DAREMUS
Dansk Selskab for Forskning i Multipel Sklerose

Nationalt MS Forskningsmøde 2017

Videnskabeligt symposium afholdes den 8. marts 2017 kl. 10.00-17.00

***Scandic Hotel*, Vester Søgade 6, 1601 København**

Kære kolleger

Nedenfor findes det endelige program med præsentation af nyeste resultater inden for dansk forskning i multipel sklerose.

Program

09.30-10.00	**Registrering og kaffe**
10.00-10.10	**Velkommen ved Annette Bang Oturai** (formand for DAREMUS). Lokale: **Grandball**
10.10-11.00	**DAREMUS-lecture** (Steven Goldman) Chair: Finn Sellebjerg.
11.00-12.00	**Session 1: Nominerede projekter til *DAREMUS-prisen* for MS forskere under 45 år** Chair: Annette Bang Oturai. Lokale: **Grandball**
11.00-11.15	Nellie Martin: Molecular profiling of damage and repair in the CSF
11.15-11.30	Eva Rosa Petersen: Smoking affects the interferon-beta treatment response in multiple sclerosis
11.30-11.45	Jeppe Romme: Progressive multiple sclerosis - CSF biomarkers responsive to treatment and compartmentalized inflammation
11.45-12.00	Morten Riemenschneider: Muscle fatigability in persons with multiple sclerosis – preliminary data
12.00-12.45	**Frokost**
12.45-13.00	**Uddeling af *DAREMUS-prisen* for årets bedste danske videnskabelige projekt.** Lokale: **Grandball**

Mødet er sponsoreret af
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Danish Multiple Sclerosis Center

13.00-14.30	**Parallelsession 1:** Behandlingsalgoritmer og behandlingsmål Chair: Per Soelberg Sørensen. Lokale: **Grandball**
13.00-13.30	Behandling af attakvis MS, Per Soelberg Sørensen
13.30-14.00	Behandling af progressiv MS, Helle Hvilsted Nielsen
14.00-14.30	Autolog hæmatopoietisk stamcelletransplantation, Morten Blinkenberg og Finn Sellebjerg
13.00-14.30	**Parallelsession 2**: Eksperimentel forskning Chair: Shohreh Issazadeh-Navikas. Lokale: **Christiansborg**
13.00-13.30	Keynote talk: Trevor Owens
13.30-13.42	Katrine Tækker Jensen: Post-transkriptionel regulering af tumor nekrose faktor og andre mikroglia-producerede cytokiner
13.42-13.54	Marlene Mørch: Localization of antibody deposition by sterile injury in the brain
13.54-14.06	Reza Khorooshi: The role of Angiotensin AT2-receptor stimulation and IL-10 signaling in an animal model of neuromyelitis optica spectrum disorder
14.06-14.18	Vian Wais: The role of CNS-endogenousNOD2 and TLR9 in neuroinflammatory disease in mice
14.18-14.30	Yawei Liu: Neuronal IFN-beta–induced PI3K/Akt-FoxA1 signaling is essential for generation of FoxA1+Treg cells
14.30-15.00	Kaffe
15.00-17.06	**Parallelsession 1**: Klinik og epidemiologi Chair: Jette Frederiksen. Lokale: **Grandball**
15.00-15.30	Keynote talk: Melinda Magyari
15.30-15.42	Magnus Boesen: Pediatric acute demyelinating encephalomyelitis in Denmark: A nationwide population-based study
15.42-15.54	Nils Koch-Henriksen: Excess mortality among multiple sclerosis patients in Denmark has dropped significantly over the past six decades
15.54-16.06	Tobias Sejbæk: Tolerabilitet og adhærens ved behandling med dimethylfumarate hos patienter med multiple sklerose
16.06-16.18	Anna Olsson: Influence of environmental factors on bone mineral density and trabecular bone score in Danish MS patients
16.18-16.30	Anne Katrine Bisgaard: The role of neutrophile-to-lymphocyte ratio in multiple sclerosis and optic neuritis

Fortsættes

15.00-17.06	**Parallelsession 1**: Klinik og epidemiologi
	Fortsat
16.30-16.42	Jette Frederiksen: Neural Cell Adhesion Molecules in acute Optic Neuritis: Relation to clinical and paraclinical findings
16.42-16.54	Jacob Callesen: Reliability of the Six Spot Step Test
16.54-17.06	Henrik Lundell: Characterization of axonal microstructure and transmission in MS with combined 7T MRI and electrophysiology
15.00-17:06	**Parallelsession 2**: Immunologi og sygdomsmekanismer
	Chair: Bente Finsen. Lokale: **Christiansborg**
15.00-15.30	Keynote talk: Zsolt Illes
15.30-15.42	Marie Louise Elkjær: Transcriptome profiling of brain lesion evolution in MS
15.42-15.54	Carsten Tue Berg: Influence of Type I IFN signalling on anti-MOG-mediated demyelination
15.54-16.06	Simone Hjæresen: En reduktion af Macrophage Migration Inhibitory factor (MIF) kan være skadelig for regenerationen af CNS efter et MS attack
16.06-16.18	Jacob Talbot: Bone marrow derived mesenchymal stem cells suppress Th1 and enhance Th17 differentiation of antigen activated CD4+ T cells in vitro
16.18-16.30	Marina von Essen: Discovering a new pathogenic CD20+ T cell population implicated in multiple sclerosis
16.30-16.42	Kerstin Soelberg: MRI findings in patients in patients with acute optic neuritis. A prospective study
16.42-16.54	Nasrin Asgari: Aquaporin-4-autoimmunity in patients with systemic lupus erythematosus: A predominantly population-based study
16.54-17.06	Anouk Benmamar-Badel: Neonatal microglia transplantation ameliorates EAE

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Title: Molecular profiling of damage and repair in the CSF

Authors: Martin NA[a], Nawrocki A[a,b], Larsen MR[b], Molnar V[c], Sellebjerg F[d], Alcaraz N[e], Baumbach J[e], Illes Z[a],
[a]Department of Neurology, OUH; [b]Department of Biochemistry and Molecular Biology, SDU; [c]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary; [d]Department of Neurology, Rigshospital, CU; [e]Computational Biology Group, Department of Mathematics and Computer Science, SDU

Hypothesis: Comparing the transcriptome of MS lesions to transcriptome of experimental de- and remyelination relate gene expression to damage and repair. Quantifying proteins of these homologous genes in the CSF proteome may identify novel biomarkers.
Methods: De- and remyelination was induced in the cuprizone mouse model. Lesions were examined by 4x44K Agilent Whole Mouse Genome Microarray. Expression of proteins was examined by immunohistochemistry. Proteomics was done by mass spectrometry (LC-MS/MS), and parallel reaction monitoring (PRM). Cytokines were examined by Mesoscale array.
Results: 644 differentially expressed genes related to de- and remyelination were identified, and compared to the transcriptome of MS lesions: 91 overlapping homologous genes were found. Presence of their corresponding proteins was examined by LC-MS/MS in pooled CSF of 10 controls and 10 patients with PPMS, RRMS and SPMS, respectively. We could detect 20 of the 91 proteins, and selected additional 32 out of the 91 proteins based on biological function. To quantify these 52 proteins, we designed 2-3 peptides representing each 52 protein: for peptide design, we explored brain, blood and CSF proteome databases. We quantitatively screened the 132 peptides by PRM in 98 individual CSF samples of 30 PPMS, 27 SPMS and 41 RRMS. Peptides of 4 proteins (Axl, TIMP1, ApoC-II, beta-2-microglobulin) were quantitatively different, and had predicting potentials to differentiate between MS subgroups. Gene expression of these 4 proteins indicated up-regulation during acute remyelination in the mouse brain; immunohistochemistry indicated TIMP1 expression by oligodendrocyte precursor cells, while Axl and ApoCII by macrophages/microglia within demyelinating lesions. ApoC II reactivity was more pronounced during acute remyelination. Correlation analysis between protein and cytokine levels in the CSF is under way.
Discussion: This proof of concept study using translational multiomics identified a number of genes in MS lesions that may be related to de- and remyelination. Protein products of 4 genes could be differentially detected in the CSF proteome, and quantified as potential biomarker. The 4 genes were upregulated in acute remyelinating lesions, and their protein was expressed by oligodendrocytes and microglia. *(Supported by Scleroseforeningen, Lundbeckfonden, Region of Southern Denmark).*

