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## The Year in Multiple Sclerosis Research 2009

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# Foreword

## The best of multiple sclerosis research in 2009

A selection of the published research

### *2009 has been a curious year for multiple sclerosis research.*

We have seen several disappointing results from trials of interferon-beta or glatiramer, including a negative result from the first analysis of the UK risk-sharing scheme. It does seem that the halcyon days of these drugs are over, and all eyes are on new agents just around the corner..

At conferences, much of the discussion has been around the results of the phase 3 trials of two pills for the treatment of multiple sclerosis: cladribine and fingolimod. Patients are enthusiastic for the convenience of oral treatments and the presented data suggest both are more efficacious than interferon-beta and glatiramer. But the trials have yet to be published. So we have not had the opportunity to rummage in the crannies of the data; in particular, the safety profile of these two treatments needs scrutinising. But watch this space. I anticipate two or three high-profile publications in the first few months of 2010!

As the year has gone on, increasing numbers of people have been reported to have developed progressive multifocal leukoencephalopathy (PML) on natalizumab (Tysabri) treatment. We are now up to 27 cases amongst the 63,000 people who have taken the drug. Hopefully, 2010 will bring some clarity on the risk of PML, especially the important question of how it varies with duration of exposure to natalizumab.

The ACNR Prize For Wackiest MS Research In 2009 goes to the work of Prof Zamboni and his "liberation procedure" of angioplasty of the venous strictures he found in 65 Italian patients with multiple sclerosis, including his wife. This has generated much excitement amongst patient groups, but sadly his work to date is full of all the usual errors of lack of control and blinding. More sober researchers in Buffalo are going to attempt a replication with larger numbers.

Whilst all this gossipy stuff has dominated the internet chat rooms, some very important research has slipped into the journals, particularly in the fields of genetics, immunology and pathology. For this ACNR supplement, my research group have chosen our particular favourites. We have decided to include only studies that have been published, rather than summarise abstracts or conference proceedings, which can be difficult to critically analyse.

The ACNR Prize For The Most Important Piece Of MS Research In 2009 goes to the International MS Genetics Consortium's paper in Nature Genetics. I should declare an interest, as I share an office with one of the authors! But it is a seriously impressive bit of team work and I predict this will have the most far-reaching impact of any other research of 2009.

We are very grateful to Merck Serono who have kindly funded this supplement. But the choice of papers and prizes, and any mistakes, are entirely my responsibility.

*Alasdair Coles, Cambridge, UK.*

## A year in MS: Simple Summary for the Stressed

> **The thirteen genes now known to be associated with multiple sclerosis are: CD6, CD58, CLEC16A, HLA-B, HLA-DRB1, IRF8, IL2RA, IL7R, IL12A, OLIG3-TNFAIP3, PTGER4, RGS1 and TNFRSF1A) [MSG, Nature Genetics]** > Progressive multiple sclerosis may be caused by inflammation, but of a different nature from that which causes relapsing-remitting disease. Instead of being mediated by T cells from the periphery, progressive MS may be driven by plasma B cells contained within the brain, [Frischer, Brain] > **Obese girls, but not snuff sniffers, have an increased risk of getting multiple sclerosis [Munger, Neurology & Hedström, Neurology]** >

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Unlike last year, Epstein-Barr virus is now not considered to be found in the brains of all people with multiple sclerosis. [Willis, Brain] > Th17 cells are looking like the real bad guys of the immune response in multiple sclerosis [Brucklacher-Waldert, Brain] > In the UK risk-sharing scheme, people on interferon-beta actually did worse than would be predicted from the untreated natural history data, although this may reflect failings in the study design rather than the drug. [Boggild, BMJ] > Soon, we will see the phase 3 trial publications on cladribine and fingolimod, the first pills to be considered for licensing as multiple sclerosis treatments.

