INTRODUCTION

It is a pleasure to present the annual report from The Danish Multiple Sclerosis Research Centre, Department of Neurology, Neuroscience Center Copenhagen University Hospital, Rigshospitalet.
The Danish Multiple Sclerosis Research Centre is composed of the MS Clinic and the MS Research Unit.
The Danish Multiple Sclerosis Research Centre constitutes one of the "European MS Centers of Excellence".

The MS Clinic is the largest in the country with responsibility for the care of more than 1000 patients with multiple sclerosis. It is a multidisciplinary MS Clinic with the mission of providing total care for patients with multiple sclerosis. An important activity is clinical research with emphasis on development of new therapies. A number of clinical trials, both national and international have been initiated and managed from the MS Clinic, and, in addition, the clinic offers the possibility for patients to take part in clinical controlled trials sponsored by pharmaceutical companies.

The MS Research Unit including the Neuroimmunology Laboratory has had a busy year with initiation of new activities and continuation of former initiated research projects. The research embraces 3 major areas: Research in the neurogenetics of multiple sclerosis, research in the immunology of multiple sclerosis, and experimental studies.

We hope that you will enjoy reading the annual report for the year 2004.

On behalf of the Danish Multiple Sclerosis Research Centre

Mads Ravnborg Morten Blinkenberg Poul Erik Hyldgaard Jensen

Per Soelberg Sørensen

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1. ORGANISATION AND STAFF

1.1. Personnel at Copenhagen MS Clinic

Head of the MS Group:

Professor Per Soelberg Sørensen, MD, DMSc

Executive manager of the MS Clinic:

Mads Ravnborg, MD, DMSc, chief consultant

Senior physicians:

Susanne Helweg-Larsen, MD, DMSc, consultant
Morten Blinkenberg, MD, PhD, consultant
Finn Sellebjerg, MD, PhD, DMSc, registrar
Annette Oturai, MD, PhD, senior registrar
Jesper Rønager, MD, senior registrar
Ingelise Christiansen, MD, senior registrar

Junior physicians:

Jens Christian Faber-Rod, MD
Pameli Datta, MD

Psychologist:

Agnete Jønsson, chief psychologist

MS-nurses:

Anne Hansen
Merete Mogensen
Anne Sund

Physiotherapist:

Lis Albrechtsen

Medical social counselor:

Merete Møller Olsen

Secretaries:

Ruth Bartholin
Gunilla Hambe
Annette Larsen

1.2. MS Clinic

One thousand-two hundred MS patients are treated regularly in the MS Clinic. Most of these (70%) are from the H:S area. The rest comes from all over Denmark, often referred for specialized immunosuppressive treatment.
The MS Clinic is a subunit of the Neuroscience Center that was established in 1994. The clinical work is taken care of by a professor, an executive manager, four consultants, three MS nurses and three secretaries. Apart from these a neuropsychologist, a physiotherapist and a social worker are members of the MS team. Working in close relationship to these are two project nurses and a secretary whose appointments are based on external funding.
The MS Clinic is located at departments N2082 and N2084 and comprises a secretariat, a chief consultant office, two nurses-offices, two consultation rooms, a nurse consultation room, a room for intravenous infusions, and a procedure room for Baklofen pump refilling and lumbar punctures.
The yearly number of visits to the clinic is about 2700. For the regular visits the schedule allows 45 minutes. The consultation comprises a thorough and goal directed interview, a quantitative neurological examination, and a conclusion in terms of goals and efforts. The visit frequency varies from 1 - 4 times a year depending on disease activity and treatment regimen. In addition to the scheduled visits, the patients are encouraged to show up spontaneously if acute needs arise.
The primary goal of the MS clinic is to offer the best possible treatment of the disease, immunological as well as symptomatic treatments, while considering the overall impact of the disease on the patients´ lives. The disease modifying treatments used currently are interferon beta, glatiramer acetate, Methotrexat, azathioprin, and mitoxantron. More than 400 patients are treated with one or more of these drugs.
Other specialist tasks are the treatment of bladder and bowel dysfunction, neurogenic pain and spasticity. About 80 patients with severe spasticity are

treated with intrathecal Baklofen pumps. This treatment implies 4-6 controls per year for refilling and adjustments.
A secondary goal of the MS clinic is to involve as many patients as possible in clinical trials. A number of multicenter investigator-driven randomized trials are organized and directed by the Copenhagen MS Center (see below). In addition, the MS Clinic is enrolling patients in a number of clinical trials sponsored by the pharmaceutical industries. In 2004 patients from the MS Clinic have been participating in trials comparing a new immunomodulatory product, natalizumab (Antegren®/Tysabri®), in a placebo-controlled trial and in a trial in which natalizumab has been used as add-on therapy to patients already treated with interferon-beta 1a. Natalizumab is a monoclonal antibody against adhesion molecules and prevents leucocytes from entering the central nervous system. It is administered as an intravenous infusion once monthly. In another large randomized trial a new compound given orally, FTY720 (Novartis) is tested against placebo.
All documentation, ordering and planning related to clinical activities are executed in the context of the MS clinic database, a multi-user on-line facility to which all members of the MS team have access.
The MS nurses are working increasingly independent of the doctors. The nurses instruct patients and relatives in injection techniques, give infusions of immunosuppressive drugs, make ultra-sound bladder scans, instruct patients in clean intermittent self-catheterizations, and guide patients through daily life obstacles. A nurse line is open for patient counseling weekdays from 9 to 11 a.m.

1.3. Personnel at MS Research Unit and Neuroimmunology Laboratory

Head of the MS Research Group:

Professor Per Soelberg Sørensen, MD, DMSc

Senior research fellows:

Mads Ravnborg, MD, DMSc, chief consultant
Morten Blinkenberg, MD, PhD, consultant
Poul Erik Hyldgaard Jensen, MSc, DMSc, Head of Neuroimmunology Laboratory
Signe Humle Jørgensen, MSc, PhD

Agnete Jønsson, chief neuropsychologist

PhD-students and junior research fellows:

Thomas Tscherning, MD
Lars Storr, MD
Pameli Datta, MD
Bodil Petersen, MD
Martin Krakauer, MD
Jens Christian Faber-Rod, MD

Part-time research fellows:

Annette Oturai, MD, PhD
Susanne Helweg-Larsen, MD, DMSc
Finn Sellebjerg, MD, PhD, DMSc

Technical staff:

Susanne Velgaard, laboratory technician
Henriette Egeblad, laboratory technician
Rikke Kroager, laboratory technician
Vibeke Jespersen, project nurse
Joan Pietraszek, project nurse
Annette Larsen, scientific secretary

1.4. MS Research Unit

The MS Research Unit is located at Juliane Maries Vej 24 in an old villa named building 92 at the Rigshospitalet campus.
The MS Research Unit Neuroimmunology Laboratory resides in building 93, Juliane Maries Vej 20, just opposite building 92. The ground floor is shared with the Neurobiology Research Unit Experimental Laboratory and the Cardiovascular Laboratory. One room is allocated to the Neuroimmunology Laboratory and it shares another 3 rooms with the other groups mentioned above. The head of the Neuroimmunology Laboratory is Poul Erik Hyldgaard Jensen, MSc, DMSc. In the Neuroimmunology Laboratory the MS Research Unit conducts research on the neuroimmunology and biochemistry of multiple sclerosis and experimental studies using the experimental autoimmune encephalomyelitis (EAE) as a model of MS. Fur-

ther, the Neuroimmunology Laboratory offers examination of cerebrospinal fluid for oligoclonal bands employing isoelectric focusing electrophoresis. In addition the laboratory carries out measurements of antibodies against acethylcoline receptors. The laboratory also contains a bio-bank of plasma, CSF and DNA for experimental use.

Research areas

The main research areas are:

- Clinical research
- Pathogenesis of MS
- Genetics in MS
- Experimental model of MS

2. RESEARCH PROJECTS

2.1. Clinical research

2.1.1. Clinical randomized trials

Treatment of acute relapses with intravenous immunoglobulin in multiple sclerosis (TARIMS)

An investigator driven international, multicenter study initiated and organized by Copenhagen MS Center. Principal investigator is professor Per Soelberg Sørensen.

Objectives: The main objective of this study was to test the hypothesis that treatment with IVIG 1 g/kg bodyweight i.v. followed by 1 g methylprednisolone i.v. daily for 3 three days resulted in a better recovery of targeted deficits in MS-relapses compared to methylprednisolone alone.

Study design and patients: The study was a multicenter, double-blind, placebo-controlled prospective trial with parallel groups. Seventy-six patients were enrolled.

Results: The study gave only hints for a slightly better remission of acute relapses by a combined treatment of a single dose IVIG and standard therapy with methylprednisolone compared with placebo and methylprednisolone, but did not provide a statistical proof for this hypothesis. However, the relapse-rate in IVIG treated patients was reduced by more than 10%, and also the time to first relapse appeared to be prolonged when compared to placebo.

