assay.

Results
CD49d and CD106 were similarly expressed on freshly isolated or bulk NK from healthy donors (HD) and their expression did not differ between HD and

untreated MS patients. Both ligand and receptor were expressed on melanoma cells. Upon exposure to NTZ, CD49d expression on bulk HD NK decreased, but their cytotoxicity towards melanoma cells was not affected, either when NTZ was added to bulk NK/melanoma co-cultures or when bulk NK were pre-exposed to NTZ for 12 hours. Some new preliminary results showed that when bulk NK and melanoma cells were pre-exposed to NTZ for 48 hours the NK cytotoxicity was decreased. CD49d expression by NK was decreased in NTZ-treated MS patients and such a decrease persisted upon in-vitro expansion. These expanded cells were as cytotoxic for melanoma cells as NK from untreated patients or HD. In the migration assay, melanoma cells, in the lower chamber, can attract NK freshly isolated cells from upper chamber during a migration time of 12 hours. Preliminary data show that NTZ exposure of HD NK and of melanoma cells during all the time of migration assay (12 hours) can influence the migration rate.

Discussion

Preliminary data show that the exposure to NTZ for a long time can influence in vitro cross-talk between NK and melanoma cells through a semipermeable barrier. Altogether, these data suggest that a long NTZ incubation of NK bulk can influence their cytotoxicity. Our ongoing studies will investigate if in treated patients NTZ could affect NK cytotoxicity or their migration towards melanoma cells.

Author Index