## A. Genetics

### More and more patients, more and more genes

Having discovered the association of multiple sclerosis with certain HLA types, in the 1970s, we heard nothing from the geneticists for decades. But the last four years or so have given us a rich seam of new genes to mull over. Two papers in 2009, published back to back in Nature Genetics, push the field further on.

The largest single genome-wide association study to date was published, from the Antipodes, in 2009: the ANZgene study included, after replication, 3,874 cases and 5,723 controls. The usual culprits were rounded up: HLA-DR15 and CD58, with less strong evidence for EVI5-RPL5, IL2RA, CLEC16A, IL7R and TYK2.

The news is that two novel risk-associated SNPs were identified: one on chromosome 12q13-14 and one upstream of CD40 on chromosome 20q13. Under the umbrella of the chromosome 12q13-14 SNPs lays a SNP associated also with rheumatoid arthritis and type 1 diabetes. The "best guess" as to the responsible gene in this area is CYP27B1, which encodes the enzyme 25-hydroxyvitamin D-1 alpha hydroxylase, which activates the inactive form of vitamin D. This is yet another line of circumstantial evidence that vitamin D and sunlight have something to do with the pathogenesis of multiple sclerosis. The SNP upstream of CD40 on chromosome 20q13 is in the same region as SNPs associated with rheumatoid arthritis and Graves' disease.

The other big genetics publication this year was the meta-analysis of previously published genome-wide association scans for multiple sclerosis susceptibility from the International MS Genetics Consortium. This is an awesome assembly of talent, databases, technical expertise, and analyses that has produced an over-rich meal of data that will take some years to digest. The sample size was an initial set of 2,624 subjects and 7,220 controls, followed by replication with 2,215 subjects and 2,116 controls. This study came up with the ACNR award for the most outstanding p value of 2009:  $3.8 \times 10^{-25}$  for the association of MS with HLA-DRB1!

The "usual suspects" in this screen included HLA-B, CD58, IL2RA, CLEC16A and IL7R. Then followed three newly identified

### *The ACNR most important paper of 2009 prize goes to genetic research from the IMMSG*

loci with genome-wide significance and 7 loci with "suggestive evidence". Of the three definite new loci, one was at TNFRSF1A, which encodes the tumour necrosis factor receptor superfamily member 1A that can activate NF- $\kappa$ B and so promote inflammation. Another was at IRF8 which encodes interferon response factor 8, which is a transcription factor of the interferon regulatory factor family. And the final definite gene was CD6, which is a marker of T cell activation.

Of other genes with less statistical evidence for an association with multiple sclerosis, there was strong supportive evidence for a true biological link through their association with other autoimmune diseases. For instance, IL12A and RGS1 are in strong linkage disequilibrium with coeliac disease susceptibility alleles. Also the PTGER4 allele is similar to those associated with Crohn's disease. This led the authors to compare and contrast their top 100 hits with the 76 highest Crohn's disease loci. As a result, seven loci were identified with substantial evidence of association in both diseases (IL12B, PTGER4, IRF8, BCL2, NEDD4L, PPA2 and STAT3).

The IMMSG authors then go on to show us what we should be doing with data of this kind. They focused on the interferon response genes, and probed the data from expression studies of the peripheral blood mononuclear cells from 240 subjects with either remitting-relapsing MS or a clinically isolated demyelinating syndrome who were untreated (n=82), on interferon b (n=94) or glatiramer acetate (n=64). They managed to map gene sets whose expression was linked to the IRF8 SNP in both the untreated and interferon beta-treated samples. They were then able to draw nice interlinked pathways of interferon-upregulation in multiple sclerosis..... But, if you stand back, that is a slightly odd thing to do, unless you believe that interferon-beta does absolutely nothing in multiple sclerosis. The authors seemed disappointed to find no correlation of

interferon-response genes between untreated multiple sclerosis and those on glatiramer acetate; but that could just mean that glatiramer acetate was having an effect! Just to prove that it is not all plain sailing, the SNP associated with IRF8 and MS did not appear to correlate with the expression of the IRF8 gene itself!

