Dear Colleague,

Denmark may be the country with the highest prevalence of Multiple Sclerosis (MS) in the world. MS being the most common non-traumatic neurologic disorder among young people, there is a significant unmet medical need to better understand the cause and potential treatments helping MS patients. This is why Biogen Idec hosted the first MS research symposium in Denmark earlier this year.

We were truly overwhelmed by the breadth and depth of dedicated MS research in Denmark. More than 20 PhD and Post Doc projects were shared between more than 80 participants. Participants’ feedback indicates that the meeting may spark off new collaboration initiatives and to capture the knowledge, share ideas and inspire further work we hope you find this booklet useful.

We are proud to be part of this journey and confident there is hope for a fuller life for all MS patients.

Thank you

Biogen Idec
Jens Leander Johansen, Medical Advisor
Christoffer Jensen, Head of Medical Affairs

The content has been approved by the speakers, respectively, however it does not necessarily represent the viewpoints of Biogen Idec.
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The impact of genetic mutations is difficult to predict. Genetic mutations, that exert a large effect, can be deleterious – whereas mutations with only a small effect can be passed on – but enriched over time in specific populations. The fact that a heritable trait can be detected leaves only little understanding of the clinical impact of this particular trait or mutation. In fact, genetically inherited diseases have a variety of genetic traits – and some will be enriched in a specific disease population such as the MS population. Genome-wide association studies (GWAS) have successfully identified genetic variants associated with MS but the key to understanding the risk of developing the disease lies in functional studies that aim at uncovering the causal and hence the clinical impact of a specific variant.

A single nucleotide polymorphism (SNP) in the TNFRSF1A gene encoding for tumour necrosis factor receptor 1 (TNFR1), has been discovered through GWAS to be associated with MS. This particular SNP is, however, not associated with other autoimmune conditions such as rheumatoid arthritis, psoriasis, or Crohn’s disease. The SNP, rs1800693, has now been identified as the causal variant in the TNFRSF1A region. Functional studies have demonstrated that the MS risk allele directs expression of a novel and soluble form of TNFR1 that blocks TNF. A clinically important impact of this finding is that TNF-blocking drugs can provoke the onset of an exacerbation of MS while simultaneously efficaciously treating a number of other autoimmune diseases without this SNP association. These functional studies support the clinical experience with these drugs, implying the disease association of rs1800693, because the MS-associated TNFR1 variant mimics the effect of TNF-blocking drugs.

Patient selection for TNF-blocking drugs can therefore be aided by comparing GWAS across common autoimmune diseases and by investigating the functional consequences of the disease-associated genetic variation.

Keynote talk

Genome-wide association studies in MS – Recognizing clinical utility

Lars Fugger, University of Oxford

The illustrations herein have kindly been provided by Lars Fugger

Key points

- Genome-wide association studies (GWAS) have successfully identified genetic variants associated with MS
- A single nucleotide polymorphism (SNP) in the TNFRSF1A gene encoding for tumour necrosis factor receptor 1 (TNFR1), has been discovered through GWAS to be associated with MS
- A clinically important impact of this finding is that TNF-blocking drugs can provoke the onset of an exacerbation of MS while simultaneously efficaciously treating a number of other autoimmune diseases without this SNP association

Reference

The morning parallel session was chaired by Professor Jette Frederiksen from Glostrup University Hospital and our scientists presented their work on eye diseases related to the CNS. Nasrin Asgari, Reza Khorooshi, and Helle Hvilsted – all from University of Southern Denmark, are studying neuromyelitis optica (NMO) and presented completed and ongoing research projects in clinical NMO and experimental models for NMO.

In addition MRI in optic neuritis appears to have a highly predictive value for developing multiple sclerosis. This was one of the findings of a literature study conducted at Glostrup University Hospital.
Neuromyelitis optica (NMO): An autoimmune disease of the CNS

Nasrin Asgari, University of Southern Denmark

Definition
- NMO (also known as Devic’s Syndrome) is an inflammatory, demyelinating disease of the CNS
- NMO is an autoimmune disease of the CNS
- NMO is not multiple sclerosis and not even a variant of MS

Clinical characteristics: Patients typically present with optic neuritis (ON), transverse myelitis (TM) and with heterogeneous brain lesions. The majority of patients are seropositive for anti-aquaporin 4 (AQP4) antibodies. Longitudinal extensive transverse myelitis (LETM) lesions including three or more vertebral segments or more, limited TM starting in the cervical spine and reaching into the brainstem may lead to respiratory failure and/or persistent intractable hiccups and nausea, both of which are regarded as typical for NMO. The brain lesions are often localized at sites of high AQP4 expression.

Key points
- Incidence rates among Caucasians were 0.4 / 100,000
- NMO patients had frequent co-existence of other autoimmune diseases and frequent family occurrence of NMO and MS
- HLA-DQB1*0402 was associated with NMO, but no association of the PTPN22 (1858 C/T) SNP in NMO
- A PD-1 mutation in NMO is a novel finding, suggesting an involvement of the PD1-PD-L pathway in the pathogenesis of NMO
- Intrathecal injection of autoantibody with complement induced pathology when directly introduced into the CSF

Aim
- Develop translational research methods for NMO by integrating clinical, immunological, and experimental studies
- Investigate whether autoimmunity contributes to the pathogenesis of NMO