Financial support: Bayer Vital

Principal investigator: Per Soelberg Sørensen
Project group: Per Soelberg Sørensen, Mads Ravnborg, Vibeke Jespersen, Joan Pietraszek
Publication: Sorensen PS, Haas J, Sellebjerg F, Olsson T, Ravnborg M; TARIMS Study Group. IV immunoglobulins as add-on treatment to methylprednisolone for acute relapses in MS. Neurology. 2004;63(11):2028-33

Nordic trial of methylprednisolone as add-on therapy to interferon-beta for treatment of relapsing-remitting multiple sclerosis (NORMIMS)
An investigator driven international, multicenter study initiated and organized by Copenhagen MS Center. Principal investigator is professor Per Soelberg Sørensen.
Objectives: The main objective is to compare the effect of methylprednisolon given at 4 weeks intervals with the effect of placebo in patients treated with interferon-beta 1a (Rebif) who during therapy have shown clinical activity.
Study design and patients: Patients with relapsing-remitting multiple sclerosis treated with interferon-beta 1a (Rebif) for at least 1 year are eligible if they have shown clinical activity defined as at least one relapse during the last 12 months. Two treatment groups with 150 patients in each as planned. Eligible patients are randomized to treatment with methylprednisolone 200 mg daily for 5 days at 4 weeks intervals in combination with interferon-beta 1a (Rebif) or placebo in combination with interferon-beta 1a (Rebif) for 24 months. The patients will be examined clinically at six months intervals. MRI is performed before start of treatment and after 24 months.
Outcome measures: The primary outcome measure is the number of documented relapses per year, and the secondary outcome measures include the occurrence of neutralizing antibodies at 24 months, the time to a permanent increase in disability of 1 point on the EDSS, and the number of active lesions on T2 weighted MRI.
Timelines:
Start of patient enrolment: January 2004
End of patient enrolment: June 2005
End of study: June 2007
Financial support: Serono
Principal investigator: Per Soelberg Sørensen
Project group: Thomas Tscherning, Bodil Petersen, Pameli Datta, Jens Christian Faber-Rod, Anne Hansen, Per Soelberg Sørensen

A prospective multicenter, double-blind, randomized placebo-controlled parallel group trial investigating immunoglobulin G intravenously as add-on therapy to interferon-beta 1a for the treatment of relapsing-remitting multiple sclerosis (IVIMS)

An investigator driven international, multicenter study initiated and organized by Copenhagen MS Center. Principal investigator is professor Per Soelberg Sørensen.

Objectives: The primary objectives is to assess the add-on effect on intravenous immunologbulin G (IVIG) given at 30 days intervals versus placebo on the mean number of documented relapses per year in patients who prior to study entry shown clinical activity during treatment with interferon-beta 1a (Avonex).

Study design and patients: Patients with definite relapsing-remitting multiple sclerosis who during therapy for at least one year with interferon-beta 1a (Avonex) have shown clinical activity during the last year on therapy.

The study design is a placebo-controlled, randomized, parallel group trial, comprising two treatment groups with 140 subjects in each group. Eligible patients on treatment with interferon-beta 1a (Avonex) are randomized to treatment with either 0.2 g/kg intravenous immunoglobulin G as add-on therapy at 30 thirty days intervals or placebo for 24 months.

Outcome measures: The primary outcome measure is the number of documented relapses per year. Secondary outcome measures include time to confirmed progression of 1 point on the EDSS, and brain atrophy measured as the brain parenchymal fraction.

Timelines:

Start of patient enrolment: January 2004

End of patient enrolment: June 2005

End of study: June 2007

Financial support: Baxter and Biogen Idec

Principal investigator: Per Soelberg Sørensen

Study group: Per Soelberg Sørensen, Mads Ravnborg, Jens Christian Faber-Rod, Pameli Datta, Joan Pietraszek

A prospective multicenter, double-blind, randomized, parallel-group trial investigating the combination of interferon-beta 1a and monthly methylprednisolone versus interferon-beta 1a and placebo for treatment of relapsing-remitting multiple sclerosis (MECOMBIN)

An investigator driven international, multicenter study initiated and organized by Copenhagen MS Center. The study was initiated by professor Per

Soelberg Sørensen and chief physician Mads Ravnborg. Principal investigator is Mads Ravnborg.
Objectives: The primary objectives is to assess the effect of interferon-beta 1a (Avonex) in combination with mehylprednisolone given at 4 weeks intervals compared to interferon-beta 1a (Avonex) and placebo on progression of disability in patients with relapsing-remitting multiple sclerosis.
Study design and patients: Patients with definite relapsing-remitting multiple sclerosis who have not previously been treated with immunomodulatory therapy. The study is a prospective multicenter, double-blind, placebo-controlled, randomized, parallel-group trial comprising 2 treatment groups of 150 subjects in each group. Eligible patients are randomized to treatment with either interferon-beta 1a (Avonex) 30 micrograms weekly and monthly methylprednisolone 500 mg orally on 3 consecutive days for 36 months.
Outcome measures: The primary outcome measure is confirmed progression of 1 step on EDSS. Secondary outcome measures include the annual relapse rate and the lesion load on T2-weighed MRI.
Time lines:
Start of patient enrolment: Jan- 2003
End of patient enrolment: June- 2005
End of study: June- 2008
Financial support: Biogen Idec
Responsible investigator: Mads Ravnborg
Study group: Mads Ravnborg, Per Soelberg Sørensen, Morten Blinkenberg, Bodil Petersen, Joan Pietraszek
For further information visit www.mecombin.dk

A multinational, multicenter, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the effect of early glatiramer-actetate treatment in delaying the conversion to clinically definite multiple sclerosis of subjects presenting with a clinically isolated syndrome.
Objectives: to assess the effect of treatment with glatiramer acetate compared to placebo on the time to conversion to CDMS over a three-year double-blind period.
Study design and patients: patients with the first episode of focal neurological symptoms that are compatible with CNS inflammation may be recruited. The symptoms must be mono-focal which practically means optic neuritis, brainstem syndromes and spinal cord syndromes. The patients must be included within the first two months after symptom presentation,

and the patients must be on study medication within 90 days after symptom presentation.
To be included the patient should have at least two MRI lesions with a size of at least 6 mm
If the MRI shows more than 10 lesions they are offered Avonex treatment, which is approved for early treatment in patients with such MRI changes.
Outcome measure: primary endpoint is time to conversion to CDMS. Secondary endpoints are a series of MRI parameters.
Inclusion start: June 2004
Inclusion ends: Dec 2005
End of study: Dec 2008 (three year double-blind period, which is then followed by a two-year follow-up)
Sponsor Company and owner of the study: TEVA/Sanofi-Aventis
Responsible investigator: Mads Ravnborg
Study group: Morten Blinkenberg, Susanne Helweg Larsen, Jens Christian Faber Rod, Joan Pietraszek

2.1.2. Other clinical studies of immunomodulatory therapy

Long-term effect on disability of interferon-beta therapy in relapsing-remitting multiple sclerosis
A Danish Multiple Sclerosis Group (DMSG) project initiated and organized by Copenhagen MS Center.
Objectives: The main objective is to investigate whether interferon-beta treatment in relapsing-remitting patients for more than 5 years results in a delay of disease progression when compared to untreated controls.
Study design and patients: A cohort of 1774 patients with relapsing-remitting multiple sclerosis who between December 1995 and December 2001 started on treatment with interferon-beta. The duration of IFN-beta treatment is median 4,3 years (range 1 day - 9,0 years). Status for duration of clinical follow-up from the first IFN-beta treatment start: Median 4,4 years (range 2 months - 8,7 years). For 135 patients alive in Denmark the last clinical follow-up is prior to January 2003. The cohort is compared with a historical control cohort from the London Ontario Multiple Sclerosis Registry.
Outcome measures: The primary efficacy outcome measure is time to EDSS 6 and 7 respectively.
Financial support: Danish Multiple Sclerosis Society, Warwara Larsen Foundation

Responsible investigator: Bodil Petersen
Project group: Bodil Petersen, Nils Koch-Henriksen, Jette Frederiksen, Mads Ravnborg, Thomas Hansen, George Ebers, Per Soelberg Sørensen

Rebound effect of the discontinuation of interferon-beta therapy in patients with relapsing-remitting MS
A Danish Multiple Sclerosis Group (DMSG) project initiated and organized by Copenhagen MS Center.
Objectives: The main objective is to investigate whether the annual relapse rate increases in patients who discontinue therapy after at least 1 years treatment with interferon-beta.
Study design and patients: Clinical information on relapses and disability is gathered for at least 6 months following secession of interferon-beta therapy. 118 patients with ≥ 1 clinical report following IFN-beta therapy discontinuation are included. IFN-beta treatment duration: Median 2,8 years (range 1,0 - 6,6 years). Time from IFN-beta treatment discontinuation to last clinical report: Median 1,5 years (range 0,5 - 5,9 years).
Outcome measures: The primary efficacy outcome is the annualized relapse rate.
Results: Relapse rate at least 6 months after IFN-beta treatment discontinuation: Mean 0,3/year, (range 0,0 - 3,1/year) compared to relapse rate during the first year of IFN-beta treatment: Mean 0,7/year (range 0,0 - 4,0/year) and relapse rate during the last 2 years before the first IFN-beta treatment start: Mean 1,4/year (range 0,0-4/year) respectively.
Financial support: Danish Multiple Sclerosis Society, Warwara Larsen Foundation
Responsible investigator: Bodil Petersen
Project group: Bodil Petersen, Nils Koch-Henriksen, Mads Ravnborg, Per Soelberg Sørensen

2.1.3. Clinical immunology

Neutralizing antibodies against interferon-beta

Clinical importance of neutralizing antibodies
A Danish Multiple Sclerosis Group (DMSG) project initiated and organized by Copenhagen MS Center.
Objectives: The main objective of this study was to follow the development of neutralizing antibodies (NAb) during treatment with interferon-beta and to evaluate the clinical impact of NAb.