Title: Smoking affects the interferon-beta treatment response in multiple sclerosis

Authors: Petersen ER, Oturai AB, Koch-Henriksen N, Magyari M, Sørensen PS, Sellebjerg F, Søndergaard HB

Objective
To investigate whether smoking before and during treatment with interferon-beta (IFN-beta) is associated with the relapse rate, and whether there is an interaction between smoking and *HLA-DRB1*15:01*, *HLA-A*02:01* and the *NAT1* variant rs7388368A previously reported to interact with smoking in determining multiple sclerosis (MS) susceptibility.

Methods
DNA from IFN-beta-treated relapsing-remitting (RR)MS patients from the Danish Multiple Sclerosis Biobank was extracted for genotyping of single nucleotide polymorphism (SNP)s for *HLA-DRB1*15:01* and *HLA-A*02:01* alleles and the *NAT1* rs7388368A variant. Information about relapses from two years before treatment start to either treatment stop or to the last follow-up visit was obtained from the Danish Multiple Sclerosis Treatment Register and smoking information from a comprehensive questionnaire.

Results
995 relapsing-remitting MS (RRMS) patients were included and evaluated before and during IFN-beta treatment. During IFN-beta treatment, smoking was significantly associated with the treatment response: smokers compared to non-smokers with an incidence rate ratio (IRR) of 1.19 (95% CI: 1.009-1.407, p=0. 039) and smoking intensity with an IRR of 1.27 (95% CI: 1.051-1.535, p=0. 013) per one additional pack of cigarettes per day. Analysis of the two years before treatment start showed no association between smoking and the number of relapses. No *HLA* association or interaction between smoking, the *NAT1* variant or disease activity was found during treatment.

Conclusion
Smoking is associated with increased disease activity in RRMS patients treated with IFN-beta. No *HLA* association was found and no interaction was observed between smoking, the *NAT1* gene variant and disease activity. Our results confirm that lifestyle factors are important in MS.

Title: Progressive multiple sclerosis - CSF biomarkers responsive to treatment and compartmentalized inflammation

Authors: Jeppe Romme Christensen, Mika Komori, Marina Rode von Essen, Rikke Ratzer, Lars Börnsen, Bibi Bielekova, Finn Sellebjerg

Hypothesis: The first positive progressive multiple sclerosis (MS) phase 3 trials have recently been presented, however the clinical effects were moderate. Thus, an emerging question is whether the modest effect on clinical outcomes in progressive MS are explained by continued neurodegeneration independent of inflammation or continued residual inflammation with associated neurodegeneration. However, biomarkers reflecting the complex pathology of progressive MS, including low-grade and compartmentalized intrathecal inflammation, are lacking. Cerebrospinal fluid (CSF) biomarker studies have demonstrated high sensitivity for intrathecal inflammation by soluble CD27 (sCD27, a marker of T cell inflammation). We hypothesized that the high sensitivity of CSF sCD27 would translate into responsiveness to anti-inflammatory treatment and sensitivity for residual inflammation after treatment.

Methods: Baseline and week 60 follow-up CSF samples from progressive MS patients from two open-label trials of natalizumab or methylprednisolone treatment were included. CSF concentrations of soluble surface markers, a panel of chemokines and cytokines, and neurofilament light chain (NFL, marker of axonal damage) and myelin basic protein (MBP, marker of demyelination) were analysed by electrochemiluminescent and ELISA assays.

Results: Natalizumab treatment (N=17) significantly reduced CSF concentrations of sCD27, sCD21, IL10, IL12p40, TNF-alpha and CXCL10. Methylprednisolone treatment (N=23) significantly reduced sCD27, sCD21 and IL12p40. For both treatments, CSF sCD27 showed the most significant decreases and superior signal-to-noise ratios for change during treatment, but remained above reference levels for healthy donors. Correlation analyses of CSF markers of inflammation and axonal damage in baseline samples (N=52), change during treatment (N=39) and in post-treatment samples (natalizumab or methylprednisolone) revealed sCD27 as the only CSF inflammatory biomarker consistently showing strong correlation with NFL concentrations.

Discussion: Treatment of progressive MS with natalizumab or methylprednisolone results in significant decreases of CSF inflammatory markers, and validates CSF sCD27 as a highly sensitive biomarker of intrathecal inflammation which captures residual inflammation after anti-inflammatory treatment. Importantly, CSF sCD27 consistently correlates with axonal damage, emphasizing that intrathecal inflammation are associated with tissue damage also after anti-inflammatory treatment. These findings support the concept of compartmentalized inflammation in progressive MS and the use of CSF sCD27 in small phase 2 trials for identifying drug candidates for progressive MS.

Title: Muscle fatigability in persons with multiple sclerosis – preliminary data.

Authors: M. Riemenschneider[1], T. Kjølhede[1], K. Overgaard[1], Lars G. Hvid[1], Egon Stenager[2,3], U. Dalgas[1].

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3: Institute of Regional Health Research, University of Southern Denmark, Odense, Denmark

Objective and hypothesis: The purpose of this study is to 1) compare fatigability of m. quadriceps induced by either isokinetic or isometric muscle contractions in persons with multiple sclerosis (pwMS) and in healthy controls, and 2) evaluate the potential involvement of central and peripheral mechanisms. It was hypothesized that muscle fatigability would be greater in pwMS after both types of fatiguing contraction protocols when compared to healthy controls, and furthermore that this difference would be due to excessive central fatigability in pwMS.

Methods: 20 MS patients and 10 HC subjects had their m. quadriceps maximal strength and voluntary muscular activation determined, along with muscle fatigability during maximal isometric and isokinetic contraction protocols. Fatigability is expressed as the relative decline in torque, whereas peripheral- and central fatigability was determined by the twitch-interpolation technique.

Results: Our preliminary data showed that muscle fatigability was greater in pwMS than HC after the isokinetic protocol (31 vs. 20 %, $p<0.05$), and tended to be greater after the isometric protocol (63 vs. 54 %, $p=0.07$). Additionally, muscle fatigability was greater after the isometric protocol than the isokinetic protocol ($p< 0.05$ in both groups). Central fatigability tended to be higher in pwMS than in HC (isokinetic: 13 vs. 1 %, $p=0.13$ and isometric: 38 vs. 22 %, $p=0.08$). No significant differences in peripheral fatigability was observed.

Discussion: This is the first study to investigate muscle fatigability in pwMS in lower extremities during both isometric and isokinetic contractions. Muscle fatigability is more pronounced in pwMS when compared to HC. A plausible explanation to this difference is the higher level of central fatigability observed in pwMS. Although, these findings are preliminary they emphasize the importance of physical rehabilitation as physical exercise has been shown to improve muscular functionality.

Titel: Post-transkriptionel regulering af tumor nekrose faktor og andre mikroglia-producerede cytokiner.

Forfattere: Jensen KT[1], Nielsen HH[2], Kjems J[3], Finsen B[1]. [1]Neurobiologisk Forskning, Institut for Molekylær Medicin, Syddansk Universitet, [2]Neurologisk Afdeling, Odense Universitetshospital, [3]Institut for Molekylærbiologi og Genetik, Interdisciplinary Nanoscience Center iNANO, Aarhus Universitet.