How
- Conduct a population based study of NMO in Caucasians through clinical files, web-based questionnaire, clinical database, clinical examination and re-evaluation, additional MRI of CNS, and measurement of anti-AQP4 antibodies
- To investigate immunogenetic and autoimmune aspects of NMO
- Based on the knowledge of the distribution of AQP4 expression in mammalian CNS and clinical observations in NMO patients, purified immunoglobulin G (IgG) from an AQP4 antibody-positive NMO patient (NMO-IgG) and human complement (huC’) was given intrathecally as a single injection to naïve mice

References
Background

- Neuromyelitis optica (NMO) is a severe inflammatory demyelinating disease of the CNS.
- Most NMO patients have immunoglobulin (IgG) auto-antibodies in their serum that bind to the water channel protein aquaporin-4 (AQP4), expressed by astrocytes.
- Auto-antibody mediated astrocyte injury is implicated as a primary event in NMO.
- The link between astrocyte damage and demyelination is yet to be identified.
- IFN-β, a member of type I IFN family, is a first line therapy for treatment of multiple sclerosis.
- Treatment of NMO patients with IFN-β has no effect or leads to a worsening of symptoms.
- The role of type I IFN signalling in NMO is unknown.

Animal models for NMO

Allows the study of mechanisms behind NMO.

How

By injecting IgG from NMO patients.

- Intravenously; it does not cause lesions in normal animals, but exacerbates CNS inflammation in animals when pre-existing CNS inflammation is present.

- Intracerebrally (+ human complement); it causes lesions in normal animals.
- Intracerebrally (+ human complement); it causes lesions in normal animals.

Characteristic histological features of NMO

- Loss of AQP4 expression
- Loss of glial fibrillary acidic protein (GFAP) expression
- Deposition of activated complement component
- Inflammatory cell infiltration
- Demyelination

Findings and conclusion

Intracerebral co-injection of NMO-IgG with huC' induced loss of AQP4 and GFAP.

The loss of AQP4 and GFAP induced by intracerebral co-injection of NMO-IgG with huC' was markedly less pronounced in IFNAR deficient than in wild type mice (Image 1).

* The animal model suggests that NMO-like pathology is dependent on Type I interferon response.

Aim

To investigate the involvement of Type I IFN signalling in the formation of NMO-like lesions in the brain of mice by intracerebral co-injection of NMO-IgG + human complement.

References

Neuromyelitis optica (NMO) - identifying biomarkers and potential treatment targets

Helle Hvilsted, University of Southern Denmark

**Background**
- Neuromyelitis optica (NMO) is characterized by inflammation of the optic nerve (optic neuritis) and the spinal cord (transverse myelitis)
- 60-80% of NMO patients have antibodies directed against the water channel Aquaporin 4 (AQP4)
- Differentiation from MS is vital, since MS treatments can exacerbate NMO and cause severe disability

**Terminology**
- NMO can manifest itself as NMO-spectrum disorders (NMO-SD) including bilateral/relapsing inflammatory optic neuritis (BON/RION) and longitudinal extensive transverse myelitis (LETM)
- NMO-SD can mimic early MS

**What**
- Identify potential biomarkers in the cellular and molecular composition of cerebrospinal fluid, blood and urine
- Investigate auto-antibodies in sera of NMO, NMO-SD, MS and healthy subjects (HS)
- Investigate urine proteome, post translational modifications and metabolome by liquid chromatography, based mass spectroscopy and nuclear magnetic resonance spectroscopy

**How**
- Investigate samples from a Hungarian biobank + database in Pecs including 120 AQP4+ NMO/NMO-SD patients, 48% NMO-SD, and >300 MS patients with paired samples of urine, sera and CSF
- Database and biobank established in Odense

**Key points**
- Proteome analysis of urine revealed 274 proteins differentially expressed in AQP4+ NMO, MS compared to HS
- Future analyses involve testing for proteins differentially expressed in AQP4+ NMO compared to MS
- Validation by testing in plasma/sera, CSF of AQP4+ NMO and NMO-SD also in AQP4- NMO and NMO-SD

Helle Hvilsted conveyed the perspectives on identification of biomarkers for NMO that may help early diagnosis of NMO and especially NMO-SD, allowing early differentiation from MS, enable early intervention, and prevention of disability. She finally addressed the possibility that biomarkers may help identify molecules, which can be potential targets of future treatments.
Background
■ Optic neuritis (ON) is the first symptom of MS in 20% of cases
■ Far from all patients with ON develop MS

What
■ A literature review was performed by medical student Nadia Poulsen under the supervision of Professor Jette Frederiksen in order to investigate the predictive value for MS with MRI in ON patients

Questions
■ How many ON patients have abnormal findings in cerebrum MRI and develop MS at a later stage?
■ What is the time span between onset of ON and onset of MS?

Key points
■ Proteome analysis of urine revealed 274 proteins differentially expressed in AQP4+ NMO, MS compared to HS
■ Future analyses involve testing for proteins differentially expressed in AQP4+ NMO compared to MS
■ Validation by testing in plasma/sera, CSF of AQP4+ NMO and NMO-SD also in AQP4- NMO and NMO-SD

The predictive value of MRI of the brain in patients with optic neuritis for developing MS

Jette Frederiksen, Glostrup University Hospital
Professor Zsolt Illes from University of Southern Denmark served as the chair of the morning parallel session of the symposium and presented parts of his work discussing the hypotheses involved in demyelination. Furthermore, Bente Finsen and Christina Fenger – also from University of Southern Denmark – presented their research contributing to a better understanding of the oligodendrogenesis.