#### **So, what have we learnt from all of this?**

- Firstly, genetics is a highly complex business that requires a great deal of cooperation. For all the fuss about the number of samples needed to do these studies, we should remember that the limiting factor is our efforts as scientists and neurologists to collect them. There is no lack of people with multiple sclerosis willing to give up their DNA....
- Secondly, for all the talk of the possible non-immunological aetiologies of multiple sclerosis, the most validated multiple sclerosis susceptibility alleles are all immunological.
- Thirdly, there seem to be risk alleles shared between autoimmune diseases. So, we should be vigilant to gene discoveries in fields outside of multiple sclerosis.
- Finally, for all the excitement over gene discoveries, the next stage of analysis: stitching the genes to their protein expression, is likely to be more complicated, more difficult to do and more fun! – Alasdair Coles

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Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene).  
Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20.  
NATURE GENETICS  
2009 Jul;41(7):824-8.

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International MS Genetics Consortium.  
Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci.  
NATURE GENETICS  
2009 Jul;41(7):776-82.

## B. Epidemiology

### Obese teenage girls have increased risk of multiple sclerosis

So says Dr Munger, Dr Ascherio and colleague, a team from Boston that has made many an important epidemiological observation on multiple sclerosis.

Here they looked at the original data in the Women in the Nurses' Health Study (n=121,700) and Nurses' Health Study II (n=116,671) from when the women were aged 18. Over 40 years later, 593 of these patients had been diagnosed with multiple sclerosis. The headline result was that obesity at age 18 (body mass index > 30 kg/m<sup>2</sup>) doubled the risk of multiple sclerosis (multivariate relative risk pooled = 2.25, 95% CI: 1.50-3.37, p trend <0.001). There was no association between adult body mass and MS risk.

How can this be explained? Do obese teenage girls not go out into the sun, to top up those vitamin D levels? Or is there some fancy immunological connection between leptins and regulatory T cells (I read about that somewhere). Or is it something to do with texting? – Alasdair Coles

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Munger KL, Chitnis T, Ascherio A.  
Body size and risk of MS in two cohorts of US women.  
NEUROLOGY  
2009 Nov 10;73(19):1543-50.

### Stop smoking: sniff snus!

The Karolinska is a serious institute and can be relied upon to do important research. Here, for instance, is the pivotal study of Swedish snuff as a cause of multiple sclerosis. Thankfully, for those of us who like

to snort a line or two of the good stuff, the result was reassuring.

The background to this all was the release last year of a couple of studies which showed that smoking tobacco increased the risk of multiple sclerosis, and – for those who already had multiple sclerosis – smoking increases the rate of progressive accumulation of disability.

So, the Karolinska gathered up 902 cases of multiple sclerosis and 1,855 controls. Smokers had an increased risk of developing MS (odds ratio [OR] 1.4, 95% confidence interval [CI] 1.2-1.7 for women, and OR 1.8, 95% CI 1.3-2.5 for men). The risk increased with increasing cumulative dose (p < 0.0001) and remained for up to 5 years after stopping smoking.

In contrast, taking Swedish snuff for more than 15 years decreased the risk of developing MS (OR 0.3, 95% CI 0.1-0.8).

Hoorah! As a service to our readers, here is a recommended supplier of snus, <http://www.swedish-snus.com/>. I am a particular fan of Skruf Stark. – Alasdair Coles

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Hedström AK, Bäärnhielm M, Olsson T, Alfredsson L.  
Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis.  
NEUROLOGY  
2009 Sep 1;73(9):696-701.

### Multiple sclerosis is caused by bad veins

This has to take the biscuit. An Italian Professor of vascular diseases has discovered the cause of multiple sclerosis! And he can treat it!