Hypotese: Multipel sklerose (MS) associeres ofte med tilstedeværelsen af autoimmune, myelinspecifikke T celler, som forårsager demyelinering og aksonal skade. Der er dog beviser for, at disse celler også kan have fordelagtige egenskaber og fremme regenerative processer så som remyelinering. I en musemodel for MS har vi vist, at interferon gamma (IFN-γ) og interleukin (IL)-17-producerende, myelinspecifikke T celler stimulerer translationen af transkriptet for det pro-inflammatoriske cytokin tumor nekrose faktor (TNF) i hjernens mikroglia. Hypotesen er, at dette foregår gennem op- eller nedregulering af specifikke, ikke-kodende RNA molekyler betegnet cirkulær RNA (circRNA), lange ikke-kodende RNA (lncRNA) og mikroRNA (miRNA), som er kendt for at være involveret i post-transkriptionel genregulering.

Metoder: Udtrykket af TNF undersøges i IFN-γ og IL-17-stimulerede (0-100ng/ml) primære mikrogliakulturer etableret fra p0-p3 C57BL/6 mus. mRNA og protein måles hhv. ved brug af q-PCR og ELISA. RNA sekvensering anvendes til at analysere mikroglias udtryk af circRNA/lncRNA/miRNA, og prøverne udgør mikroglia stimuleret med dén koncentration af IFN-γ/IL-17, som resulterer i den højeste TNF koncentration. Via bioinformatik udvælges en gruppe af circRNA/lncRNA/miRNA kandidater, og deres cellulære ekspression undersøges vha. *in situ* hybridisering i 1) en musemodel, der kombinerer adoptiv T celle transfer med aksonal læsion af perforant pathway og 2) i vævsprøver fra afdøde MS patienter. Ved brug af *in situ* hybridisering og immunhistokemiske farvninger korreleres udtrykket af circRNA/lncRNA/miRNA med tilstede-værelsen af myelinspecifikke T celler, mikroglia og udtrykket af TNF.

Resultater: Foreløbige resultater viser, at stimulering med 10ng/ml IFN-γ resulterer i den højeste mikrogliale produktion af TNF mRNA og protein, hvorimod lave koncentrationer af IL-17 (0-10ng/ml) ikke viser nogen effekt. Effekten af højere koncentrationer (10-100ng/ml) af både IFN-γ og IL-17 undersøges i øjeblikket.

Diskussion: Ikke-kodende RNA'er er potentielt gode terapeutiske targets, som kan være cellespecifikke, og en forbedret viden om deres regulering af mikroglial cytokinproduktion kan vise sig betydningsfuld for udviklingen af nye og bedre behandlingsmuligheder for patienter med MS.

TIlte: Localization of antibody deposition by sterile injury in the brain

Authors: Marlene Thorsen Mørch, Sofie Forsberg Sørensen, Reza Khorooshi, Nasrin Asgari and Trevor Owens

Hypothesis: Sterile injury in the brain determines localization of antibody deposition

Method: A sterile injury was created in the brain of female C57Bl/6 mice by stereotactic injection. At 1, 2, 4 or 7 days post injury mice received an intrathecal injection into the cerebrospinal fluid (CSF) via cisterna magna of 300µg of immunoglobulin-G (IgG) either from neuromyelitis optica patients (NMO-IgG) or from healthy donors. To determine where antibodies were localized, brain sections from perfused mice were evaluated for human IgG deposition 1 day after intrathecal injection. NMO-like pathology was evaluated in mice that received NMO-IgG with complement by measuring loss of aquaporin 4 and glial fibrillary acidic protein staining, and deposition of complement.

Results: Significant deposition of IgGs was observed at the sterile injury, compared to low level non-specific deposition elsewhere. The level of deposition at the injury site peaked transiently at 1 day, with low level deposition still present 7 days post injury. Complement-dependent astrocyte pathology was observed at the injury site but only in mice receiving NMO-IgG.

Discussion: Injury-directed distribution of antibodies from the CSF may have relevance to autoimmune etiology and pathology, as well as responses to inflammation and in repair. . Encounter of autoantibodies with specific antigen can lead to hypersensitivity reactions and pathology. Antibodies in the CSF and their distribution are of interest in the context of multiple sclerosis and NMO, as IgG antibodies are present in CSF as well as in pathological lesions of both diseases.
This study demonstrates that a prior injury can influence IgG trafficking in CSF. The molecular mechanism behind site-specific deposition and its dependence on time after injury is still being elucidated. Together these findings suggest that prior, possible subclinical events represent an important basis for patterns of disease in the CNS.

Titel: The role of Angiotensin AT2-receptor stimulation and IL-10 signaling in an animal model of neuromyelitis optica spectrum disorder

Authors: Reza Khorooshi, Emil Ulrikkaholm Tofte-Hansen, Camilla Hermansen, Roser Montanana Rosell, Hannah Liska Limberg, Nasrin Asgari, Ulrike Muscha Steckelings and Trevor Owens

Hypothesis: Angiotensin AT2-receptor (AT2R) stimulation plays a protective role in neuromyelitis optica spectrum disorder (NMOSD)-like pathology via induction of interleukin 10 (IL-10).

Methods: NMOSD-like pathology was induced in wild type, AT2R - and IL-10 deficient mice by intracerebral co-injection of immunoglobulin G (IgG) purified from an NMOSD patient and human complement. Treatment consisted of intracerebral co-injection with the AT2R agonist Compound 21 (C21) at day 0 followed by intrathecal administration at day 2. Mice were sacrificed at day 4, and NMOSD-like pathology was evaluated by quantification of astrocyte damage.

Results: Intracerebral injection of patient derived-IgG and human complement produced NMOSD-like pathology, which was significantly attenuated by treatment with C21. The protective effect of AT2R stimulation was absent in mice lacking AT2R, confirming that the observed effect was elicited through AT2R. The stimulation of AT2R by central administration of C21 led to induction of IL-10 in the central nervous system (CNS). This finding suggested the involvement of IL-10 in the therapeutic effect of C21. Histopathology revealed exacerbated NMOSD-like pathology in IL-10 deficient mice, indicating a potential protective role of IL-10 signaling in NMOSD. However, treatment with AT2R agonist reduced pathology in IL-10 deficient mice with similar effect to that observed in wild type mice. This indicates that the protective effect of AT2R stimulation was independent of IL-10.

Discussion: NMOSD is a primary astrocyte disease of the CNS. IgG antibody specific for the astrocyte water channel aquaporin 4 is identified as a biomarker for NMOSD and is thought to initiate astrocyte pathology. Currently, there is no cure for NMOSD. AT2R is proposed to play a protective role in CNS diseases that may involve induction of the anti-inflammatory cytokine IL-10. This study shows that both AT2R stimulation and IL-10 signaling can play a protective role in NMOSD. Our findings identify AT2R as a new potential target in NMOSD therapy and IL-10 as an important cytokine that regulates pathology in NMOSD.

Title: The role of CNS-endogenous NOD2 and TLR9 in neuroinflammatory disease in mice

Authors: Reza Khorooshi, Vian Wais, Ruthe Storgaard Dieu, Gill Webster, Trevor Owens

Hypothesis: Synergistic interaction between NOD2 and TLR9 within the CNS induces type I IFN and suppresses EAE

Methods: Microparticle immune stimulator (MIS) 416 comprises ligands for innate receptors NOD2 and TLR9. Ligands for NOD2 (MDP) and TLR9 (CpG) were used to test our hypothesis. MIS416 and CpG were administrated into the cerebrospinal fluid of mice and CNS tissue was analyzed by RT-qPCR for immunomodulators. Cellular sources of IFNβ were investigated in IFNβ-yellow fluorescent protein reporter mice. Myeloid cell infiltration in CNS was determined by flow cytometry. To study the effect of MIS416 or CpG on EAE, these were intrathecally injected at the onset of symptoms.