SESSION 1B – DEMYELINATION / REMYELINATION
The neglected part of MS: Systems biology of demyelination
Zsolt Illes, University of Southern Denmark

Background
- Examination of molecular mechanisms of de- and remyelination is difficult in the classical model of MS, experimental autoimmune encephalomyelitis (EAE), due to the heterogeneous pathology.
- The molecular pathways of cuprizone-induced oligodendrocyte death and demyelination are similar to those observed in pattern III MS plaques.
- MicroRNAs (miRNAs) regulate gene expression by mRNA degradation or repression of translation.

What
Investigation of the expression of 627 murine miRNA by Agilent array in the isolated corpus callosum during cuprizone-induced demyelination as well as early and late remyelination following suspension of cuprizone.

How
- Differential expression of three miRNAs was validated by real-time RT-PCR. Up-regulated expression of one miRNA was also observed during postnatal myelination.
- The effect of cuprizone treatment was CNS-specific, although expression of two miRNAs is characteristic of the spleen and thymus, respectively.
- Potential target mRNAs was evaluated in two ways: data base search and examination of differentially expressed genes using Agilent gene expression array.

Key points
- Pathway analysis of predictive targets identified axonal guidance among others. Despite of the preserved oligodendrocytes in the normal appearing white matter (NAWM), miRNA expression was altered similar to corpus callosum, which may indicate different sensitivity of oligodendrocytes to mitochondrial cell death.
- At present, miRNA functional silencing experiment, proteomics to examine translation repression and examination of gene expression during EAE are underway to identify molecular regulatory networks involved in myelination, which can be potential future treatment targets.
The function of Nkx2.2 in oligodendrocytes and their progenitor cells

Christina Fenger, University of Southern Denmark

Background

Nkx2.2 contains both an activator and a repressor domain – and has the ability to act as transcriptional activator and repressor depending on co-expressed transcription factors and co-factors.

Questions

Does Nkx2.2 promote remyelination of demyelinated axons? Does Nkx2.2 act as a repressor and an activator at different stages of oligodendrogenesis, at a still incompletely known set of target genes?

Thus the objective of the study is to identify the Nkx2.2 target genes in oligodendrocytes progenitor cells (OPCs) and oligodendrocytes (Ols), and to clarify whether Nkx2.2 activates or represses the expression of these genes.

How

Through chromatin immunoprecipitation combined with DNA sequencing, the primary Nkx2.2 target genes in murine OPCs and in vitro differentiated Ols are identified. The transcriptome in OPCs/Ols containing CNS structures of postnatal Nkx2.2 deficient and wild type mice are identified by microarray, qPCR and in situ hybridization.

(A) In situ detection of Nkx2.2 mRNA and one of the Nkx2.2 target genes encoding for myelin basic protein (Mbp) in medulla of postnatal wild type (first column) and nearly absence in Nkx2.2 deficient (second column). Combined in situ mRNA detection and Mbp immunostaining in wild type mice (third column) shows that Nkx2.2, like Mbp, is expressed in pre-myelinating/myelinating Ols with Mbp+ cell processes. Md, medulla. Scale bars: 20 µm.

(B) Nkx2.2 ChIP-Seq results for part of the Mbp gene in oligodendrocytes (Ols) after in vitro differentiation and in their progenitor cells from postnatal wild type mice. Significantly enriched sequences corresponding to the peaks marked by stars suggest that Nkx2.2 has several binding sites in the Mbp gene, whereby Nkx2.2, in agreement with panel (A), contributes to the regulation of the Mbp expression at transcriptional level.

The illustrations herein have kindly been provided by Christina Fenger

Key points

The preliminary findings are that Nkx2.2 contributes to the regulation of the transcription in several genes encoding for proteins involved in:

- OPC cell division
- Myelination, myelin compaction, and axonal protection by oligodendrocytes
Stimulation of adult oligodendrogenesis by myelin reactive T-cells

Bente Finsen, University of Southern Denmark

Background

- Remyelination of demyelinated axons in plaques in patients with MS reinstates nerve conduction and protects against secondary axonal degeneration
- Remyelination occurs in acute plaques and in experimental models of de- and remyelination
- Remyelination is often limited or absent in plaques in patients with progressive and chronic MS

What

Investigate possible mechanisms of regeneration of myelin in MS-experimental models

How

Experimental modelling of axonal transection has been used based on the perforant pathway or ECL lesion paradigm. This model is based on the well-characterized anatomy of the dentate gyrus, which is part of the hippocampal formation, and the perforant pathway which is the main afferent projection to the dentate gyrus. Adoptive transfer of T cells reactive to the myelin basic protein or protein proteolipid protein into the area with degenerating fibers and terminals stimulated oligodendrocyte precursor cell (OPC) proliferation and the formation of new oligodendrocytes, microglial clearance of myelin debris, and axonal sprouting. These results demonstrate that infiltrating myelin reactive T cells in addition to their damage-inducing effect, also have significant trophic effects. A microarray study was performed to identify genes with trophic effect on OPC and oligodendrocytes.

Key points

- Stimulation of OPC proliferation and oligodendrogenesis can be mediated by myelin reactive T cells
- T cells enhance microglial clearance of myelin debris
- T cells increase axonal-lesion induced sprouting
- Characterization of four genes of putative interest is ongoing
Regulation of inflammation in the CNS

Trevor Owens, University of Southern Denmark

Multiple sclerosis is an inflammatory demyelinating disease. 73% of lesions have an autoimmune appearance and the pathology and the response to immunotherapy suggest an autoimmune aetiology, involving CD4, CD8 T-cells, antibodies and macrophages as both effectors and regulators of pathology. MS therapies focus generally on either blocking access to the CNS or immunoregulation.