Operating out of Ferrara, Prof Zamboni has shown that every single one of the 65 patients with multiple sclerosis that he has seen has had abnormal flow in cervical, thoracic or even abdominal veins, compared to none of the healthy controls. The azygous vein, in particular, was obstructed in multiple

sclerosis patients. Furthermore, different patterns of venous anomaly were associated with relapsing-remitting, primary and secondary progressive forms of the disease.

This paper in the JNNP raises an unorthodox proposal. So, Prof Zamboni needs to provide blue-chip evidence for his claims, which he fails to do. For instance, it would be standard practice in MRI assessments of MS clinical trials, to have the scans read by a blinded assessor. That has not happened with Prof Zamboni's venograms. We have to remember the unintentional bias of the enthusiast.

Not reported in the JNNP, but apparent from Zamboni's article in the Journal of Vascular Surgery, is that he treated all 65 patients with multiple sclerosis using percutaneous angioplasty. And the results are as impressive as you would find in any other uncontrolled, unblinded trial in multiple sclerosis.

I learnt from an article in Canada's Globe and Mail that Elena, Prof Zamboni's wife, has multiple sclerosis and has undergone the "liberation procedure" and her illness has "for all intents and purposes, gone".

– Alasdair Coles

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Zamboni P, Galeotti R, Menegatti E, Malagoni AM, Giancesini S, Bartolomei I, Mascoli F, Salvi F.  
A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency.  
JOURNAL OF VASCULAR SURGERY  
2009 Dec;50(6):1348-58.

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Zamboni P, Galeotti R, Menegatti E, Malagoni AM, Tacconi G, Dall'Ara S, Bartolomei I, Salvi F.  
Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis.  
J NEUROL NEUROSURG PSYCHIATRY  
2009 Apr;80(4):392-9.

## C. Pathology

### Where has all the EBV gone?

There has been a long history of implicating viruses in the aetiology of multiple sclerosis. And Epstein-Barr virus is Public Enemy No. 1 culprit at present. We thought its case had been truly cooked by Francesca Aloisi's assertion (Serafini J Exp Med. 2007 26;204(12):2899-912) that EBV could be found in brain-infiltrating B cells and meningeal follicles of nearly every person with MS and not in controls.

*Obese teenage girls have an increased risk of MS; but sniffing Swedish snuff reduces the risk!*

It all seemed to fit. The trouble is, other labs did not agree. There has been a lot of scurrying behind the scenes, ending up with this paper in *Brain* from the Brigham & Women in Boston, which shows by multiple methodologies (in situ hybridization, immune-histochemistry and two independent real-time PCRs for genomic EBV or EBV encoded RNA) that EBV is only rarely detected in the brains of patients with multiple sclerosis.

Oh dear. This has become a bit like a pantomime "Oh yes it is... Oh no it isn't". The explanation for the discrepant results must lie in the details of laboratory protocols. For what it is worth, I would put money on these Bostonians in *Brain*, but we will see. – Alasdair Coles

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Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, Roughton JE, Almendinger SE, Blewett MM, Brück W, Hafler DA and O'Connor KC.

Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain.

BRAIN

2009;132(Pt 12):3318-28.

## There is more inflammation in progressive multiple sclerosis than you might have thought.....

This paper very nearly won the ACNR best research of 2009 prize. I am sure this will disappoint Hans Lassmann, senior author and already winner of the Hoechst Prize, the Prize of the Sobek Foundation for Outstanding Multiple Sclerosis Research, and the Charcot Award of the Multiple Sclerosis International Federation for Life Long Achievements in Multiple Sclerosis Research.....still, you can't win 'em all.