Results: MIS416 and CpG both triggered IFNβ induction in the CNS as well as IFNγ, IRF7, CCL2, CXCL10, iNOS, IL-6 and IL-10. Both ligands induced CNS infiltration of $CD45^+$ myeloid cells. Some $CD45^+$ extraparenchymal myeloid cells were cellular sources of IFNβ. Furthermore, injection of fluorescent-tagged MIS416 showed co-localization with $CD45^+$ myeloid cells as well as parenchymal $Iba1^+$ cells, suggesting these cells produce IFNβ as a response to MIS416 phagotosis. Treatment of EAE with MIS416 or CpG inhibited disease progression, this did not occur in IFNAR1-deficient mice. This suggests that CNS-endogenous induction of IFNβ is one mechanism by which MIS416 and CpG act to suppress MS-like disease in mice.

Discussion: Our findings support a general model whereby innate immune stimulation can be triggered to suppress inflammation. Previous work supporting this concept showed that stimulating an innate TLR3-driven Type I IFN response in CNS of mice with EAE inhibited disease progression[1]. Our findings here extend this to the TLR9 ligand CpG and to a TLR9+NOD2 bispecific particulate construct MIS416, which is currently in phase 2b clinical trial for secondary progressive multiple sclerosis. Intra-CNS effect has not been studied nor has a role for Type I IFN been shown before. By studying the effect of innate ligands individually and in synergy we will obtain a better understanding of CNS homeostasis and its regulation for therapy.

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[1] Khorooshi, R., et al., *Induction of endogenous Type I interferon within the central nervous system plays a protective role in experimental autoimmune encephalomyelitis.* Acta Neuropathol, 2015.

Title: Neuronal IFN-beta–induced PI3K/Akt-FoxA1 signaling is essential for generation of $FoxA1^{+}T_{reg}$ cells

Authors: Yawei Liu[1], Andrea Marin[1], Patrick Ejlerskov[1], Louise Munk Rasmussen[1], Marco Prinz[2] & Shohreh Issazadeh-Navikas[1,*]

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Neurons reprogram encephalitogenic T cells ($T_{(enc)}$) to become regulatory $T_{(reg)}$ cells either $FoxP3^{+}T_{regs}$ or $FoxA1^{+}T_{regs}$. We reported previously that neuronal ability to generate $FoxA1^{+}T_{regs}$ was central to preventing neuroinflammation in experimental autoimmune encephalomyelitis. Mice lacking the cytokine interferon (IFN)β were defective in generating $FoxA1^{+}T_{regs}$ in the brain. Neuron-induced $FoxA1^{+}T_{regs}$ were capable of preventing chronic and demyelinating EAE in mice lacking IFNβ. Here we show that lack of neuronal IFNβ-signaling was associated with lack of neuronal expression of program death-ligand1 (PDL1), which also prevented their ability to reprogram T_{enc} cells to $FoxA1^{+}T_{regs}$. Transfer of IFNβ competent encephalitogenic T cells to mice lacking IFNβ or its receptor; IFNAR in the brain (Nes^{Cre}:$Ifnar^{fl/fl}$) led to the absence of $FoxA1^{+}T_{regs}$-generation and aggravated neuroinflammation. We identified that IFNβ activated neuronal PI3K/Akt signaling. Phosphorylated Akt consequently bound to transcription factor FoxA1, which upon translocation to the nucleus induced neuronal PDL1 expression. Conversely, inhibition of PI3K/Akt, or FoxA1 and PDL1 knock-down blocked neuronal ability to generate $FoxA1^{+}T_{regs}$. Our study identified crucial molecular players central for neuronal ability to reprogram pathogenic T-cells and to generate $FoxA1^{+}T_{regs}$, which could be a therapeutic target to prevent neuroinflammation.

Title: Pediatric acute demyelinating encephalomyelitis in Denmark: a nationwide population-based study

Authors: Magnus Spangsberg Boesen (1,2), Melinda Magyari (2,3), Morten Blinkenberg (3), Nils Koch-Henriksen (2,4), Peter Vilhelm Uldall (1), Alfred Peter Born (1).

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2. The Danish Multiple Sclerosis Registry, Department of Neurology, Rigshospitalet, University of Copenhagen
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4. Department of Clinical Epidemiology, University of Aarhus, Clinical Institute, Aarhus

Background: We assessed the consequences of the consensus criteria of acute demyelinating encephalomyelitis (ADEM) proposed by the International Pediatric Multiple Sclerosis Study Group (IPMSSG), in particular regarding disease progression to MS.

Methods: Data were sourced from the National Patient Register, providing potential cases of ADEM during 2008–2015. This led to the following diagnostic codes (ICD-10 in brackets): optic neuritis (H46), transverse myelitis (G37.3), neuromyelitis optica (G36.0), and other acute disseminated demyelination (G04.0, G36, G36.8, G36.9, G37.8, G37.9). All medical records were reviewed to validate the register-based diagnoses and to collect clinical and paraclinical data.

Results: We identified 49 cases of pediatric ADEM (incidence rate 0.51 per 100,000 person-years) who all had an abnormal MRI. However, only 17 (35%) of these patients fulfilled the IPMSSG criteria regarding encephalopathy and polyfocal neurological deficits. The latter group was similar in all aspects, except for a younger age at onset and a trend towards a worse clinical outcome. After a mean follow-up time of 4.6 years (range 0.2–8.3 years) 31% had still clinical sequelae from the initial ADEM; one child with non-encephalopathic ADEM had progressed to MS; one child with encephalopathic ADEM developed radiological MS, but no further clinical events; none had relapse of ADEM.

Conclusion: Danish pediatricians primarily diagnose ADEM based on an MRI suggestive of ADEM. Accordingly, applying the IPMSSG consensus criteria regarding encephalopathy and polyfocal neurological deficits may considerably underestimate pediatric ADEM.

Title: Excess mortality among multiple sclerosis patients in Denmark has dropped significantly over the past six decades

Authors: Nils Koch-Henriksen[1,2], Bjarne Laursen[2,3], Egon Stenager[2,4] Melinda Magyari[2,5]

[1]Clinical Institute, Department of Clinical Epidemiology, University of Aarhus, Aarhus, Denmark, [2]The Danish Multiple Sclerosis Registry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [3]National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark, [4]Institute of Regional Health Research, University of Southern Denmark, Odense, Denmark.

[5]Danish Multiple Sclerosis Research Centre, Department of Neurology, Neuroscience Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

Background: Numerous studies have shown reduced life time expectancies among people with MS, but few studies have had sufficient follow-up or sufficient number of patients to assess if survival has improved with time. However, a recently published meta-analysis found no time-dependent change in MS excess mortality across studies over recent decades.

Objective: To investigate whether short-term all-cause excess mortality in MS patients in the total Danish population has changed over the last six decades.

Patients and methods: All patients recorded in the nationwide Danish MS Registry with definite or probable MS and onset from 1950 through 1999 were included. Date of death and vital status were provided by the Danish Civil Registration System with follow-up in 2015. Sex-, age-, and calendar-year specific population mortality was supplied by Statistics Denmark. We used life table methods, and excess mortality was calculated as excess death rate (EDR), *i.e.* excess number of deaths per 1000 person years, and standardized mortality ratio (SMR), *i.e.* observed divided by expected number of deaths.