A T-cell model of the immunopathogenesis in MS underlines the immunological dogma behind MS. T-cells must migrate from lymph nodes and be re-activated, and hence they require co-stimulation. Entry to the CNS depends on an antigen presenting step which occurs on a perivascular basis. Cells in perivascular locations are not always detectable by gadolinium-enhanced MRI.1

In vivo mouse models are useful in the exploration of inflammation in the CNS. Although mouse models for progressive MS are essentially not available, findings from the EAE model yield valuable information about the inflammatory processes occurring during MS.

The role of microglia

Microglial cells play an important role in the inflammatory processes in MS. Activated microglial cells do not look very different from macrophages; however, they can be distinguished by flow cytometry. Microglia can present antigen in the CNS. CD11c positive or negative microglia can be distinguished and show functional differences from blood-derived dendritic cells (DC) in CNS. There are increased numbers of PD1 positive microglia in the inflamed – and demyelinated CNS. The ability of these cells to present antigen appears equivalent to DC, especially for secondary T-cell responses.2

Type 1 interferons, including IFN-β, appear to be natural endogenous regulators of neuroinflammation and are produced by glial cells. They show opposite effect in neumyelitis optica, which we have re-produced in mice lacking the IFNAR receptor.3 The endogenous cytokine regulator IL18 binding protein (IL18bp) is also produced in the CNS by microglia and macrophages. Since overexpression of IL18bp inhibited Th17 responses and EAE, this represents a mechanism whereby microglia regulate inflammatory cytokines.4 Furthermore, it is well-known that, in the absence of IFN-γ, mice get worse EAE, indicating that IFN-γ also inhibits CNS inflammation. This conflicts with observations in a trial of IFN-γ in MS, and represents a disconnection between this animal model and MS.

Neuroprotection

With respect to neuroprotective effects the CD11c positive cells and regulatory T-cells, especially those that act via production of anti-inflammatory cytokines, may play a role in neuroprotection.

CD8+ T-cells are reported to be more numerous than CD4+ T-cells in MS lesions. Amplification of TCR gene rearrangements and sequence analysis has revealed that the majority of the CD8+ cells belong to a few clones with signs of selection. Animal experiments allow isolation of CD8+ and CD4+ T-cells from the CNS for further analysis. IL17 producing CD8+ cells have not been identified by us, whereas IFN-γ producing CD8+ cells are numerous, data were also presented on chemokine receptor expression by CNS-infiltrating T-cells, suggesting greater heterogeneity in association of CXCR3 and CCR6 with CD4+ T-cell subsets than currently appreciated. More severe EAE in mice immunized with recombinant MOG1-125 than in mice immunized with MOG35-55 correlated better with IFN-γ-producing CD4+ T-cells than with Th1 or CCR6+ cells.

References

Associate Professor Thor Petersen from Aarhus University Hospital was the chair on the afternoon parallel session where epidemiology, aetiology, and risk factors associated with multiple sclerosis was discussed. Claudia Pfleger from Aalborg University presented a large register study investigating how civil status, children, and employment affect the social prognosis of MS patients. Stephanie Binzer from University of Southern Denmark discussed the hypothesis behind an ongoing research project aiming at investigating MS at the Faroe Islands as an example of how the disease develops in isolated populations. Finally, Thor Petersen himself gave an overview of the hypothesis behind viral infections as a risk factor for developing MS.
The social prognosis for MS patients

Claudia Pfleger, Aalborg University Hospital

Background

- MS is frequent and often affect young patients
- MS causes cumulative handicap and we have detailed knowledge concerning clinically course but our knowledge about social prognosis is rather poor

What

A historically prospective cohort study was performed, including 2,538 MS patients with onset between 1980 and 1989. The study investigated change over time from disease onset till reaching significant social events defined by preservation of partnership, staying employed, and sustained level of income. The control group consisted of 20 persons per patient, matched by age and gender.

How

Linked with IDA (Integrated Database for Employment market – Denmark Statistics)

Statistics: Actuarial life table methods and Cox proportional-hazards Regression for survival data

Key points

Partnerships:

- Proportion of MS patients who end their partnership is higher
- Patients with late onset have higher risk of breaking up
- Among patients with early onset, male gender and absence of little children are associated with worse outcome

Employment:

- Risk factors among MS patients for early drop out from work force:
  - Onset symptom from efferent nerve fibres (HR 0.61)
  - Physically work (HR 0.70)
  - Female gender (0.71)

Income:

- All patients compared to control persons:
  - Income starts on same level, but patients’ income decrease due to low rate of disability pension
- Comparing only active working patients and control persons:
  - same income development

Kaplan-Meier curve depicting the cumulative probability of MS patients remaining in the work force after MS onset. 30 % of MS patients receive disability pension after five years, and after 24 years 83 % of MS patients receive disability pension. Among controls 9 % and 17 % receive disability pension after a period of five and 24 years, respectively.

The illustration is kindly provided by Claudia Pfleger.
Background

- Multiple sclerosis has the highest incidence in the temperate zones.
- Epstein-Barr virus (EBV) is associated with MS, is independent of vitamin D levels, and seems to be particularly true for symptomatic forms of EBV infections.
- Recent data support that the proportion of EBV seropositives is related to the latitude, possibly explaining the higher incidence of MS in temperate zones of the world.