The reason this paper is so good is firstly that it is extremely thorough. It is a painstaking pathological study of 67 post mortem multiple sclerosis brains, compared to 28 controls. Secondly, it addresses a key question in multiple sclerosis: what is the relationship between inflammation and neurodegeneration? There are various views on this already out there. One extreme, shall we say the "Lyon position" after Christian Confavreux, is that they are independent; so progressive multiple sclerosis inevitably

## *Progressive MS may be treatable by B-cell immunotherapies which cross the blood-brain barrier*

appears around the age of 40, whether or not there have been any preceding relapses, due to neurodegeneration. Another view, an ancient view, is that all phases of multiple sclerosis are due to on-going inflammation. A nuanced position (the "Cambridge position" after Alastair Compston) is that the early relapsing-remitting phase of multiple sclerosis is due to inflammation, which injures neurones in some way that they become vulnerable to long-term neurodegeneration which – once started – proceeds even when inflammation dies down. The implication of each for immunotherapy is very different: for those in Lyon, there is little point; for the "all is inflammation" crowd, people should be on immunotherapy till the grave; and for those in Cambridge, there is a window of opportunity early on in the relapsing-remitting phase of the disease to have a long-term impact.

So, Hans Lassmann and his Viennese group have systematically studied the brains of patients in various phases of the disease with 15 different antibodies to identify lymphocyte subgroups, myelin and neuronal loss (disturbed fast axonal transport and axonal swellings) by immunohistochemistry. What they found was surprising: inflammatory cells are not only present in early relapsing-remitting disease but also in progressive multiple sclerosis. The nature of the inflammation changes though, with T and B cells dominating relapsing – remitting brains and plasma cell infiltrates being found in those from both primary and secondary progressive patients. Throughout there was a tight correlation between the amount of inflammation and the degree of axonal injury. By the by, they also confirmed the old view that multiple sclerosis can "burn out"; in patients in their 70s with disease of 30 years' duration, there was no more inflammation than age-matched controls.

So, if inflammation drives all phases of multiple sclerosis, except at the very end, why is it that MRI markers of inflammation are infrequent, and why are immunotherapies so ineffective, in progressive disease? Lassmann suggests that in progressive multiple sclerosis "inflammation becomes trapped within the brain compartment behind a closed or repaired blood-brain barrier". Perhaps the secondary lymphoid follicles in the meninges of these patients become autonomous producers of plasma cells.

If that is correct, then the therapeutic implications are enormous. We should continue to treat progressive multiple sclerosis with immunotherapies, but only with agents that cross the blood brain barrier (or else give drugs intrathecally) and they should particularly target plasma cells (which makes rituximab redundant).

Ummm... now that puts the cat amongst the pigeons. – Alasdair Coles

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Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H.

The relation between inflammation and neurodegeneration in multiple sclerosis brains.

BRAIN

2009 May;132(Pt 5):1175-89.

## D. Immunology

### Which cell is the bad guy?

Multiple sclerosis (MS) is believed to be an autoimmune disease which starts with activation and proliferation of autoreactive CD4+ T helper cells in the periphery.

Originally Th1 cells were thought to be the pathogenic T helper subset. However there is growing evidence that 'Th17 cells' which secrete the pro-inflammatory cytokine interleukin-17 are key, at least to the mouse with EAE. Pathologically, MS lesions contain interleukin-17, interleukin-22 and interleukin 23 (Th17 related cytokines). But the evidence that IL-17 secretion is important to disease in humans has been lacking.

But two papers this year have established the importance of Th17 cells in people with multiple sclerosis.

Luca Durelli's team from Turin investigated the Th1/Th17 balance in relapsing-remitting MS patients with active or inactive disease and the effect of IFN- $\beta$ . Peripheral blood was taken from 22 healthy subjects (HS), 30 active MS (AMS) and 32 inactive MS patients (IMS). Th1 and Th17 cells were identified by flow cytometry. 'Active MS' (AMS) was defined as an exacerbation within 10 days confirmed clinically. Patients with 'inactive MS' (IMS) had no clinical or radiological disease activity. Patient groups were similar though the inactive group had longer disease duration than the active group (3.9 v. 1.4 years).