Results: We included 18,847 patients. Within the follow-up period 6,102 patients had died as opposed to 2,492 expected deaths giving a SMR of 2.45 (95% CI 2.39-2.51), and EDR was 10.63 (95% CI 10.19 - 11.09). Median time from onset to death was 35.0 years (matched population: 49.1 years) and median age at death was 69.1 years (matched population 80.2 years). The 15-year EDR dropped gradually from 11.29 (95% CI: 9.95-12.73) in the 1950-1959-onset cohort to 2.56 (95% CI: 1.98-3.18) in the 1990-1999 onset cohort. The corresponding numbers for SMR was 4.48 (95% CI: 4.06-4.92) to 1.80 (95% CI: 1.62-1.99).

Conclusion: The decline in excess mortality in MS patients may have several causes. Notably, the decline started decades before disease modifying treatment of MS became available, before use of MRI became widespread, and before the McDonald criteria were introduced. A change in the natural history of MS with fewer malignant cases may be a significant contributor.

Titel: Tolerabilitet og adhærens ved behandling med dimethylfumarate hos patienter med multiple sklerose

Forfattere: Tobias Sejbæk[1,2], Mads Nybo[3], Thor Petersen[4] Zsolt Illes[1,2]

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Hypotese: Postmarketing data på tolerabilitet og adhærens ift. nye orale MS behandlinger er fortsat begrænset. Lav komplians er associeret med højere attakrate og flere sygehuskontakter. Studiet gennemgår forskelle i tolerabilitet og adhærens hos dimethylfumarate (DMF) behandlede MS patienter i Aarhus (Klinik 1) og Odense (Klinik 2).

Metode: Retrospektivt tværsnitsstudie med inklusion af alle patienter påbegyndt DMF i perioden marts 2014 til november 2015 (n=253, Klinik 1=103 og Klinik 2=150). Dataindsamling inkluderer demografiske data, bivirkningsprofiler, blodprøveresultater og behandlingshistorik. Lymfocytters biologisk variation er beregnet jf. International Council for Standardization in Haematology.

Resultater: Den gennemsnitlige behandlingslængde for patienter som ophørte behandling var 140.5±114.4 dage i Klinik 1 og 305.3±186.3 dage i Klinik 2 ($p<0.001$). Generelt ophørte flere patienter i behandling i Klinik 1 sammenlignet med Klinik 2 medførerende en Odds Ratio (OR) for diskontinuation på 3.56 (95% CI: 2.0-6.3, $p<0.001$) i Klinik 1 sammenlignet med Klinik 2. OR for diskontinuation på baggrund af en bivirkning (attak og graviditet ekskluderet) var 6.13 (95% CI: 3.0-12.7, $p<0.001$) i Klinik 1 sammenlignet med Klinik 2. Behandlingsnaive patienter havde en OR på 11.24 (95 % CI 2.51-50.42 $p<0.01$) for diskontinuation i Klinik 1 sammenlignet med Klinik 2. OR for diskontinuation hos patienter tidligere behandlet med immunologisk medicin var 4.56 (95 % CI 2.15-9.68 $p<0.0001$) i Klinik 1 sammenlignet med Klinik 2. Patienter i begge klinikker som tidligere var behandlet med 2 disease modifying treatments (DMTs) eller 3 DMTs havde en risiko for diskontinuation på henholdsvis 49.2% ($p<0.001$) og 70.4% ($p<0.0001$) sammenlignet med behandlingsnaive patienter. Ingen patienter i Klinik 1 udviklede grad III lymfopeni, 2.4 % udviklede grad III lymfopeni i Klinik 2.

Diskussion: Studiet viser store forskelle i tolerabilitet imellem skleroseklinikkerne. OR for discontinuation var 3.56 når man sammenlignede Klinik 1 og 2. OR for diskontinuation pga. bivirkninger (attakker og graviditet ekskluderet) var 6.13. Forskellene imellem klinik 1 og 2 var størst ved sammenligning af behandlingsnaive patienter. Patienter som tidligere var behandlet med én DMT tolererede bedre DMF end behandlingsnaive patienter. Risikoen for diskontinuation stiger jo flere præparater patienten tidligere er behandlet med. Kliniske vurdering af lymfopeni bør foretages ud fra repetitive analyser grundet biologisk varians.

Title: Influence of environmental factors on bone mineral density and trabecular bone score in Danish MS patients

Authors: Anna Olsson[1], Helle Bach Søndergaard[1], Finn Sellebjerg[1]

Peter Sandor Oturai[2], Annette Bang Oturai[1].

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Background: MS patients are at increased risk of reduced bone mineral density (BMD) and fractures. To date, the etiology of bone loss in MS is unclear. As we previously reported (*Olsson et al. MS Journal 2015)*, age, body mass index (BMI) and disease severity were the principal factors associated with reduced BMD in our cohort. Trabecular bone score (TBS) is a recently developed analytical tool that provides a measurement of the three-dimensional bone microarchitecture. Decreased TBS predicts an increased fracture risk independently of BMD. To date, no studies have investigated the TBS in MS patients.

Objectives: To assess bone quality in MS patients by TBS and to evaluate potential risk factors that may affect BMD and TBS in patients with MS.

Methods: 260 patients from the Danish MS Center were included. Data on BMD - measured by dual x-ray absorptiometry (DXA) in the period 2012 to 2013 – were previously published. The antero-posterior spine images from these scans were reanalyzed and TBS was calculated using the TBS iNsight software (MediMaps®). T-test and regression analyses were performed with information on smoking, alcohol, sun exposure, physical activity, diet, BMI at year 20, and vitamin D supplements - all obtained from questionnaires.

Results: The TBS values were not significantly different from those of an age-matched reference population. Low TBS was associated with high age ($p<0.001$) and high EDSS ($p=0.021$). Low BMD in both lumbar spine ($p=0.016$) and femur ($p<0.005$) was associated with low BMI at 20 years of age. When dichotomized into *never smokers* and *ever smokers*, lower BMD in lumbar spine was found in the group of ever smokers ($p=0.018$). Patients reporting regular physical activity between age 15-19 years had higher BMD in lumbar spine than patients reporting inactivity or occasional activity in this period ($p=0.021$).

Discussion: Reduced TBS was not prevalent in patients with MS, suggesting that BMD alone, and not the bone microarchitecture, is affected in patients with MS. As in the background population avoiding smoking, performing regular physical activity and maintaining a normal BMI in late adolescence likely contribute to a better bone health in MS.

Title: The role of neutrophile-to-lymphocyte ratio in multiple sclerosis and optic neuritis

Authors: Anne Katrine Bisgaard, Gorm Pihl-Jensen, Jette L. Frederiksen

Hypothesis: The blood neutrophil-to-lymphocyte ratio (NLR) has recently been identified as a potential predictor of systemic inflammation in several diseases. In multiple sclerosis (MS) and optic neuritis (ON) the NLR has been found significantly higher than in healthy controls (HC) and higher for patients in relapse over patients in remission. This study evaluates the significance of NLR in the different courses of MS; relapsing-remitting MS (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS) and ON. The NLR is also measured in relation to relapse and remission and Expanded Disability Status Scale (EDSS).

Methods: A total of 382 patients suffering from RRMS (n=138), SPMS (n=30), PPMS (n=55), CIS (n=19) or ON (n=140) and 813 HC were included. Complete blood count, demographic, and clinical data from MS patients were evaluated retrospectively. The NLRs were calculated and compared for all participants by Student's t-test. Logistic regression models were constructed for EDSS ≥ 4,0 as outcome and age, gender, NLR, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), erythrocyte distribution width (ERYDRW) and disease duration as predictor variables.

Results: The NLR is significant higher (p<0.001) in MS and ON patients compared to HC. Patients in relapse had a significant higher NLR (p<0.005) than patients in remission, but no significant difference in the CRP, ESR, ERYDRW and EDSS. SPMS and PPMS patients showed no significant difference in NLR and no significant variance between RRMS and progressive MS patients were found. No significance was found between any of the predicting variables and an EDSS score ≥4.0.