Antibodies to specific EBNA-1 domains and HLA DRB1* interact as risk factors for MS. EBV appears to be related to MS disease activity and to affect the clinical response of interferon-β therapy. The pathogenic mechanisms of EBV in the development of MS could involve: molecular mimicry, a change in B-cell function, or immune dysregulation.

Expression levels of HERV antigens have been suggested as markers for MS activity as they are up-regulated in MS PBMNCs and anti-HERV antibodies are up-regulated in MS serum.

There is a significant association between multiple sclerosis and the locus on chromosome X harbouring the human endogenous retrovirus HERV-Fc. This band contains no other known coding capabilities, suggesting that this virus plays a role in the etiology of multiple sclerosis.

Key point

- Human endogenous retrovirus and certain members of the herpes-virus group, as well as the antiviral immune response, may play a role in MS development.
- The combination of HLADRB1*, smoking, vitamin D deficiency, and symptomatic EBV infection increases the risk of developing MS.

Vaccinations against EBV has not been demonstrated to be 100% - preventive of mononucleosis.

References

Multiple sclerosis in isolated populations
Stephanie Binzer, University of Southern Denmark

Background
- Only 30% of the disease heritability in MS is accounted for
- Isolated populations are more genetically homogeneous and characterised by founder effects and consanguineous marriages

What
An ongoing research project at the University of Southern Denmark in collaboration with Karolinska Institute, Stockholm, is investigating the genetics of multiple sclerosis on the Faroe Islands.

Why
- The prevalence of MS in isolated populations is often increased
- Founder effect may result in rare gene variants inherited through generations, and detecting these rare variants is easier in isolated populations
- Consanguineous marriages result in amplification of those genes

How
- Large genome-wide association studies can detect common variants
- In the ongoing study, 47 Faroese MS patients and matched controls are studied
- Analysed with illumina chip ~ 1 million SNPs
- Detailed questionnaire about lifestyle and environmental exposures
Head of Laboratory, PhD, Poul Erik H. Jensen from Danish Multiple Sclerosis Center at the Rigshospitalet chaired an afternoon parallel session on biomarkers and inflammation. Stig Præstekjær from Glostrup University Hospital presented work on how to investigate the blood-brain-barrier permeability in MS by means of MRI and Poul Erik H. Jensen himself presented and discussed the impact of anti-drug antibodies during treatment of MS patients.
Abnormal blood-brain barrier (BBB) permeability in MS investigated by MRI

Stig Praestekjaer Cramer, Glostrup University Hospital

Background
- Active MS lesion formation is associated with blood-brain barrier breakdown
- Post mortem microscopic data have revealed decreased TJ protein expression in inactive MS lesions
- Several MRI techniques have indicated structural abnormalities in normal appearing grey and white matter

Key points
- There is significantly increased BBB permeability in normal appearing white (NAWM) of MS patients compared to controls, but not in other tissue types
- Blood flow is not decreased in this subgroup of relatively young relapsing remitting MS patients
- Decreased permeability was found in non-enhancing lesions compared to NAWM
- New MRI lesions implicated increased BBB permeability in non-enhancing lesions

Questions
- Is blood-brain barrier permeability increased in apparently healthy MS tissue?
- Is blood brain barrier permeability dependent on MS clinical characteristics – such as EDSS, years since MS onset, total MRI lesion load, new MRI lesion activity, and treatment status

How
- MRI T1 weighted perfusion method utilizing gadolinium-based contrast agent to assess various hemodynamic measurements such as cerebral blood volume, cerebral blood flow, mean transit time, and permeability of the blood-brain barrier
- 18 RRMS patients and 16 healthy controls matched for age and sex were included
  - MS patients were referred with intention to start 2.line treatment (natalizumab or fingolimod)
  - 15 patients were treated with IFNbeta (n=13) or glatiramer acetate (n=2) and 3 patients received no current treatment at time of investigation.

Fig. 1. Four T2-weighted MRI slices of an MS patient (top row) and corresponding maps of blood flow (bottom left) and blood-brain barrier permeability (bottom right). A contrast enhancing lesion (red arrow) is clearly visible on the permeability map, but does not exhibit higher blood flow compared to the surrounding tissue.

Fig. 2. A) Boxplot of blood-brain barrier permeability in normal appearing white matter showing significantly higher values in MS patients compared to controls (p=0.00004; 2-tailed T test). B) Boxplot of blood flow in same area as A, not showing any significant differences between MS patients and controls.

The illustrations herein have kindly been provided by Stig Praestekjaer Cramer

Stig Praestekjaer Cramer drew perspectives from his presentation on abnormal blood-brain barrier permeability in MS on whether second line treatment potentially normalizes BBB leakiness, leaving a possibility to monitor treatment efficacy
Analysis of anti-drug antibodies in multiple sclerosis patients in treatment with natalizumab or interferon-β

Poul Erik H. Jensen, Rigshospitalet

Background

- Long-term use of recombinant-based therapeutics, such as interferon beta (IFN-β) can lead to an immune response to the drug and the occurrence of binding antibodies.
- A fraction of those antibodies are NAb; neutralizing antibodies that inhibit or reduce the functional activity of the biologic drug molecule, as determined by an in vitro test method, regardless of its in vivo relevance.
- For patients on IFN-β treatment, the presence of NAb, especially in persistent high titers, is associated with reduction of clinical effectiveness of the treatment.
- All currently available IFN-β formulations induce NAb in IFN-β treated MS patients and different IFN-β formulations show different immunogenicity.