The first result was that the percentage of Th17 cells was low in healthy subjects and inactive MS, but 7 fold higher in active MS patients. That sounds impressive but, in absolute terms, the numbers are all small: less than 1% rising to fewer than 3% of CD4+CD45RO+ cells are Th17 respectively.

Th17 cells in 18 patients were followed longitudinally over 12 months; 9 active MS patients (5 of whom started IFN $\beta$ ) became 'inactive' at 6 and 12 months with a corresponding drop in their Th17 cells, 6 were inactive throughout and had consistently low Th17 cells. Levels of Th17 were raised during relapses whereas Th1 cells did not correlate with disease activity. In vitro spiking of peripheral blood cell cultures with IFN $\beta$  reduced Th17 (but not Th1) cells in a dose dependent manner, this reduction was shown to be due to apoptosis.

Eva Tolosa's group in Hamburg did something similar. They analysed Th17 cell numbers in patients with active (n=7) and inactive (n=12) relapsing-remitting multiple sclerosis, and those with a clinically isolated syndrome (9 during the episode, and 8 afterwards). They did not quite replicate Durelli's findings of Th17 cell differences in peripheral blood, although this could just be

a matter of numbers. But they did find differences in Th17 cells in the cerebrospinal fluid. They showed that there were more Th17 cells in the CSF of multiple sclerosis and CIS patients than controls. And there were more CSF Th17 cells in patients with active, than inactive, disease. However, as the authors point out, for every Th17 cell in the CSF there are about ten Th1 cells.....so it is not completely clear, numerically, that the Th17 cells will call the shots.

Tolosa's team then scrutinised a number of Th17 clones from their patients. This allows for sophisticated characterisation of the biology of individual T cells, but there is always a worry that the cloning process introduces artefact. With that proviso, it seems that human Th17 cells express higher basal levels of the activation markers CD5, CD69, CD2 and human leukocyte antigen-DR as well as of the CD28-related family of co-stimulatory molecules, when compared to Th1 clones. A particular adhesion molecule, melanoma cell adhesion molecule, is particularly more abundant on Th17 than Th1 cells, making it a potential target for new therapies.

It seems fairly convincing evidence that Th17 cells correlate with multiple sclerosis disease activity so targeted therapy against this cell population may be an effective therapy. – Claire McCarthy

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Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, Ferrero B, Eid P, and Novelli F. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. *ANNALS OF NEUROLOGY* 2009;65:499-509.

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Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthausen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. *BRAIN* 2009 Dec;132(Pt 12):3329-41.

## Regulatory cells can be bad, as well as good...

Standard thinking now is that autoimmune diseases arise when regulatory T cells (Tregs) are overcome by effector Th17 cells. The Boston multiple sclerosis immunology community has now gone and disrupted this tidy story...

Beriou et al have studied peripheral immune cells from healthy volunteers as a reference point for future investigations that may include autoimmune disorders. These were initially sorted into four T cell populations according to their expression of markers of naive T cells (CD45RA), class II HLA (DR) and the relative expression of a surface marker called CD25 (high = regulator, intermediate = activated): (i) CD45RA+ CD25- (naive responders) (ii) CD45RA+ CD25int (memory responders) (iii) CD45RA- CD25high DRneg (DRneg Tregs) and (iv) CD45RA- CD25high DRpos (DRpos Tregs). Activation and co-culture / suppression assays revealed that DRneg Tregs secreted more IL-17 than DRpos Tregs and, within DRneg Treg, IL-17 was predominantly produced by cells expressing Fox P3+. IL-17 secretion also correlated with expression of the Th17 associated chemokine receptor CCR6 and production could be influenced by the surrounding cytokine milieu. IL-1 and IL-6 had both individual and synergistic enhancement of IL-17 production from DRneg Tregs but production was negatively controlled by TGF $\beta$ . Further co-culture experimentation indicated that DRneg Treg IL-17 production was inversely correlated with suppressive activity with a shift to a non suppressive phenotype accompanying activation and IL-17 secretion. To definitively confirm that a single cell could both suppress and produce IL-17, the authors cloned DRneg and DRpos Tregs and CD25-