Study design and patients: We measured NAb every 12 months for up to 60 months in 541 MS patients using an anti-viral neutralization assay.
Results: During NAb-positive periods patients had significantly higher relapse rates compared to NAb-negative periods, yielding an odds ratio for relapses of 1.5. The time to first relapse was significantly increased by 244 days in NAb-negative patients. A trend was found towards faster progression in NAb-positive patients.
Financial support: Danish Multiple Sclerosis Society
Principal investigator: Per Soelberg Sørensen
Project group: Per Soelberg Sørensen, Mads Ravnborg
Publications: Ross C, Clemmesen KM, Svenson M, Sorensen PS, Koch-Henriksen N, Skovgaard GL, Bendtzen K. Immunogenicity of interferon-beta in multiple sclerosis patients: influence of preparation, dosage, dose frequency, and route of administration. Danish Multiple Sclerosis Study Group. Ann. Neurol. 2003; 48,5:706-12
Sorensen PS, Ross C, Clemmesen KM, Bendtzen K, Frederiksen JL, Jensen K, Kristensen O, Petersen T, Rasmussen S, Ravnborg M, Stenager E, Koch-Henriksen N, and the Danish Multiple Sclerosis Study Group. Clinical importance of neutralizing antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis. Lancet. 2003;362:1184-91

Biologic response to interferon-beta in patients with and without neutralizing antibodies
Objectives: The main objective of this study is to assess the in vivo biologic response to an interferon-beta injection by measuring changes in neopterin and beta2-microglobulin.
Study design and patients: Thirty-two patients have been included in the study. Neutralizing antibodies are measured using an anti-viral neutralization assay. Neopterin and beta2-microglobulin are measured with in-house assays.
Outcome measures: The primary outcome measure is the response to interferon-beta injection documented as an increase in neopterin and beta2-microglobuline. The secondary measure is the number of gadolinium enhancing lesions per scan in patients with a biologic response compared to patients without a biologic response.
Timelines: The study has been terminated and data are submitted for publication.
Principal investigator: Per Soelberg Sørensen
Project group: Per Soelberg Sørensen, Thomas Tscherning, Mads Ravnborg

The reversibility of neutralizing antibodies against interferon-beta

Objectives: The main objective of this study is to assess if neutralizing antibodies against interferon-beta developed during therapy can be reduced or removed by treatment with azathioprine and cyclic methylprednisolone.

Study design and patients: Ten patients with high concentration of neutralizing antibodies and no in vivo biologic response to interferon-beta injection are treated with azathioprine 2.5 mg/kg daily and cyclic methylprednisolone 1 g monthly for 6 months. After 6 months the NAb concentration is measured and the biologic in vivo response to interferon-beta injection is assessed by measuring the increase in blood levels of neopterin, beta2-microglobulin and MxA protein after an interferon-beta injection.

Outcome measure: The primary outcome measure is the proportion of patients with reestablishment of a biologic response to interferon-beta and a decrease in NAb concentration.

Timelines:

Start of enrolment of patients: January 2005

End of patient enrolment: June 2005

End of study: November 2005

Principal investigator: Per Soelberg Sørensen

Project group: Per Soelberg Sørensen, Mads Ravnborg

Persistence of neutralizing antibodies against interferon-beta after discontinuation of therapy

A Danish Multiple Sclerosis Group (DMSG) project initiated and organized by Copenhagen MS Center.

Objectives: The main objective is to follow concentrations of neutralizing antibodies against interferon-beta after discontinuation of interferon-beta therapy.

Study design and patients: Concentrations of neutralizing antibodies (NAb) against interferon-beta are measured using an antiviral neutralization assay. The concentration of neutralizing antibodies (neutralizing capacity) is followed in patients who have been treated with interferon-beta for more than 1 year, and thereafter have discontinued therapy or switched therapy to glatiramer acetate (Copaxone). Of 89 patients who had NAb analysis done during and after IFN-beta treatment, 28 patients were NAb-positive with NAb% ≥ 20% at least during IFN-beta therapy. January 2005 we are having Kawade-titration done for patients with NAb% ≥ 20% following IFN-beta treatment discontinuation.

Outcome measure: The primary outcome measures are change in neutralizing capacity and Kawade-titer.
Results: Of 28 patients with median NAb 97% (16-100%) at last test during IFN-beta treatment, after median 45 treatment months (12-83), median 4 months (0-34 months), before treatment stop. All were still NAb-positive with median NAb 96% (21-100%) median 15 months (5,5-57 months after IFN-beta treatment secession).
Financial support: Danish Multiple Sclerosis Society, Warwara Larsen Foundation
Responsible investigator: Bodil Petersen
Project group: Bodil Petersen, Nils Koch-Henriksen, Klaus Bendtzen, Mads Ravnborg, Per Soelberg Sørensen

2.1.4. Neuro-imaging

Positron Emission Tomography (PET), MRI and cognitive dysfunction in patients with newly diagnosed relapsing-remitting multiple sclerosis (MS).
Objectives: The aim of the study is to examine the cross-sectional and longitudinal changes in positron emission tomography (PET) measurements of cerebral glucose metabolism, in patients with newly diagnosed MS. The results are compared with pathological changes in magnetic resonance imaging (MRI), and a selected panel of neuropsychological tests evaluating cognitive dysfunction.
Study design and patients: 21 patients were included in the initial evaluation with PET, MRI and neuropsychological evaluation, and the results of this initial cross-sectional study have been presented elsewhere. Currently, the studied population is undergoing final MRI, PET and neuropsychological evaluation after a two year time period, in order to evaluate the longitudinal pathological changes in the early course of MS.
Timelines:
Time of enrolment of patients: August 2000 and following.
End of study: December 2005.
Financial support: Copenhagen University
Responsible investigator: Thomas Tscherning
Project group: Agnete Jønsson, Henrik Kahr Mathiesen, Thomas Tscherning, Per Soelberg Sørensen, Morten Blinkenberg, Lars Hanson, Egill Rostrup, Olaf B. Paulson

Multi-slice echo planar spectroscopic MR imaging in MS patients with cognitive impairment

Objectives: To test the hypothesis that global brain NAA/creatine (Cr) is a better predictor of cognitive dysfunction in multiple sclerosis (MS) than conventional magnetic resonance imaging (MRI) measures.

Study design and patients: Twenty patients, 16 females and 4 males, with relapsing remitting MS (RRMS). of less than 5 years duration were included. Multi-slice echo planar spectroscopic imaging (EPSI), which is a spectroscopic method able to provide information on axonal loss or dysfunction in MS lesions, NAWM, and cortical grey matter, and also provide global estimates of different metabolites was applied together with conventional MRI techniques. To evaluate cognitive dysfunctions, the patients completed a battery of neuropsychological tests including 29 measures. A Cognitive Dysfunction Factor including the 16 measures, which best distinguished between MS patients and normal controls were constructed.

Results: Cognitively impaired patients (9) had significantly (P=0.036) lower global NAA/Cr than unimpaired patients (11), while there was no relationship between the brain parenchymal fraction (BPF) or lesion volume (LV) and CDF. A significant partial correlation between global NAA/Cr and CDF (partial r=0.62, P=0.045) was found. There was no significant correlation between EDSS or treatment and any of the MRI measures.

Financial support: Danish Multiple Sclerosis Society,

A collaborative study with *Danish Research Center for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Denmark*

Responsible investigator: Henrik Kahr Mathiesen

Project group (MS Research Unit): Agnete Jonsson, Thomas Tscherning, Morten Blinkenberg, Per Soelberg Sorensen.

Publications: Mathiesen HK, Tscherning T, Sorensen PS, Larsson HBW, Rostrup E, Paulson OB, Hanson LG. Multi-slice echo-planar spectroscopic MR imaging provides both global and local metabolite measures in multiple sclerosis. Magn Reson Med 2005;53:750-9.

2.1.5. Rehabilitation and neuropsychology

Cognitive dysfunction in MS

A longitudinal study of cognitive dysfunctions in newly diagnosed multiple sclerosis patients

Objectives: The aims of the study are 1) to examine frequency, type and severity of cognitive dysfunctions in newly diagnosed MS, 2) to follow the development of cognitive dysfunctions over a period of 5 years with re-examination once a year and 3) to correlate cognitive dysfunctions with neurological impairment and psychosocial factors in order to optimize relevant advise and rehabilitation.

Study design and patients: Eighty consecutive patients diagnosed with MS within one year have been included. The last patient was included in the middle of 2001. Seventy-one of 80 patients have passed the second examination and 68 patients passed the third examination. These data are being analyzed. Sixty-four patients have passed the forth examination, 43 patients the fifth examination and 27 patients have passed the sixth and last examination.