How

- The luciferase assay is based on luciferase induction (luminescence) in response to IFN-β stimulation of cells manipulated to express the luciferase gene; if NAb is present in serum, luciferase induction is blocked and NAb titer can be determined.
- MxA is endogenously produced in cells in response to IFN, and can thus be used as a biomarker for IFN-β response in patients; samples for analysis are taken 4-12 hrs after IFN-β administration and MxA mRNA is extracted and quantified by qPCR and an MxA index is determined.

NAb from natalizumab

- Among Danish patients treated with natalizumab, 4-6% became persistent positive for antibodies to this drug.
- It was noted that high antibody titers were often associated with the generation of persistent antibodies, and conversely, low titers were associated with the generation of transient antibodies.

Key points

- Clinical impact of NAb:
  - 28-47% of patients on IFN-β-1b treatment became positive for persistent NAb; 12-28% of patients treated with IFN-β-1a (sc) and 2-6% treated with IFN-β-1a (im) developed persistent antibodies to the drug.
  - All patients in IFN-β treatment should be analyzed for NAb every 6 months after initiation of treatment for up to 24 months, positive samples should be confirmed at 3-6 months interval, and two consecutive positive samples should lead to treatment shift.
  - 4-6% of patients on natalizumab became persistent positive for antibodies to this drug.
  - All patients in natalizumab treatment should be analyzed for NAb after 3, 6 and 12 months after initiation of treatment, but for patients positive after 3 months, an extra test should be performed after 5 months. Patients at least positive for NAb twice should after 6 months have treatment shift.

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  - All patients in IFN-β treatment should be analyzed for NAb every 6 months after initiation of treatment for up to 24 months, positive samples should be confirmed at 3-6 months interval, and two consecutive positive samples should lead to treatment shift.
  - 4-6% of patients on natalizumab became persistent positive for antibodies to this drug.
  - All patients in natalizumab treatment should be analyzed for NAb after 3, 6 and 12 months after initiation of treatment, but for patients positive after 3 months, an extra test should be performed after 5 months. Patients at least positive for NAb twice should after 6 months have treatment shift.

Analysis of anti-drug antibodies in multiple sclerosis patients in treatment with natalizumab or interferon-β

Poul Erik H. Jensen, Rigshospitalet

Background

- Long-term use of recombinant-based therapeutics, such as interferon beta (IFN-β) can lead to an immune response to the drug and the occurrence of binding antibodies.
- A fraction of those antibodies are NAb; neutralizing antibodies that inhibit or reduce the functional activity of the biologic drug molecule, as determined by an in vitro test method, regardless of its in vivo relevance.
- For patients on IFN-β treatment, the presence of NAb, especially in persistent high titers, is associated with reduction of clinical effectiveness of the treatment.
- All currently available IFN-β formulations induce NAb in IFN-β treated MS patients and different IFN-β formulations show different immunogenicity.

How

- The luciferase assay is based on luciferase induction (luminescence) in response to IFN-β stimulation of cells manipulated to express the luciferase gene; if NAb is present in serum, luciferase induction is blocked and NAb titer can be determined.
- MxA is endogenously produced in cells in response to IFN, and can thus be used as a biomarker for IFN-β response in patients; samples for analysis are taken 4-12 hrs after IFN-β administration and MxA mRNA is extracted and quantified by qPCR and an MxA index is determined.

NAb from natalizumab

- Among Danish patients treated with natalizumab, 4-6% became persistent positive for antibodies to this drug.
- It was noted that high antibody titers were often associated with the generation of persistent antibodies, and conversely, low titers were associated with the generation of transient antibodies.

Key points

- Clinical impact of NAb:
  - 28-47% of patients on IFN-β-1b treatment became positive for persistent NAb; 12-28% of patients treated with IFN-β-1a (sc) and 2-6% treated with IFN-β-1a (im) developed persistent antibodies to the drug.
  - All patients in IFN-β treatment should be analyzed for NAb every 6 months after initiation of treatment for up to 24 months, positive samples should be confirmed at 3-6 months interval, and two consecutive positive samples should lead to treatment shift.
  - 4-6% of patients on natalizumab became persistent positive for antibodies to this drug.
  - All patients in natalizumab treatment should be analyzed for NAb after 3, 6 and 12 months after initiation of treatment, but for patients positive after 3 months, an extra test should be performed after 5 months. Patients at least positive for NAb twice should after 6 months have treatment shift.
Multiple sclerosis (MS) is generally divided into subtypes of relapsing and non-relapsing forms. The individual role of inflammation versus degeneration during the progression of the disease is controversial. MS has a multifactorial aetiology with environmental (e.g. smoking and viral infections), immunological (e.g. inflammation, immune activation and regulation), and genetic factors individually playing a role and possibly affecting each other. Genetics help us understand why and how therapy works. Genome-wide association analysis of MS has revealed that variations near genes, and CD4+ T-cell responses to MBP are also associated with low expression of IFN-β induced with a positive effect in MS. Similarly an induction of IL10 has been demonstrated by exogenous IFN-β. IL10 is an anti-inflammatory cytokine, mediated by the induction of IL10 and, possibly, IL27 autoreactive CD4+ T-cells. It is possible that this is regulated by the activation of potentially pathogenic, blocking TNF-α and MS genetic studies suggest association between VCAM-1 and MS in MS patients who are disease free and patients who have activity, however, IL10 is expressed slightly higher after IFN-β when there is no disease activity. The CD4+ T-cell response to myelin basic protein (MBP) is associated with low expression of IFN-β induced genes, and CD4+ T-cell responses to MBP are also decreased in IFN-β treated MS. These findings suggest that both endogenous and therapeutic IFN-beta regulates the activation of potentially pathogenic, autoreactive CD4+ T-cells. It is possible that this is mediated by the induction of IL10 and, possibly, IL27 in monocytosis.