Discussion: MS and ON patients have a significantly higher NLR than HC, indicating the occurrence of chronic inflammation compared to healthy persons. NLR may be measured as a marker of disease activity, because of the significantly higher NLR in patients with relapse compared to patients in remission. This needs confirmation in future trials.

Title: Neural Cell Adhesion Molecules in acute Optic Neuritis: Relation to clinical and paraclinical findings.

Authors: Jette L Frederiksen, Kiren Farooq, Sharmilee Gnanapavan

Hypothesis: Acute optic neuritis (AON) is an inflammatory condition of the optic nerve presumably of autoimmune origin. More than 50 % develop multiple sclerosis (MS). No studies have thus far investigated the levels of Neural Cell Adhesion Molecules (NCAM) in the CSF in AON to determine whether NCAM is associated with demographic and paraclinical findings suggestive of MS. AON patients were chosen to achieve a homogenous patient group in which complete diagnostic work up was performed within one month from onset.
Methods: We performed a prospective study of NCAM level in the CSF in 50 adults with AON, median age 32 (range 18 to 62) years and healthy controls. Associations between NCAM levels and age, gender, results of brain MRI at 3.0 Tesla and routinely measured biomarkers in the CSF in patients with AON were assessed.
Results: The median level of NCAM in the CSF was 348 ng/ml in AON, compared to a mean value 412 +/- 109 ng/ml in healthy controls. There was no age and gender difference. There was neither significant association between NCAM and presence of elevated leucocyte count in the CSF, nor elevated IgG index, nor presence of oligoclonal IgG bands in the CSF. No significant association was found between brain MRI and NCAM level, but patients with a normal NCAM level tended to be more likely to also have a normal brain MRI ($p=0.057$, Fisher exact test). The results showed a trend towards increased NCAM and the presence of Gadolinium enhancing lesions on brain MRI albeit not significant.
Discussion: The study showed no significant association between NCAM level in the CSF and the results of routinely measured biomarkers in the CSF and brain MRI without and with Gadolinium DTPA in 50 consecutive patients with AON. In a follow up study we will examine whether NCAM levels predict development of MS.

Title: Reliability of the Six Spot Step Test

Authors: Callesen J, Rasmussen C.L., Richter C.R., Sunesen I.L., Naesby M.C., Skjerbaek A.G.

Introduction: The Six Spot Step Test (SSST) is a complex measure of gait function developed to test people with multiple sclerosis (MS). In addition to fast walking, it also encompasses acceleration, balance and coordination. Despite increasing use in clinical practice and research, no studies have so far investigated the within- and day-to-day variability.
Purpose: This study investigated the reliability in repeated measures of the SSST test within and between days in persons with MS.

Method: 38 MS-inpatients (EDSS 1-6, age 34-76, female: 68%) underwent a SSST and 5 min. later a re-test. This procedure was repeated two days later under the same conditions. Time was measured on a handheld stopwatch. Bland-Altman analyses were used to estimate relative and absolute 95% Limits of Agreement (LOA). Sub-analyzes with stratification for gait speed and assistive devices were conducted.

Results: Within-day and between-day agreements relative to the test time were ±15% and ±19% respectively (n=38). Variation in repeated measures tended to increase as test time increased. For tests performed in less than 20 sec. (n=32), absolute LOA within days and between days were ±1.5 sec. and ±2.2 sec. respectively. In tests performed without assistive devices LOA were for both outcomes ±1.7 sec. A tendency towards a learning effect from repeated measures was observed with an estimated effect of < 0.4 sec.

Conclusion: The SSST test has an acceptable within- and day-to-day reliability, despite a potential minor learning effect. For interventional purposes a change of >19% can generally be regarded as a real change. Absolute reliability tends to depend on disability level and cautious use is recommended when assistive devices is required or when the test is performed at a very slow speed.

Title: Characterization of axonal microstructure and transmission in MS with combined 7T MRI and electrophysiology

Authors: Henrik Lundell[1], Anke Karabanov[1], Vincent Boer[1], Irina Akopian[1], Morten Blinkenberg[2], Finn Selleberg[2], Itamar Ronen[4], Esben Thade Petersen[1], Tim Dyrby[1,4] and Hartwig Roman Siebner[1,5]

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7T MRI offers new insight into the pathogenesis and structure of MS lesions in gray and white matter (1,2). The increased field strength also improves quantitative imaging methods for characterization of subtle changes in normal appearing tissue. Diffusion weighted spectroscopy (DWS), unlike diffusion tensor imaging (DTI) offers cell-specific readouts sensitive to microstructural

changes in MS (3). In this ongoing study we hypothesize that the mobility of water and the metabolite N-acetylaspartate (NAA) independently reflect demyelination and axonal damage. Furthermore, we hypothesize that those markers, detectable with DWS, will describe neural transmission latency and amplitude respectively.

We plan to recruit 15 healthy controls and 30 individuals with relapsing remitting and secondary progressive MS. All participants undergo a conventional structural 3T MRI protocol for lesion characterization, white matter tract segmentation and to evaluate the degree of spinal cord involvement (4). An extensive electrophysiological examination with transcranial magnetic stimulation (TMS) is performed with focus on the transmission in corticospinal and transcallosal pathways. Finally, our DWS assessment of cell specific metabolite mobility borrows new principles from state of the art diffusion MRI techniques and is performed on the national danish 7T MRI scanner (5, 6).

Our preliminary results obtained in healthy individuals suggest that our improved 7T DWS methods allow faster and more accurate measurements of intra-axonal integrity throughout white matter.

Improved neuroimaging tools will be needed to understand the reciprocal causality between tissue de/re-generation and functional impairment and adaptation. We anticipate that our integrative approach will shed light on some basic relationships between function and structure in MS.

This work is kindly supported by The Danish Sclerosis Foundation and by a Sapere Aude Grant from the Danish Research Council for Independent Research – Production and Technology.

Title: Transcriptome profiling of brain lesion evolution in MS

Authors: Elkjaer ML[1], Burton M[2], Martin N[1], Reynolds R[3], Kruse T[2], Baumbach J[4], Thomassen M[2], Illes Z[1]
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Hypothesis: We hypothesize that (i) lesion evolution in the MS brain can be characterized by specific transcriptome signature that can be used for stage-specific biomarker discovery; (ii) persisting endogenous and exogenous viruses are activated in the brain and maintain inflammation.
Methods: We characterized 75 lesions from brain of 10 patients with secondary progressive MS (SPMS) for cellular changes (i.e. microglia/macrophage activity), myelin integrity and phagocytosis of myelin proteins by using standard histology and immunohistochemistry. The lesions covered the full spectrum of lesions evolution: normal-appearing white matter (NAWM), active, chronic active, inactive, and repairing lesions. As controls, we chose 25 white matter (WM) areas from five brains without neurological disease. We microdissected the selected lesions and extracted the RNA. Next-generation RNA sequencing was performed by using paired end sequencing of 2x80 bases on Illumina's NextSeq500/550. The transcriptome assembly of the RNA reads was done by using reference sequences from Ensembl and alignment program from TopHat2. We analyzed the data by using HT-seq, a edgeR package, and KEGG pathway enrichment.
Results: From the preliminary data comparing the chronic active MS lesions and the white matter control areas, 1301 significantly differentially expressed genes and 62 significantly differentially regulated pathways were found. The most changed genes belong to the immunoglobulin family. Other differentially expressed genes and pathways confirmed the known key factors playing a role in MS such as the presence of the $CD8^+$ T cells and $CD20^+$ B cells, oxidative stress markers, Ca^{2+}/Na^+-induced K-channels and metabolic pathways. Differentially expressed genes involved in degeneration and cell death/survival were also found, such as growth factors and components for axonal regeneration. Known HERV sequences were not found in the chronic active lesion transcriptome, but repetitive sequences may suggest novel retroviral repeats. We also found upregulated genes belonging to viral carcinogenesis pathways.
Discussion: Our data support lesion type specific transcriptome signature. We use *de novo* network enrichment analysis to identify new genes, and apply a novel software designed to detect cryptic virus-host fusion and low-abundance transcripts to investigate the viral theories of MS.