Timelines:

Time of enrolment of patients: March 1997

End of study: April 2007

Financial support: Rigshospitalets Forskningsudvalg, Fonden for Neurologisk Forskning

Responsible investigator: Agnete Jønsson

Study group: Agnete Jønsson, Jente Andresen, Mads Ravnborg, Lars Storr, Thomas Tscherning, Per Soelberg Sørensen

2.1.6. Rehabilitation in MS

Rehabilitation of MS patients with acute relapse and incomplete remission after treatment with methylprednisolone

Objectives: To determine if MS patients with incomplete remission after methylprednisolone-treated relapse, benefit from multidisciplinary rehabilitation.

Study design and patients: A national multicenter study with a randomized and controlled design. We plan to include 120 patients with acute MS relapse, which have incomplete remission after methylprednisolone treatment. The patients are randomized to multidisciplinary rehabilitation at Haslev Rehabilitation Center or follow the usual guidelines for rehabilitation in their local neurological department/physiotherapy. Follow up examination is planned after 3 and 6 months.

Timelines:
Time of enrolment of patients: During 2005/2006
End of study: 2007/2008
Financial support: Unknown
Responsible investigator: Morten Blinkenberg
Project group: Per Soelberg Sørensen, Morten Blinkenberg, Mads Ravnborg

2.2. Pathogenesis of MS

Identifying patterns and extent of remyelination in MS
Objectives: The aims of the study are 1) study the extent of remyelination in grey and white matter of MS-lesions in brain and spinal cord. 2) describe morphological patterns of remyelination and relate this to MS subtype, diseases duration and disability. 3) Examination of factors that promote or inhibit oligodendrocyt proliferation and axonal susceptibility for remyelination.
Study design and patients: Histopathological evaluation of autopsy lesions of 46 patients (225 blocks) with SP and PP MS. 39 patients have not been treated with any immunosuppressives and the mean disease duration is 23 years.
Outcome measures: Systematic investigation of remyelination of MS lesions in patients with long disease duration is essential if treatment that stimulates remyelination is to be developed. Especially factors that influence remyelination in the spinal cord are important, as lesions in spinal cord have large impact on disability.
Financial support: Danish Multiple Sclerosis Society
Responsible investigator: Jens Christian Faber-Rod
Project group: Jens Christian Faber-Rod, Peter Patrikios, Hans Lassmann and Per Soelberg Sørensen
This project is done in collaboration with department of neuropathology, Rigshospitalet (Henning Laursen), The Danish MS Registry (Nils Koch-Henriksen) and Center for Brain Research, Medical University of Vienna, Austria (prof. Hans Lassmann, Alexandra Kutzelnigg, Peter Patrikios).

Immunomodulatory drugs in MS: Effects on subtypes of blood mononuclear cells in vivo and in vitro
The mechanisms of action of the immunomodulatory drugs currently used in the treatment of MS are still unclear. The drugs act on several levels including systemic activation/suppression of immune cells, cytokine produc-

tion, and leukocyte trafficking across the blood brain barrier. Better understanding of these mechanisms is required in order to devise future treatment regimens and rational drug combination treatment. Furthermore, identifying biological markers of disease activity could lead to better-individualized treatment.

Objectives: We aim to assess the effects of immunomodulatory treatment (interferon-β, glatiramer acetate, and natalizumab) on peripheral blood mononuclear cells. Among others, we are testing the hypothesis that interferon-β treatment leads to a reduction in the number of CD4+CD26+ T-cells, CD8+CD26+ T-cells and monocytes that produce proinflammatory cytokines. Moreover, that treatment leads to an increase in the number of CD4+CD25+ regulatory T-cells producing immunoregulatory and/or neurotrophic cytokines.

Methods: Blood samples are collected from MS patients initiating immunomodulatory treatment. Patients are re-sampled after 3 months and 6 months of treatment. Inflammatory activity is monitored by means of serial MRI scans and clinical parameters and is correlated to the immunological findings. Blood mononuclear cells are stained for flow cytometry with an extensive panel of surface and intracellular antigens, including markers of activation, differentiation, co-stimulation, co-inhibition, chemokine receptors, adhesion molecules and mediators of apoptosis. In addition, production of specific mRNA in response to treatment is assessed by micro array analysis and real time reverse transcriptase PCR (quantitative PCR). In vitro cell culture experiments are employed to verify results obtained in vivo.

Financial support: University of Copenhagen, Faculty of Health Sciences, Danish Multiple Sclerosis Society, Rigshospitalet, Lily Benthine Lund Foundation, Dagmar Marshall Foundation, Foght Trust Fund, Bent Bøgh and Inge Bøghs Foundation

Responsible investigator: Martin Krakauer

Project group: Martin Krakauer, Finn Sellebjerg, Henriette Egeblad, Susanne Velgaard, Pameli Datta, Annette Bang Oturai, Per Soelberg Sørensen

Monitoring in vivo interferon-β responses in MS

Treatment of relapsing remitting MS with interferon-β generally reduces relapse rates by one third and slows disease progression. However, in individual MS patients there is a wide spectrum of interferon-β treatment responses, spanning from no clinical or paraclinical (MRI) effect at all to almost complete abrogation of CNS inflammation and markedly prolonged

relapse-free intervals. Some of the individual differences in interferon-β response can be explained by the long-term development of neutralizing antibodies to interferon-β (NAb). There is, however, also a subgroup of patients with little or no initial response to interferon-β, which cannot be explained by NAb's. These patients probably have a defective cellular response to interferon-β.

Objectives: Interferon-β treatment is expensive and associated with multiple side effects. Identification of non-responders is crucial. This will enable doctors to stop an ineffective treatment and offer the patient alternative treatment options (i.e. glatiramer acetate).

We aim to develop a simple assay to evaluate in vivo interferon-β responses in individual MS patients. Present assays for NAb (cytopathic bioassays) are laborious and difficult to standardize. Moreover, these assays do not identify NAb-negative non-responders.

In addition we plan to investigate the cellular mechanism responsible for NAb-negative non-responders.

Methods: We have developed a real time reverse transcriptase PCR method to quantify in vivo interferon-β induced MxA protein mRNA in blood mononuclear cells. MxA protein is exclusively induced by type 1 interferons and it's mRNA serves as a marker of interferon-β response. MxA mRNA will be correlated to the presence of Nab's and clinical and paraclinical markers of interferon-β response.

Mechanisms conferring interferon-β non-responsiveness on a cellular level are also studied.

Financial support: University of Copenhagen, Faculty of Health Sciences, Danish Multiple Sclerosis Society, Rigshospitalet

Responsible investigator: Per Soelberg Sørensen

Project group: Per Soelberg Sørensen, Martin Krakauer, Rikke Kroager, Henriette Egeblad, Finn Sellebjerg

Characterization of immunoregulatory mechanisms in MS

Researchers in the immunological field have recently rekindled interest in a subset of CD4+ T lymphocytes expressing high levels of CD25. These cells have immunoregulatory properties and are able to attenuate immunoproliferative responses in vitro and in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). The CD4+CD25+ cells are a putative targets for treatment in autoimmune diseases such as MS, due to their immunoregulatory properties. Their mechanism of action is as yet unknown.

Objectives: We aim to clarify the role of the CD4+CD25+ T lymphocyte subset in vivo and in vitro in terms of responses to treatment with immunomodulatory MS drugs and the mechanism involved in regulating immune responses of other lymphocyte subsets.
Methods: Peripheral blood mononuclear cells are isolated from treated and untreated MS patients and stained for flow-cytometry identifying the relevant subsets of T lymphocytes. Magnetic Beads are employed to isolate the putative regulatory CD4+CD25+ T-lymphocytes. In vitro cell cultures of these cells are studied in order to ascertain their effect on a cellular level.
Financial support: University of Copenhagen, Faculty of Health Sciences, Danish Multiple Sclerosis Society, Rigshospitalet, Lily Benthine Lund Foundation, Dagmar Marshall Foundation, Foght Trust Fund, Bent Bøgh and Inge Bøghs Foundation
Responsible investigator: Martin Krakauer
Project group: Martin Krakauer, Finn Sellebjerg, Susanne Velgaard, Rikke Kroager, Per Soelberg Sørensen

Examination of complement factor C3 activation in cerebrospinal fluid and serum of MS-patients

Objectives are to determine changes in the activation of complement factor C3 in blood and cerebrospinal fluids of patients with MS, and to relate these to the disease course and pathogenetic subgroups of patients.
Design: By use ELISA, the concentrations of the final C3-activation product are measured in CSF and serum samples for a group of MS patients in comparison to a control group. Furthermore the concentrations of total C3 in CSF and in serum have been measured by use of radial immunodiffusion. Fractions of activated to total C3 have been calculated for each individual in both MS-patients and controls. By measurements of the corresponding albumin concentrations in the samples, calculations of activated C3 and C3 indexes for the various groups have been determined.
Timeline: end April 2005
Financial support: Direktør Ib Henriksens Fond, Hestehandler Ole Jakobsens Mindelegat
Responsible investigator: Poul Erik Hyldgaard Jensen
Project group: Signe Humle Jørgensen, Per Soelberg Sørensen, Susanne Velgaard, Henriette Egeblad, and Poul Erik Hyldgaard Jensen

Conformational changed α2-Macroglobulin in patients with MS

Objectives are 1) to demonstrate correlation between the proteinases MMP-1, -2, -3, –9 and α_2M of MS-patients in both plasma and CSF, 2) to identify possible abnormality in α_2M, and 3) to identify the influence of α_2M on oligodendroglial cell responses, induced by the cytokines TNF-α, interferon-γ, Il-6, Il-10, and TGF-β.