Second line therapy in MS with natalizumab and fingolimod targets cell migration of the pathogenic T-cells that are activated in the lymph nodes. Natalizumab decreases VLA-4 expression, CD134+,CD4+ T-cells blocking their migration into the CNS, and on top of this IL10 is upregulated while IFNy is downregulated inside the CNS, resulting in less inflammation. Fingolimod prevents lymphocyte egress from lymph nodes by resulting in an internalisation of the S1P receptor, and inhibition of lymphocyte egress along the S1P gradient.

The illustrations herein have kindly been provided by Finn Seiliebjerg.

Natalizumab is a humanized monoclonal antibody against alpha-4 (α4) integrin, which is required for T-cell adhesion and migration across the blood brain barrier. Among many effects, treatment recovery, and the impact of medical intervention on disability progression varies from 12-42% over 12 weeks and 24-53 % over 24 weeks in reduction of sustained disability progression1-5. Differences of effect rates may relate to the different modes of actions. Immunosuppression, immunomodulation, and selective intervention serve the purpose of first line therapy whereas current second line therapies primarily focus on anti-migration effects.

First and second line therapy INF-β is a first line therapy in MS. A time dependent induction of IL10 has been demonstrated by exogenous INF-β. IL10 is an anti-inflammatory cytokine of a positive effect in MS. Similarly an induction of IL27 is seen after exogenous INF-β. There is no clear difference between gene expressions pattern from patients who are disease free and patients who have activity; however, IL10 is expressed slightly higher after IFN-β when there is no disease activity. The CD4+ T-cell response to myelin basic protein (MBP) is associated with low expression of INF-β induced genes, and CD4+ T-cell responses to MBP are also decreased in INF-β treated MS. These findings suggest that both endogenous and therapeutic INF-beta future promises for treating MS lies in treating the relapsing-remitting and the secondary progressive forms with relapse. Little is known so far about the effect of medical intervention on progressive-relapsing MS. Overall relapse rate reductions reported from individual clinical trials span between 29-58 %1-4 and the impact of medical intervention on disability progression varies from 12-42% over 12 weeks and 24-53 % over 24 weeks in reduction of sustained disability progression1-5. Differences of effect rates may relate to the different modes of actions. Immunosuppression, immunomodulation, and selective intervention serve the purpose of first line therapy whereas current second line therapies primarily focus on anti-migration effects.

References
Associate Professor Finn Sellebjerg at the Rigshospitalet was the chair of the final session of the MS symposium rounding off with scientists presenting completed and ongoing studies on how to treat MS. Jeppe Romme from the Rigshospitalet presented the preliminary work on a phase 2 study in progressive multiple sclerosis. Henrik Boye Jensen from Odense University Hospital discussed the effects of medical intervention on walking capacity and muscle strength in MS patients. Ulrik Dalgas from Aarhus University Hospital advocated for exercise therapy both with the audience and in MS patients and presented a research project investigating this. Finally, Roberto Oliveri from the Rigshospitalet discussed how stem cells may represent a future treatment possibility in MS with a clinical trial on mesenchymal stem cell treatment recently being initiated.
Progressive multiple sclerosis
New views on pathogenesis and treatment development

Jeppe Romme, Rigshospitalet

Characteristics of progressive MS
- Primary progressive MS or secondary progressive MS
- Progressive development of irreversible neurological disability
- Gradual and accelerated disability development
- Relapses are rare or absent

The pathogenetic substrate underlying progressive MS disease activity is believed to be axonal damage and demyelination. It is however, controversial whether axonal damage is dependent of inflammation, and it is continuously discussed whether intrathecal inflammation is dependent on recruitment of immune cells from the systemic immune compartment.

Intrathecal inflammation
Pathology studies have demonstrated that widespread inflammation in cortical lesions, meningeal inflammation and inflammation in normal appearing white matter (NAWM) correlate with axonal damage and disease progression. These findings are supported by increased levels of biomarkers of inflammation (osteopontin (OPN), CXCL13, and matrix metalloproteinase-9 (MMP9) axonal damage (neurofilament light chain), and demyelination (myelin basic protein) in cerebrospinal fluid (CSF) and the inflammation found in the CSF correlates with the severity of axonal damage.

Systemic inflammation
Progressive MS patients have increased systemic inflammation and this is associated with disease activity and is characterized by activated follicular T-helper cells and Th17-cells.

Hypothesis
Systemic inflammation may contribute to intrathecal inflammation and disease activity, and a phase 2A clinical trial investigated specifically the effect of blocking the migration of systemic immune cells into the brain with natalizumab in progressive MS. 30 patients were screened, 12 SPMS and 12 PPMS patients were included. Of these 7 SPMS and 10 PPMS patients completed the study.

Results
The primary endpoint, CSF osteopontin concentration, was significantly reduced after 60 weeks of natalizumab treatment compared to baseline and the level of CSF osteopontin in non-inflammatory neurological disease controls. This indicates a beneficial effect on intrathecal inflammation, which remained significant in the subgroup analyses of both SPMS and PPMS patients.