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Title: Influence of Type I IFN signalling on anti-MOG-mediated demyelination

Authors: Carsten Tue Berg[1], Reza M. H. Khorooshi[1], Nasrin Asgari[1,2], Trevor Owens[1],

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Hypothesis: Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Although considered a T cell-mediated disease, many MS lesions show deposition of antibodies and activated complement. Myelin oligodendrocyte glycoprotein (MOG) is a candidate autoantigen in MS. Although antibodies with specificity for MOG are implicated in some forms of MS and related diseases, the pathogenic importance of anti-MOG antibody in demyelinating pathology remains poorly characterized. Interferon beta (IFN-beta**),** a Type I IFN, is used to treat MS and is effective against experimental autoimmune encephalomyelitis (EAE), an MS-like disease in mice. By contrast, IFN-beta is ineffective for the related disease Neuromyelitis Optica (NMO), and we have shown that Type I IFN signaling is necessary for NMO-like pathology in mice. We hypothesized that anti-MOG antibodies could induce complement-dependent demyelinating pathology, and that anti-MOG-mediated demyelinating pathology is influenced by Type I IFN signaling.

Methods: Purified IgG2a anti-MOG antibody and mouse complement were stereotactically injected into the corpus callosum of wild type and type I IFN receptor deficient mice (IFNAR1-KO) with and without pre-established EAE. Demyelination (loss of luxol fast blue staining) was quantitated using ImageJ.

Results: Anti-MOG induced complement-dependent demyelination in corpus callosum of wild type mice, without EAE, and did not occur in mice that received control IgG2a. Demyelination was similarly induced in NOD-scid mice that lack T or B cells. Deposition of activated complement coincided with demyelination, and this was significantly reduced in IFNAR1-KO mice. Co-injection of anti-MOG and complement at onset of symptoms of EAE in wild type and IFNAR1-KO mice induced a similar level of callosal demyelination.

Discussion: Anti-MOG antibody and complement was sufficient to induce callosal demyelination and pathology was dependent on type I IFN. Induction of EAE in IFNAR1-KO mice overcame the dependence on type I IFN for anti-MOG and complement mediated demyelination. The effect of Type I IFN signaling on antibody-mediated demyelinating pathology is influenced by the CNS inflammatory environment. These findings are novel, representing direct demonstration of pathogenicity of anti-MOG antibody.

Titel: En reduktion af Macrophage Migration Inhibitory factor (MIF) kan være skadelig for regenerationen af CNS efter et MS attack.

Forfattere: Simone Hjæresen[1], Åsa Fex Svenningsen[1]
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Hypotese: Macrophage Migration Inhibitory factor (MIF) samt dens nyligt identificeret bindingspartner – Serine proteasen HtrA1, kan have en central rolle for sygdomspatologien i multiple sklerose (MS).
Metoder: Koncentrationerne af MIF og HtrA1 i serum og cerebrospinalvæske (CSF) fra relapsing-remitting (RRMS) patientprøver samt raske kontroller blev undersøgt ved brug af enzyme-linked immunosorbent assay (ELISA). MIF og HtrA1s funktion i generelle regenerations processer blev undersøgt ved brug af migration- (scrath assay) og proliferations (BrdU inkoporation) assays samt western blotting.
Resultater: Vi fandt at niveauet af både MIF og HtrA1 var uændret i serum fra RRMS patienter sammenlignet med raske kontroller. Til gengæld så vi en klar signifikant forskel på koncentrationerne af begge proteiner i CSF fra RRMS patienter i forhold til de raske kontroller. Her viste vi at koncentrationen af MIF er signifikant reduceret, mens koncentrationen af HtrA1 er signifikant øget. Vi viste også en klar korrelation mellem koncentrationerne af begge proteiner i de raske kontroller i både serum og CSF. Denne korrelation var til gengæld ikke at se i CSF fra RRMS patienter, hvilket indikerer en klar dysregulering af begge proteiner i disse patienter.
Ud over dette viste vi at både MIF og HtrA1 har en gavnlig effekt for flere af de glia celle styret regenerations processer som er essentielle efter et MS attack.
Diskussion: Dette studie viste en klar dysregulering af både MIF og HtrA1 i CSF fra RRMS patienter. HtrA1 er et protein med enzymatisk aktivitet og flere af dens substrater er kendt. Disse inkluderer flere forskellige fibroblast growth factors (FGF), transforming growth factors (TGF) og ekstra cellulære matriks komponenter. Vi har tidligere vist at MIF binder HtrA1 og derved hæmmer dennes proteolytiske aktivitet. En dysregulering af bindingsforholdet for disse proteiner kan derfor være essentielt for tilgængeligheden af vækstfaktorer som kan være afgørende under både de- og regeneration. Den direkte såvel som den indirekte effekt af MIF på generelle glia celle processer som er fundamentale for regenerationen indikerer ligeledes en central rolle for dette bindingspar i MS sygdomspatologien.

Title: Bone marrow derived mesenchymal stem cells suppress Th1 and enhance Th17 differentiation of antigen activated $CD4^+$ T cells in vitro

Authors: Jacob L. Talbot,[1] Lars Börnsen,[4] Marina R. Von Essen,[1] Anne Fisher-Nielsen,[3] Morten Blinkenberg,[2] and Finn Sellebjerg[1]

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Introduction: Ongoing clinical trials have yet to show whether mesenchymal stem cells (MSCs) have a place in the treatment of MS.
MSCs modulate immune cell proliferation, activation and differentiation. While MSCs have been found to inhibit proliferation and Th1 differentiation of activated CD4+ T-cells, the impact of MSCs on Th17 differentiation is less understood.
Hypothesis: Whether MSCs enhance or suppress the proliferation of immune cell responses depends on the specific activating antigens and which T helper cell response they induce.
Materials/methods: Peripheral blood mononuclear cells (PBMCs) from 6 healthy individuals were incubated with bone-marrow(BM)-MSCs from one healthy donor and one multiple sclerosis (MS) patient using PBMC:MSC ratios ranging from 100:1 to 10:1. PBMCs were stimulated with 6 different proteins known to induce different $CD4^+$ T cell responses. After incubation CXCR3- and CCR6-expression was assessed on proliferating $CD4^+$ T-cells by flow cytometry. Furthermore, $CD4^+$ T cells incubated with BM-MSCs were analyzed by intracellular staining of cytokines representing different T helper cell pathways.
Results: BM-MSC significantly suppressed the proliferation of VZV specific CD4+ T cells but significantly increased the proliferation of candida albicans specific CD4+ T cells. We observed reduced Th1- and enhanced Th17-like chemokine receptor expression correlating with the ratio of PBMC to MSCs in all assays. No differences were observed between BM-MSCs from the MS patient and the healthy donor. Intracellular analysis showed increased expression of Th17 cytokines and decreased expression of Th1 cytokines in response to MSCs.
Discussion: Our results show that the modulation of immune cell responses by BM-MSCs is not suppressive in an absolute manner. Whether BM-MSCs induce or suppress CD4+ T cell proliferation depends on the distinct T helper cell response induced by the specific antigen. Irrespective of antigen, MSCs seem to suppress Th1 responses and enhance Th17 responses in CD4 T cells.