Design: *Project 1: Binding of MMP-1, -2, -3, and -9 to α_2M in MS-patienter.* Due to increased concentrations in MS-patients of the MMP-proteinases, we examine if any correlations exist between the concentrations of MMP's and the α_2M (total and transformed) in 200 patients of the RR/SP types. The measurements will be performed in serum/plasma and CSF. The concentrations of α_2M will be measured by ELISA and binding of MMP to α_2M will be studied by zymography and Western blots.

Project 2: Studies of structural changes in α_2M from MS-patient plasma. An article was published from our group in 2004 concerning the changed concentrations of α_2M in plasma from MS-patients and controls. To examine if the α_2M is abnormal, it will be purified from CSF and plasma of RR/SP MS-patients, and then compared to α_2M from healthy controls. Functional sites of α_2M will be examined, and carbohydrates structures will be examined by binding of ligands are in progress. Interestingly we have identified different isoelectric points of α_2M in blood and CSF of individuals.

Project 3: Determination of the concentrations of Il-8, Il-10, TGF-βand TNF-αin plasma and CSF from MS-patients, and correlation with corresponding α_2M concentrations. Studies have demonstrated changed concentrations of various cytokines in MS-patients. Since α_2M can bind and neutralize a wide range of cytokines we will study the correlation between their concentrations. In MS-patients serum and CSF samples will be tested by use of ELISA.

Project 4: Examination of the α_2M influence on oligodendroglial cell responses, induced by the cytokines TNF-α, interferon-γ, Il-6, Il-10, and TGF-β. Oligodendrocytes are the source to myelin, and apoptosis has been shown to be induced in a humane oligodendrocyt cell line (MO3.13) by the toxic effects of the cytokines TNF-α and INF-γ. These effects may indicate, that in MS-patients oligodendrocytes can be damaged by direct attack of the cytokines TNF-α and INF-γ. Synergistic effects of both cytokines result in increased cytotoxicity and more apoptosis. The project will

examine the effects of α_2M on the apoptotic regulation of the cells by different concentrations of cytokines (TGF-β, TNF-α and INF–γ). α_2M does not demonstrate binding to oligodendrocytes directly. Further the effects of α_2M on Il-6 and Il-10 will be examined. These cytokines have positive effects on the survival of oligodendrocytes.
Timelines: Projects 1 and 3 ELISA's will be performed before May 2005. Immunodiffusion of samples before June 2005. Project 2 will be running during the year 2005. Project 4 will start after summer 2005 and will continue until summer 2006.
Financial support: Fonden til Lægevidenskabens Fremme, A.P. Møller og Hustru Chastine McKinney Møllers Fond til Almene Formål, Oda og Hans Svenningsens Fond, Jacob Madsens og Hustru Olga Madsens Fond
Responsible investigator: Poul Erik Hyldgaard Jensen
Project group: Signe Limborg, Per Soelberg Sørensen, Henriette Egeblad, Rikke Kroager, Susanne Velgaard and Poul Erik Hyldgaard Jensen

Determination of concentrations of the soluble receptor CD163 in cerebrospinal and serum samples from MS-patients
Objectives: are to study if sCD163 is increased in CSF and serum from patients with MS, and to examine the possibility of using sCD163 as an inflammation parameter.
Design: by use of ELISA the concentrations of the soluble receptor CD163 were measured in serum and CSF from control groups and MS-patients.
Financial support: None
Responsible investigator: Poul Erik Hyldgaard Jensen
Project group: Poul Erik Hyldgaard Jensen, Per Soelberg Sørensen, Søren Moestrup (Aarhus), Holger Jon Møller (Aarhus), Babs Fabriek (The Netherlands)

Determination of concentrations of sUPAR (soluble urokinase-type plasminogen activator receptor) in cerebrospinal and serum samples from MS-patients
Objectives: are to study if increased concentrations of sUPAR are found in CSF and serum from patients with MS, and to examine the possibility of using sCD163 as an disease parameter.
Design: by use of ELISA the concentrations of the soluble receptor CD163 are measured in 200 samples of serum and CSF from control groups and MS-patients.
Financial support: None
Responsible investigator: Jesper Eugen-Olsen and Liselotte Kahns

Project group: Poul Erik Hyldgaard Jensen, Mads Ravnborg, Per Soelberg Sørensen, Jesper Eugen-Olsen, Liselotte Kahns

Immune activation in subsets of blood mononuclear cells in MS patients before and during immunomodulatory treatment

Background: The mechanisms of action of the immunomodulatory drugs currently used in the treatment of MS are still unclear, but include immunomodulatory effects on T cells and other mononuclear cells in peripheral blood have been found in previous studies. A better understanding of these mechanisms is required to develop new treatment regimens based on rational drug combination regimens.

Objectives: We assess the cellular effects of immunomodulatory treatment with interferon-beta, glatiramer acetate, methylprednisolone, intravenous immunoglobulin, and mitoxantrone on peripheral blood mononuclear cells.

Methods: Blood samples are collected from MS patients beginning an immunomodulatory drug treatment and from patients who are treated with combinations of immunomodulatory drugs in ongoing clinical trials.

Patients initiated on immunomodulatory treatments are studied again after 3 months and 6 months of treatment, disease activity in the brain is monitored by means of serial MRI scans, and the patients are scored clinically. Mononuclear cell expression of an extensive panel of surface and intracellular molecules is studied by flow cytometry. In addition, production of specific mRNA in response to treatment is assessed by micro array analysis and real time reverse transcriptase PCR (quantitative PCR). In addition, basic immunoregulatory mechanisms involving the CD4+CD25+T-cell population and intracellular signaling pathways are studied.

Financial support: University of Copenhagen, Faculty of Health Sciences, Danish Multiple Sclerosis Society, Rigshospitalet, Lily Benthine Lund Foundation, Dagmar Marshall Foundation, and the Danish Hospital Foundation for medical research – Region of Copenhagen, the Faroe Islands and Greenland.

Results: Data from a series of patients initiated on treatment with interferon-beta indicate that treatment induces: 1) General changes in the phenotype of CD4 positive T cells without distinct differences in the effect on CD25-negative, CD25-intermediate, and CD25-high (regulatory) subsets. 2) Increases in the surface expression of the chemokine receptors CCR4, CCR5, and CCR7, but no change in the expression of CXCR3. 3) Changes in cell surface expression Fas and Fas-ligand and plasma concentrations of the soluble forms of these molecules. 4). Changes in the activation status of CD26-high memory CD4 T cells compared to healthy controls, with some changes in the activation of this T cell subset after treatment with interferon-beta.

Ongoing studies: Studies of chemokine, chemokine receptor, cytokine, and transcription factor expression at the mRNA level and studies of CD25-high CD4 T cell regulatory activity in the initial patient cohort are ongoing. In addition, sample collection from new patient cohorts treated with interferons and other immunomodulatory drugs as monotherapy or in combination is continued.
Responsible: Martin Krakauer
Project group: Martin Krakauer, Finn Sellebjerg, Susanne Velgaard, Henriette Egeblad, Pameli Datta, Annette Oturai, Per Soelberg Sørensen

2.3. Genetics

Identification of risk factors in Multiple Sclerosis
Background: Worldwide, genome screens have been published without identification of any major gene(s). From these studies it seems more likely that about 20 genes with small to moderate genetic effects are interacting. Large-scale patient materials are needed to identify these genes. On this background we have collected DNA from 800 MS patients and 1200 controls and since 1994 we have participated in the MS Nordic genetic group to increase the sample size. Today, the material in the Nordic collaboration consists of 4000 MS cases, 4000 controls, 1000 trio families, and 177 affected sib pairs. The collecting of DNA from MS patients is ongoing. The Nordic material is unique. The population is genetically more homogeneous compared to other populations, and the prevalence rate is among the highest in the world, whish makes is suitable for genetic analysis. Despite the Nordic collaboration we have since year 2000 been in a European collaboration.
Methods: The aim of this study is to identify susceptibility and disease modifying genes in MS.
Financial support: The Danish Multiple Sclerosis Society, Johnsen og hustrus mindelegat, Warwara Larsen Fond
Responsible investigator: Annette Oturai
Project group: Annette Oturai, Pameli Datta, Per Soelberg Sørensen
This project is done in collaboration with department of Clinical Immunology, Rigshospitalet Denmark (Lars P. Ryder, Hans O. Madsen, Arne Sveigaard)

Two genome-wide linkage disequilibrium screens in Scandinavian multiple sclerosis patients
Background & objectives: The etiology of MS is complex and includes a complicated interplay between unknown genetic and environmental factors. Worldwide, eight genome screens have been published without identi-

fication of any major gene(s). From these studies it seems more likely that about 20 genes with moderate genetic effects are interacting. The Nordic countries have participated in a large European collaboration performing genome screens, using 6000 micro-satellites in two sets of Nordic populations. Results have been published, and a European meta-analysis is ongoing. The Nordic group has been collaborating since 1994. The objective of this study was to perform genome-wide linkage disequilibrium screens in Scandinavian MS patients to identify regions encompassing putative susceptibility genes.