Secondary endpoints included CSF markers for inflammation, axonal damage and demyelination, clinical endpoints and MRI endpoints and also demonstrated effect of natalizumab treatment after 60 weeks.

Key points
- Progressive MS patients have marked intrathecal inflammation which is associated with axonal damage and disease progression
- Progressive MS patients have increased systemic inflammation, including Th17-cells and activated follicular T-helper cells
- Natalizumab treatment of progressive MS reduces CSF markers of inflammation, axonal damage and demyelination
- Natalizumab treatment of progressive MS increases MTR in cortex and NAWM likely reflecting a beneficial effect on tissue integrity
Background on neurophysiology

There are three types of ion channels in the CNS. Some are constantly open when the neuron is resting, some are ligand activated by neurotransmitters, and some are voltage gated and open up when a critical voltage difference across the membrane is reached.

The resting action potential of the axon is -60 mV, very equal to the equilibrium potential of K+. By propagation of the action potential a transient change of membrane potential occurs. This change is primarily to the threshold value of approximately -45 mV and followed by a rising phase, overshoot, repolarization, and hyperpolarization.

In MS the effect of prolonged release fampridine on the action potential resembles the action potential as it was before demyelination, and thus the drug has an effect on walking speed and muscle strength in the lower extremities. Not all patients respond, however, and it appears to be clear quite soon whether a patient is a responder or not, as there is really no intermediate effect. The treatment is reversible – so when it is discontinued the effect disappears.

Prolonged-release fampridine

Effect on walking capacity, muscle strength, and much more

Henrik Boye Jensen, University of Southern Denmark

Key points

In summary the findings of the open label enrichment phase of FAME were the following:

- PR-fampridine improved walking speed and muscle strength in the lower extremities
- The MS-population seems to be dichotomized to the effect of PR-fampridine
- PR-fampridine was well tolerated and easy to use
- SSST was more sensitive to the effect of PR-fampridine than T25FW
- It seems that PR-fampridine had an effect on the lower extremities
- Additionally, an effect was found on the upper extremities, and possibly on cognition, and fatigue

FAME was an outcome measure study aiming at evaluating the relative sensitivity of clinical tests for the identification of MS patients that respond to PR-fampridine. Furthermore, the study compared the effect size of PR-fampridine treatment when assessed with the Six Spot Step Test (SSST) and the Timed 25 Foot Walk (T25FW). The study was a double-blind, randomized, placebo controlled, parallel group design preceded by open label enrichment. 114 subjects were randomized and 109 completed the study. Muscle strength was measured by dynamometry.
Exercise therapy and MS
Ulrik Dalgas, Aarhus University

Background

- MS patients used to be discouraged to exercise because it was thought to worsen symptoms
- 40% of patients do experience an increase in some symptoms and some are more fatigued – however, the worsening is temporary and ameliorated within 30 minutes.
- MS relapse is not associated with exercise – actually the most physically active tended to experience less relapses.

The effects of exercise therapy in MS have been controversial for many years. However, it is believed that exercise therapy has beneficial effects on inactivity induced physical impairment; whether exercise also has an effect on the disease process per se remains speculative.

What type of training is needed? Aerobic training improves endurance whereas resistance training strengthens the muscles by increasing the muscle mass.

Key points
Clinical studies in MS investigating the effects of exercise therapy overall conclude:

- Endurance training and resistance training to be safe and beneficial in MS
- Individual prescription is a necessity
- Worsening of symptoms is temporary
- Some effects are identical and some differ indicating that combined ET and RT may be optimal
- Exercise improves self-reported fatigue

References
Definitions and background

- A stem cell is a unique cell that can renew itself and differentiate into other more specialized cells.
- A human being has approximately 50 trillion cells which all are derived from the single totipotent fertilized egg (zygote).
- Adult mesenchymal stem cells (MSCs) can be isolated from a number of tissues including bone marrow, adipose tissue, and umbilical cord stroma. Given the right conditions MSCs can develop into a number of different tissues from the mesodermal germ layer, and perhaps transdifferentiate into neural progenitors.
- MSCs can home, meaning that when they are injected intravenously they can travel with the blood stream, adhere to the endothelial cells and migrate to damage /inflamed area once the destination has been reached.

On top of the regenerative capacity, stem cells are believed to hold potent paracrine by-stander mechanisms such as anti-apoptotic, immunomodulatory, angiogenic, and anti-inflammatory effects that altogether understandably make stem cells represent the hypes and the hopes in a number of cell degenerative diseases. In an experimental set-up MSCs transplantation has led to a decrease demyelination, reduction in T-cell infiltration, decrease in the level of macrophages and B-cells in the CNS, and reduced axonal damage.\(^1\)

The immunomodulatory effects of MSCs in MS are currently being investigated in an international multi-centre phase 2 randomized double-blind cross-over clinical trial in MS. The aim is to enrol 160 patients globally; 25 are to be enrolled at the Rigshospitalet.

Key points to the ongoing phase 2 MSC transplant study in MS

- The trial covers autologous MSCs versus placebo.
- MSCs are harvested from the patient’s bone marrow.
- After marrow processing, ex vivo cell expansion, and quality control, the MSCs are injected intravenously to the patient.
- Primary outcome is inflammatory activity on MRI.
- The results are expected around 2015.

Reference


Procedure

The illustrations herein have kindly been provided by Roberto S. Oliveri.