Title: Discovering a new pathogenic CD20+ T cell population implicated in multiple sclerosis

Authors: Marina Rode von Essen, Cecilie Ammitzbøll, Eva Rosa Petersen, Finn Sellebjerg

Hypothesis: Recently a subgroup of T cells expressing the B cell marker CD20 was discovered. Until now, the strong efficacy of anti-CD20 antibody therapy (e.g. Retuximab, Ocrelizumab, Ofatumumab) in reducing disease progression in patients with multiple sclerosis (MS) has been attributed to the depletion of B cells. New studies have shown that anti-CD20 antibody therapies also targets CD20+ T cells and it is therefore very likely that depletion of CD20+ T cells also contribute to the treatment effect. This raises the question to what end these newly discovered T cells function. With this study, we therefore wish to describe the phenotype and effector function of CD20+ T cells and to investigate if CD20+ T cells play a role in the pathogenesis of MS. We hypothesize that CD20+ T cells are pro-inflammatory T cells implicated in the pathogenesis of MS.

Methods: To characterize the phenotype and function of CD20+ T cells and to study the prevalence of CD20+ T cells in the blood and cerebrospinal fluid (CSF) from patients with MS flow cytometri analysis was performed.

Results: Preliminary data showed that blood CD20+ T cells express both chemokine receptors and adhesion molecules known to direct T cells from the blood to the CSF. Furthermore, CD20+ T cells express large amounts of death receptor molecules on the cell surface known to induce apoptosis in the cell itself or in nearby cells. CD20+ T cells was also shown to produce pro-inflammatory cytokines (e.g. IL-17, IFN-γ) even without prior cell stimulation. Lastly, we found that CD20+ T cells were enriched in the CSF from patients with MS compared to the prevalence in the blood.

Discussion: Our early data suggest that CD20+ T cells are highly inflammatory Th17-like T cells with the ability to migrate to the CNS. In accordance, we observed an increased prevalence of CD20+ T cells in the CSF of patients with MS. Altogether, these findings suggest that CD20+ T cells are implicated in the pathogenesis of MS and substantiate the possibility that depletion of these T cells in patients treated with anti-CD20 antibody therapies contributes to the positive treatment effect.

Title: MRI findings in patients with acute optic neuritis. A prospective study

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Hypothesis: The presence of brain MRI abnormalities at the time of the first acute optic neuritis (ON) may be a relative risk factor for multiple sclerosis (MS).

Methods: A prospective cohort study of patients with idiopathic ON including acute ON evaluation with a one year follow-up was performed in the Region of Southern Denmark from 2014-2016. Patients were seen in a diagnostic investigation including clinical examination and MRI. On MRI, the optic nerves were divided into the following three segments: intra-orbital, canalicular (optic foramen back to chiasm) and chiasmal (chiasm + optic tracts) for lesion characterization. The MRIs were evaluated by a neuroradiologist, who was masked to clinical and serological information.

Results: Sixty-three patients were evaluated for ON and 52 were included in the study. Thirty-one had an MRI evaluation of brain including orbit at a single episode of acute ON. Median time between onset of symptoms and MRI was 22 days (range 3-55 days). The median age at onset was 40 years (range 17-66 years). Twenty-nine patients had unilateral and two had bilateral ON. Signal abnormalities on MRI of the optic nerve were demonstrated in 81 % (25/31) of the patients. The optic nerve lesions had intraorbital location for 48 % (12/25), canalicular for 8 % (2/25) and combined intraorbital and canalicular for 44% (11/25). Of these 84 % (21/25) had brain MRI abnormalities, 40 % (10/25) of whom met the diagnostic criteria for MS at the acute ON episode. A total of 80 % (8/10) of the MS-patients had optic nerve lesions located intraorbitally.

Discussion: ON is an inflammatory optic neuropathy that causes acute vision loss. MRI in the acute phase may help to confirm the diagnosis as well as exclude alternative diagnoses. In the present study, we observed that inflammation of the optic nerve was demonstrated by MRI in the majority of patients. Brain abnormalities in ON occurred frequently in association with MS, suggesting that brain MRI in the acute phase of a single ON can facilitate the diagnosis of MS.

Title: Aquaporin-4-autoimmunity in patients with systemic lupus erythematosus: A predominantly population-based study

Authors: Asgari N[1,2], Jarius S[3], Laustrup H[4], Skejoe HPB[5], Lillevang ST[6], Weinshenker BG[7], Voss A[4]

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Hypothesis: Co-existence between neuromyelitis optica spectrum disease (NMOSD) and Systemic Lupus Erythematosus (SLE) putatively suggests susceptibility to antibody-mediated autoimmune disease.

Methods: The study included a predominantly population-based cohort with clinical and serological investigations of 208 patients with SLE, followed prospectively since 1995. All patients received immunosuppressive treatment. NMOSD was evaluated retrospectively based on the 2015 IPND criteria. Polymorphisms in programmed death 1 (PDCD-1) PD-1.3 G/A were genotyped. Determination of aquaporin-4 (AQP4)-IgG and other autoantibodies including myelin oligodendrocyte glycoprotein (MOG) was performed blinded to clinical diagnosis.

Results: Of 208 patients with SLE 45 (22%) had neuropsychiatric (NP) SLE, and central nervous system involvement predominated in 30/45 (67%). Serum AQP4-IgG was detected in two of 30 (6.7%) NPSLE patients both of whom had myelitis and antiphospholipid syndrome; one patient also had myasthenia gravis. None had MOG-IgG. PD-1.3A allele was not associated with SLE nor NPSLE.

Dicussion: In the present study, we estimated the prevalence of NMOSD in SLE in a predominantly population-based approach and in addition investigated the potential immunogenetic background. Our data suggest that AQP4-IgG autoimmune syndrome may rarely coexist with SLE and such patients have other NMOSD-typical syndromes such as myelitis. Immunosuppressive treatment used in most patients with SLE may have influenced our results.

Title: Neonatal microglia transplantation ameliorates EAE

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Hypothesis: Microglia are the resident immune macrophages of the central nervous system (CNS), which are involved in surveillance of the brain in physiological conditions as well as in pathological contexts. In the last few years, microglia have been suggested to also be key players during neurodevelopment. Our group evidenced that neonatal microglia are distinct from adult microglia and exhibit a specific gene expression pattern including neuroectodermal markers and proliferative factors. Moreover, we showed that a subset of microglia expressing the dendritic cell marker CD11c predominate in the neonatal brain and have a role in survival, migration and differentiation of neurons, oligodendrocytes and astrocytes. On the contrary, adult microglia in the context of symptomatic experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis have an inflammatory profile. We hypothesize that the neuroprotective profile of neonatal microglia, potentially through the subset expressing CD11c, could help ameliorate EAE after transplantation.

Methods: We use magnetic-activated cell sorting (MACS) against CD11b to isolate neonatal microglia or fluorescence-activated cell sorting (FACS) to isolate CD11c+ and CD11c- microglia before injecting them in the cisterna magna of mice affected by symptomatic EAE at the onset of disease. We perform histology staining on spinal cord tissue collected from these mice to evidence myelinqtio and infiltration.

Results: We show that transplantation of neonatal microglia, whether CD11c+ or CD11c-, in the brain of mice with symptomatic EAE results in stopping progression of EAE symptoms and even reduction of symptoms. We highlight that injection of neonatal microglia in the context of symptomatic EAE leads to reduced demyelination and infiltration.

Discussion: These findings highlight neonatal microglia as neuroprotective in the context of EAE, with no peculiar contribution of CD11c expressing microglia, in spite of their unique expressioj profile. Our results suggest that neonatal microglia may have remyelinating and anti-inflammatory effects in the context of EAE, opening new avenues for multiple sclerosis therapy.