Study design and patients: 400 Scandinavian MS patients and unrelated control individuals were included in two independent screens. A set of 6000 micro satellite markers were typed on pooled DNA as described by Barcellos et al. 1997.

Results: 3331 markers were analyzed in both screens, and a comparison between them revealed 31 markers associated to MS. Among these several markers confirmed the well-established association of MS to the HLA-region on chromosome 6. The most promising regions were located on 19q13 and 11q23.

Future perspectives: To confirm the results in a new patient material by individual typing.

Financial support: The Danish Multiple Sclerosis Society, Warwara Larsens Fond, Ejner Jonasson og Hustrus mindelegat, Christenson-Cesons Familiefond

Responsible investigator: Pameli Datta

Project group: Annette Oturai, Pameli Datta, Per Soelberg Sørensen

This project is done in collaboration with:

Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet (Lars P. Ryder, Arne Svejgaard)

Institute of Immunology, Rikshospitalet, University Hospital, Oslo, Norway (Anne Spurkland, Hanne F. Harbo, Frode Vartdal)

Genetic group in Cambridge: Stephen Sawcer, Alastair Compston)

Department of Neurology, Ullevål University Hospital, Oslo, Norway (Elisabeth G Celius)

Department of Neurology, Huddinge University Hospital, Karolinska Institute, Huddinge, Sweden (Jan Hillert, Eva Åkesson, Helena Modin)

Department of Neurology, Lund University Hospital, Lund, Sweden (Magnhild Sandberg-Wollheim)

Department of Neurology, Haukeland Hospital, Bergen, Norway (Kjell-Morten Myhr)

Department of Neurology, Gothenburg University Hospital, Gothenburg, Sweden (Oluf Andersen)

Publication: Datta P, Harbo HF, Oturai A et al. Two genome-wide linkage disequilibrium screens in Scandinavian multiple sclerosis patients. J Neuroimmunol 2003; 143(1-2):101-6

Confirmation of genome-wide screen candidate regions in Nordic MS patients

Background: Several association genome-wide screens, based on pooled DNA, have been performed to determine genetic factors involved in MS. These results need to be confirmed with individual genotyping in new patient materials.

Objectives: The aim of this study was to confirm the results from three independent Nordic screens by individual typing. In this study the results from three Nordic linkage disequilibrium genome screens were investigated in new materials.

Methods and patients: Thirty-six non-HLA and four HLA micro satellite markers identified from two Scandinavian and one Icelandic linkage disequilibrium genome screens were investigated in 328 Caucasian Nordic trios from Denmark, Norway, Sweden and Iceland. The markers were individually genotyped and tested in the trios using transmission disequilibrium test. Markers, confirmed in the trios, were additionally tested in the same patients and a set of 372 unrelated controls using Fisher's exact test.

Results: Twelve (40%) out of the thirty-two markers available for statistical analysis were confirmed in the trios. Four of these 12 markers, two HLA markers (SA-99805, D6S2443) and two non-HLA markers (D11S1347, D1S2627) were supported by the case-control comparison and had significant allele frequency differences ($P<0.05$) in both transmission disequilibrium test and Fisher's test. Four additional markers D19S552, D12S1051, D6S273 (HLA-marker) and D19S888 showed suggestive evidence of association ($P<0.15$) in both statistical analyses.

Future perspectives: To confirm these regions in a larger patient material.

Financial support: The Danish Multiple Sclerosis Society, Warwara Larsens Fond, Ejner Jonasson og Hustrus mindelegat, Fonden for Neurologisk forskning, Emmy Langes, født Kramps Legat

Responsible investigator: Pameli Datta

Project group: Pameli Datta, Annette Oturai, Vibeke Jespersen, Per Soelberg Sørensen

This project is done in collaboration with:

Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet (Lars P. Ryder, Arne Svejgaard)

DeCODE, Genetic center, Reykjavik, Iceland: Jeffrey Gulcher, Kari Stefansson, Ragnheidur Fossdal, Aslaug Jonasdottir, Theodora Thorlacius
Institute of Immunology, Rikshospitalet, University Hospital, Oslo, Norway: Anne Spurkland, Hanne F. Harbo, Frode Vartdal
Department of Neurology, Ullevål University Hospital, Oslo, Norway: Elisabeth G Celius
Department of Neurology, Huddinge University Hospital, Karolinska Institute, Huddinge, Sweden: Jan Hillert, Eva Åkesson, Helena Modin
Department of Neurology, Lund University Hospital, Lund, Sweden: Magnhild Sandberg-Wollheim
Department of Neurology, Haukeland Hospital, Bergen, Norway: Kjell-Morten Myhr
Department of Neurology, Gothenburg University Hospital, Gothenburg, Sweden: Oluf Andersen
Multiple Sclerosis Rehabilitation and Diagnostic Center of the MS Society of Iceland, Reykjavik, Iceland: John Bendikz, MD

Short and long-term effect of interferon-beta in gene-expression profiling in relapsing remitting multiple sclerosis patients

Background: The biological mechanisms behind the beneficial effects exerted by interferon-beta and glatiramer-acetate in the treatment of multiple sclerosis are as yet not fully understood. Furthermore, no biological markers with information of treatment response are identified. However, prior to this, it is important to investigate the possible differences between short and long term gene-expression induced by interferon-beta.
Objectives: In this pilot study, we investigated the differentially regulated gene expression profiling with micro-arrays in MS patients starting treatment with interferon-β-1a.
Methods and patients: We collected samples from 10 patients with relapsing remitting multiple sclerosis, scheduled for treatment, at baseline; just prior to and between 14 hours after first treatment dose and after three months; just prior to injection and 14 hours after. Total RNA was isolated from peripheral blood mononuclear cells for gene-arrays analysis, screening over than 6000 human genes. Verification of results is done by real time reverse transcriptase PCR (quantitative PCR).
Financial support: The Danish Multiple Sclerosis Society, Warwara Larsens Fond, Ejner Jonasson og Hustrus mindelegat, Christian the X's foundation, Lily Benthine Lunds Foundation
Responsible investigator: Pameli Datta
Project group: Pameli Datta, Annette Oturai, Per Soelberg Sørensen

This project is done in collaboration with:
Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet: Lars P. Ryder, Jacob Larsen, Ingrid Alsing
Institute for Inflammatory research, Copenhagen University Hospital, Rigshospitalet: Klaus Rieneck, Klaus Bendtzen

Treatment induced gene expression in responding and non-responding multiple sclerosis patients

Background: MS is a heterogeneous disease in terms of clinical manifestation, etiology and pathology. The response to available treatment in MS is heterogeneous too and may roughly reflect two subgroups of patients; patients with relatively good response and patient with little or no response to treatment. However, at this point there are no biomarkers with information regarding treatment response available. Furthermore, the mechanisms behind the beneficial effect of treatment remain to be elucidated.

Objectives: To examine gene expression profiling in peripheral blood mononuclear cells induced by treatment at three time points. This in order to identify potential biomarkers containing information regarding response status in the early phase of treatment.

Patients and methods: MS patients starting treatment with low-doses (Avonex), high-doses interferon-beta (Betaferon, Rebif) and glatiramer-acetate (Copaxone) are included. Our aim is to include approximately 20 patients in each group. Response to treatment is evaluated by MRI performed at baseline, after 3 and 6 months. At the same time points peripheral blood samples are collected and total RNA isolated from mononuclear cells for gene-array analysis. Verification of results will be done by real time reverse transcriptase PCR (quantitative PCR).

Financial support: The Danish Multiple Sclerosis Society, Warwara Larsens Fond, Ejner Jonasson og Hustrus mindelegat, Christian the X's foundation, Lily Benthine Lunds Foundation

Responsible investigator: Pameli Datta

Project group: Pameli Datta, Lars Ryder, Annette Oturai, Martin Krakauer, Finn Sellebjerg, Anne Hansen, Merete Mogensen, Anne Sund, Per Soelberg Sørensen

This project is done in collaboration with:
Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet: Lars P. Ryder, Jacob Larsen, Ingrid Alsing
Department of MRI, Copenhagen University Hospital, Hvidovre: Henrik Lund, Kirsten Nielsen, Olaf Paulson

Institute for Inflammatory research, Copenhagen University Hospital, Rigshospitalet: Klaus Rieneck, Klaus Bendtzen

2.4. Experimental models of MS

Treatment of experimental autoimmune encephalomyelitis (EAE) with intravenous immunoglobulin

Project outline: Clinical trials have shown that intravenous administration of polyclonal immunoglobulin (IVIG) has the potential to reduce the disease activity in multiple sclerosis (MS). IVIG possesses multiple immunomodulatory and anti-inflammatory properties, and IVIG therapy may, therefore, represent a way of interfering with the disease process in multiple sclerosis (MS). In the present study we have evaluated IVIG treatment of experimental autoimmune encephalomyelitis (EAE), the primary animal model for MS. The objectives of the study were to assess the effects of IVIG on 1) the incidence, time course and severity of active EAE in the Dark Agouti rat and 2) the EAE pathology in the brain and spinal cord. In the Dark Agouti rat, a protracted and relapsing form of EAE was induced by inoculating animals with spinal cord homogenate obtained from syngenic donors. The EAE disease course in this model involves repeated incidents of neurological deficits, thus resembling the disease course in relapsing-remitting MS. After the induction of EAE, the animals received treatment with intravenous infusions of IVIG or placebo. It was assessed if the IVIG treatment reduced the incidence or duration of active EAE, or if treatment with IVIG influenced the severity of neurological symptoms. The outcome of IVIG treatment was also evaluated by histological investigations, where the pathological changes in the central nervous system were studied. Both the neurological EAE symptoms and the loss of body weight due to EAE were significantly reduced by prophylactic IVIG treatment. Furthermore, early immunoglobulin infusions significantly attenuated the inflammatory response in both brain and spinal cord tissues. In 2004 results from this study were accepted for publication.

Financial support: The Hørslev Foundation and Aase and Ejnar Danielsens Foundation

Responsible investigator: Signe Humle Jørgensen

Project group: Signe Humle Jørgensen, Poul-Erik Hyldgaard Jensen, Henning Laursen, Per Soelberg Sørensen

Publication: Jorgensen SH, Sorensen PS. Intravenous immunoglobulin treatment of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis. J Neurol Sci (in press)

Cytokine expression during the development of EAE – effects of immunoglobulin treatment

Project outline: Intravenous immunoglobulin (IVIG) is an established treatment of immune-mediated demyelinating neuropathy and is presently regarded as a second-line treatment of multiple sclerosis (MS). However, the mechanisms by which IVIG may interfere with the pathophysiology of MS are not yet fully understood. The objective of this study is to investigate the effects of IVIG treatment on T cell activation during the development of experimental autoimmune encephalomyelitis (EAE), an MS animal model. Moreover, we wish to investigate whether the effects of IVIG administration are primarily due to peripheral effects on the immune system, or if IVIG administration may also affect T cell reactivation within the central nervous system. The effects of IVIG on T cell function are evaluated by analyzing the gene expression of inflammatory cytokines and growth factors that contribute to demyelination and inflammation in the central nervous system during the establishment of EAE. In 2004 the project has been outlined with pilot experiments starting in spring 2005.

Financial support: The Hørslev Foundation and Aase and Ejnar Danielsens Foundation

Responsible investigator: Signe Humle Jørgensen

Project group: Signe Humle Jørgensen, Finn Sellebjerg, Henning Laursen, Per Soelberg Sørensen

Blood-brain barrier passage of ^{99m}Tc-labelled IVIG during the acute attack in EAE

Project outline: Treatment with intravenously administered immunoglobulin G (IVIG) may reduce the disease activity in multiple sclerosis (MS). The preparations of IVIG are produced by purification from large pools consisting of plasma from thousands of donors, and IVIG thus contains a variety of antibody specificities. IVIG may, therefore, accumulate non-specifically in areas of inflammation, also making IVIG a valuable tool in imaging and radioimmunodetection of infections and inflammatory foci. In the present project we have studied if IVIG enters the central nervous system by passing through the blood-brain barrier. The experiments were carried out in the MS animal model, experimental autoimmune encephalomyelitis (EAE). After inducing EAE in rats, the animals were treated with

IVIG infusions during the acute attack of the disease. It was then evaluated, if IVIG accumulated in the inflammatory infiltrates found in the brain and spinal cord. The immunoglobulin was conjugated to hydrazine nicotinamide (HYNIC) for labeling with radioactive ^{99m}Tc-pertechnetate, and the radiochemical purity of the preparation was measured using instant thin layer chromatography (iTLC). After *in vivo* administration, the ^{99m}Tc-IVIG was localized and visualized in CNS tissue sections by means of autoradiography. Studies evaluating the ^{99m}Tc-IVIG biodistribution and plasma half-life were also performed. The experiments have been carried out during 2003-2004 and after final data analysis the results are submitted for publication.

Financial support: The Hørslev Foundation and Aase and Ejnar Danielsens Foundation

Responsible investigator: Signe Humle Jørgensen

Project group: Signe Humle Jørgensen, Nicolas Storm, Poul-Erik Hyldgaard Jensen, Henning Laursen, Per Soelberg Sørensen

Micro array analysis of the gene expression profile in EAE.

Project outline: In multiple sclerosis (MS), the autoimmune disease processes are driven by immune cells that are first activated, then pass through the blood-brain barrier into the central nervous system, where reactivation takes place. For all of these processes to occur, changes in the cellular expression of proteins are required, e.g. proteins, which are involved in, cell signaling, cell adhesion, ligand binding, second messenger systems etc. Recently, development of the micro array technology has made it possible to screen thousands of gene transcripts simultaneously from small amounts of tissue. We have used the Affymetrix Gene chip to evaluate the differential regulation of genes after induction of the MS animal model experimental autoimmune encephalomyelitis (EAE) in susceptible rats. By analyzing approximately 9,000 genes, more than 500 genes were found differentially expressed after the induction of EAE. The results from analyzing the EAE gene expression profile will be related to the presence of the different classes of immune cells within the CNS, as evaluated by immunohistochemistry.

Responsible investigator: Signe Humle Jørgensen and Pameli Datta

Project group: Signe Humle Jørgensen, Jacob Larsen, Pameli Datta, Lars Ryder, Annette Oturai, Per Soelberg Sørensen

3. SCIENTIFIC PUBLICATIONS 2003 - 2004

3.1. Peer reviewed original papers

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Comi G, Clanet M, Lublin F, Martinelli V, Polman CH, **Sorensen PS**. Pharmacologic Research in Neurologic Disorders. Multiple Sclerosis and Other Demyelinating Diseases. In: du Souich P, Erill S, Orme M. The IUPHAR Compendium of Basic Principles for Pharmacological Research in Humans. International Union of Basic and Clinical Pharmacology. 2004:248-62

4. SCIENTIFIC PRESENTATIONS AND ABSTRACTS 2004

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measuring disease severity. 56th Annual meeting of the American Academy of Neurology, San Francisco, April 2004 (poster)

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Sorensen PS (invited speaker). Long-term efficacy of interferon beta and the role of neutralizing antibodies (invited speaker). 7th Congress of the European Society for Clinical Neuropharmacology, Trieste, Italy, May 2004

Jønsson A. Frontal lobe (dys)functions and assessment methods (invited speaker). Seminar for clinical psychologists and neuropsychologists. Tallinn, Estonia, May 2004

Jørgensen SH, Storm N, Jensen PEH, Laursen H, Sorensen PS. Treatment of experimental autoimmune encephalomyelitis with intravenous immunoglobulin. 34th Scandinavian neurology congress, Copenhagen, June 2004

Sorensen PS. Lessons from IVIG trials in relapsing-remitting multiple sclerosis: The IVIMS study – a combination trial of IVIG and IFN-beta. (invited speaker). ENS Congress, satellite symposium, Barcelona, Spain, June 2004 (oral presentation)

Jønsson A. Cognitive impairment in newly diagnosed multiple sclerosis patients. 8th Nordic Meeting in Neuropsychology (invited speaker). Turku, Finland, August 2004

Jorgensen SH, Storm N, Jensen PEH, Laursen H, Sorensen PS. Blood-brain barrier passage of 99mTc-labelled IVIG in EAE (poster). 7th Interna-

tional Congress of Neuroimmunology, Venice, September 2004. J Neuroimmunol 154:1-2:81 (abstract)

Larsen J, Jorgensen SH, Datta P, Oturai A, Sorensen PS, Ryder LP. Gene expression profiling in rats with experimental autoimmune encephalomyelitis: implications of intravenous immunoglobulin treatment (poster). 20th Congress of the European Committee for Treatment and Research in Multiple Sclerosis, Vienna, September 2004. Mult Scler 10:suppl.2:S198 (abstract)

Datta P. Confirmation of candidate regions 1p34 and 11q23 in Nordic MS patients. (Abstract and Poster) ECTRIMS, Vienna, Austria, October 2004

Koch-Henriksen N, Sørensen PS, Christensen T. The national Danish interferon-beta project: Head to head comparison between IFN-beta 1a 22 µg once weekly and IFN-beta 1b 250 mcg every other day in a randomized open-label study. ECTRIMS, Vienna, October 2004 (poster/abstract)

Sorensen PS, Tscherning T, Ross C, Mathiesen HK, Langkilde A, Clemmesen K, Bendtzen K. Effect of neutralizing antibodies against interferon-beta on in vivo biologic response and disease control in multiple sclerosis patients. ECTRIMS, Vienna, October 2004 (poster/abstract)

Jønsson A. Cognitive dysfunctions in newly diagnosed multiple sclerosis patients. A 5-year follow-up (invited speaker). European Charcot Foundation Symposium. Taormina, Sicily, Italy, November 2004

Sørensen PS, The relationship between MRI and PET changes and cognitive disturbances in MS (invited speaker). European Charcot Foundation Symposium. Taormina, Sicily, Italy, November 2004

Sorensen PS. Immunomodulatory Treatment in Denmark – A Nationwide Study (invited speaker). Hot Topics in MS. Oslo, Norway. December 2004

Oturai A: ”Spiller generne en rolle ved MS?” MS seminar (Schering), København, 2004 (oral presentation)

Sorensen PS. Long-term efficacy of interferon beta and the role of neutralizing antibodies. J Neural Transm 2004;III:X (Abstract)

5. COLLABORATORS

5.1. National

Neurobiology Research Unit, Copenhagen University Hospital, Rigshospitalet (Olaf Paulson, MD, DMSc, Henrik Lund, M.Sc., Kirsten Nielsen, MD)

Danish Research Center for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Denmark.(Henrik Kahr Mathiesen, MD, Lars G. Hanson, Physicist, professor Olaf Paulson, MD, DMSc, Henrik Lund, MSc, Kirsten Nielsen, MD)

The Danish Multiple Sclerosis Register, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark (Nils Koch-Henriksen, MD)

Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet (Jacob Larsen, MSc, Lars Ryder MSc, professor Arne Svejgaard, MD, DMSc, Hans O. Madsen, MSc, Bodil Jacobsen)

Institute for Inflammatory research, Copenhagen University Hospital, Rigshospitalet (K. Rieneck, MD, professor Klaus Bendtzen, MD)

Laboratory of Neuropathology, Copenhagen University Hospital (Henning Laursen, MD)

Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark (Trine Rasmussen Nielsen , MD, Henrik Hjalgrim, MD)

5.2. International

Neurobiology Research Unit, Copenhagen University Hospital

Institute of Immunology, Rikshospitalet, University Hospital, Oslo, Norway (Anne Spurkland, MD, DMSc, Hanne F. Harbo, MD, DMSc, profes-

sor Frode Vartdal, MD, DMSc)

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Department of Neurology, Haukeland Hospital, Bergen, Norway (Kjell-Morten Myhr, MD, DMSc)

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Department of Neurology, Gothenburg University Hospital, Gothenburg, Sweden (Oluf Andersen, MD, DMSc, Jan Lycke, MD, DMSc)

Multiple Sclerosis Rehabilitation and Diagnostic Center of the MS Society of Iceland, Reykjavik, Iceland (John Bendikz, MD)

DeCODE: Genetic center, Reykjavik, Iceland (Jeffrey Gulcher, MD, DMSc, Kari Stefansson, MD, DMSc, Ragnheidur Fossdal, M.Sc., Aslaug Jonasdottir, M.Sc., Theodora Thorlacius, MSc, Jeffrey Gulcher, MD, Ragnheidur Fossdal, MSc)

Genetic group in Cambridge, United Kingdom (Stephen Sawcer, MD, Ph.D, professor Alastair Compston, MD)

Department of Neurology, Tromsø University Hospital, Tromsø, Norway (professor Svein Ivar Mellgren, MD, DMSc)

Department of Neurology, Sentral Sykehuset i Akershus, Nordby Hagen, Norway (Antonie Beiske, MD, DMSc)

Department of Immunology, Umeå University, Sweden (Peter Sundström, MD, PhD., Martin Gunnarsson MD, PhD, professor Torgny Stigbrand, MD)

Neuroimmunology Unit, Karolinska Hospital, Stockholm, Sweden (Tomas Olsson, MD, DMSc)

Department of Neurology, Umeå Hospital, Sweden (Anders Svenningson, MD, DMSc)

Department of Neurology, Helsinki University Hospital, Helsinki, Finland (Markus Färkkilä, MD, DMSc)

The London, Ontario Multiple Sclerosis Database and the Department of Clinical Neurology, University of Oxford, England (professor George C. Ebers, MD)

Department of Neurology, Duesseldorf University Hospital, Duesseldorf, Germany (professor Hans-Peter Hartung, MD, DMSc, Bernd Kieseier, MD)

Department of Neurology, Tilburg, The Netherlands (Leo Visser, MD, PhD)

Department of Radiology and Neurology, Vrije Universiteit Hospital, Amsterdam, The Netherlands (professor Frederik Barkhof, MD, PhD, Bernd Uidtehaag, MD, PhD)

Department of Neurology, Charlerois Hospital, Belgium (professor Pierette Seeldrayers, MD, PhD)

Department of Neurology, Karl-Franzens-University, Graz, Austria (professor Franz Fazekas, MD, DMSc)

5.3. Collaboration with pharmaceutical companies on clinical trials

IPC Nordic, Denmark
Novartis, Denmark
Serono Nordic, Denmark, Norway and Sweden
Biogen Idec, Denmark and USA
Teva/Aventis, Israel and Denmark
Bayer Vital, Germany
Baxter, Belgium, Austria and USA
Octapharma, Austria

Schering, Germany
Astion Denmark A/S

6. ACKNOWLEDGEMENTS

The Copenhagen MS Center and Research Unit have received generous support from a number of public and private research funds:
The Hørslev Foundation
Aase and Ejnar Danielsens Foundation
Civilingeniør Bent Bøgh og hustru Inge Bøghs fond
Selskabet Danske Neuropsykologer
The Danish Multiple Sclerosis Society
Warwara Larsens Foundation
Lily Benthine Lund Foundation
Christian the X's Foundation

7. OTHER ACTIVITIES

7.1. Awards

Pameli Datta. Torben Fogs and Erik Triers Foundation, prize of 50.000 DKR. Received in January 2005 and given for the contribution in the exploration of multiple sclerosis

7.2. International and national committees

International committees:

European Charcot Foundation for Research in Multiple Sclerosis. Professor, dr.med. Per Soelberg Sørensen, member of the executive board.
Executive board: Prof. O.R. Hommes, prof. L. Kappos, prof. G. Comi, prof. H. Lassman, prof. P. Soelberg Sørensen

Sylvia Lawry Center for MS Research. Per Soelberg Sørensen, member of clinical working group

International Medical Advisory Board of the Multiple Sclerosis International Societies. Professor Per Soelberg Sørensen, member

Advisory Committee on Clinical Trials of New Drugs in MS of the American National Multiple Sclerosis Society. Professor Per Soelberg Sørensen, member

Scientific panel on Demyelinating Diseases of the European Federation of Neurological Societies. Professor Per Soelberg Sørensen, chairman

EFNS task force on Anti-interferon-beta Antibodies in Multiple Sclerosis. Professor Per Soelberg Sørensen, chairman

Publication committee of the European Federation of Neurological Societies. Professor Per Soelberg Sørensen, chairman

Rehabilitation In Multiple Sclerosis (R.I.M.S.). Chief neuropsychologist Agnete Jønsson and Mads Ravnborg, MD, members of the Executive Board

Special Interest Group (S.I.G.) on Neuropsychology and Psychology. European interest and working group within R.I.M.S. Chairman: Päivi Hämälainen, co-chairman: Agnete Jønsson

National committees:

Research Committee of the Danish Multiple Sclerosis Society: Professor Per Soelberg Sørensen, chairman

Danish Multiple Sclerosis Group: Professor Per Soelberg Sørensen, chairman

Research Committee of Copenhagen University Hospital, Rigshospitalet: Professor Per Soelberg Sørensen, vice-president

The Treatment Board of the Danish MS Society: Professor Per Soelberg Sørensen, chairman, Agnete Jønsson, member

The Expert Panel of The Danish Multiple Sclerosis Society: Agnete Jønsson, member

Danish Society for Research in Multiple Sclerosis, (DAREMUS): Annette Oturai, treasurer

7.3. Congress and meeting organization

The European Charcot Foundation Symposium 2004. Taormina, Italy, November 2004. Professor Soelberg Sørensen, member of the organizing committee

7.4. Pre- and postgraduate teaching (incl. Osval supervision)

OSVAL supervision by Morten Blinkenberg: Medical student Lars Bjerregaard with the thesis "Monoclonal α_4 β_1-antibodies for treatment of Multiple Sclerosis"

8. PROSPECTS 2005 AND ONWARDS

In the MS Clinic an increasing number of patients from all over the country will receive specialized immunosuppressive therapy with Mitoxantron. Patients will be enrolled in several randomized trials of new immunomodulatory products, and, in particular, in trials employing combination therapy for multiple sclerosis.
The main research activities will include the following domains: Management of the MECOMBIN, IVIMS and NORMIMS clinical trials. These studies are investigator driven international multi-center studies initiated and directed by senior physicians from the Copenhagen MS Center. In collaboration with the other members of the Danish Multiple Sclerosis Group the Danish Multiple Sclerosis Research Centre will study the effects of neutralizing antibodies on the biological response to interferon-beta and assess measures to prevent and the occurrence of neutralizing antibodies the possibilities of remove neutralizing antibodies.
The Neuroimmunology Laboratory has procured a real-time PCR system, and much interest will be centered on new projects employing molecular

biology. A number of studies will focus on immunological changes induced by disease modifying therapies utilizing real-time PCR, gene-chip and proteomic technologies.
The collaboration with the MRI Center at Hvidovre Hospital will be intensified in projects that will use new MRI technologies to assess treatment response in patients receiving immunomodulatory therapy and to correlate MRI changes with cognitive deficits in newly diagnosed MS patients.
The MS Research Unit will continue the collaboration with partners in the Nordic MS Genetics Consortium and extend the collaboration to the European MS Genetics Collaboration (GAMES).
Experimental studies will be made seeking new compounds with effect on experimental autoimmune encephalomyelitis as a model of MS. We anticipate increasing the number of staff members at the Copenhagen MS Center and welcoming these new colleagues. Finally we are looking forward to continue and extend our collaboration with current and new national and international collaborators.