*The bad guys in MS are "Th17" cells, not Th1 cells. And Tregs are not as good as we once thought.....*

responders and categorised them according to FoxP3 expression and IL-17 production. Clones were assessed for suppressive activity, including proliferation and cytokine production (IL-2, IFN $\gamma$ ). Finally the authors demonstrate that the suppressive activity of the Fox P3 IL-17+ population (estimated as 8.9% of the CD25<sup>high</sup> DR<sup>neg</sup> population) was reversible following expansion and restimulation in vitro.

These experiments have shown that a subset of Tregs, defined by lack of class II HLA expression, can suppress and secrete IL-17; and they can also lose suppressor function under inflammatory conditions. So the original paradigm gets more complicated. It has to be remembered however that these cells are a small proportion of normal Treg population and future studies are needed to place the significance of these cells in context. – Allison Curry

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Beriou G, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C, and Hafler DA. IL-17 producing human peripheral regulatory T cells retain suppressive function. BLOOD 2009;113:4240-9.

The T cell immunoglobulin and mucin domain-containing protein-3 (TIM-3) molecule has been hailed one of the central regulators of the immune response. However, its expression within human CD4+ subsets remains ill defined. In mice, it is expressed on Th1 cells, associates with the regulation of IFN- $\gamma$  production, tolerance induction, and the exacerbation of EAE and NOD mouse diabetes. But is it “good” or “bad”?

The work reported here by Hastings et al benefits from the production of human TIM-3 antibodies, which has allowed expression analysis of TIM-3 on the surface (and internally) of ex vivo CD4+ and CD8+ cells both in the periphery (PBMC) and in the primary immune compartment (pancreatic lymph node isolates).

TIM-3 expression was limited to activated T cells present in lymphoid areas rather than the periphery where virtually no cell expressed TIM-3, although the majority of in vitro activated CD4+ cells become TIM-3+ following stimulation via the TCR and signal 2 (i.e. CD28). Cytokine analysis in vitro and ex vivo was consistent with the hypothesis that TIM-3 is a negative regulator of T cells that have previously undergone activation and regulates specific (i.e. Th1 and Th17) but

of TIM-3 in specific T cell activation, and the consequences of this for autoimmunity, clearly requires further experimentation and clarification where the TIM-3 antibodies generated by Hastings et al will be an invaluable tool for investigating the role of TIM-3 in the regulation of a variety of autoimmune disorders. – Allison Curry

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Hastings WD, Anderson DE, Kassam N, Koguchi K, Greenfield EA, Kent SC, Zheng XX, Strom TB, Hafler DA, Kuchroo VK.

TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. EUROPEAN JOURNAL OF IMMUNOLOGY 2009;39:2492-501.

## Mimicking one's self

Hartmut Wekerle's team, from the Max Planck Institute of Neurobiology, Martinsried, are responsible for some seriously important immunological observations over the years. And this is another one...

Firstly, remember what you learnt about one possible cause of autoimmune disease at college... that an invading bacteria looks very like an ordinary part of “self” so that the appropriate immune response against the bug mistakenly leads to auto-damage. Hence “molecular mimicry”.

Now, consider this. Wekerle's team have been playing around with a mouse whose entire T cell repertoire consists of one response: to the myelin peptide MOG. In theory, it should only respond to MOG. Through the mechanism of molecular mimicry from an invading bug, it can be induced to get EAE. But, when the mouse is further trans-gened not to be able to produce MOG, you would expect that it could not get EAE, because there is no MOG target to get inflamed about. However, these animals continued to develop EAE spontaneously. After a lot of fancy purification, it turns out that T cells from these animals were targeted at two neurofilament proteins. One, NF-M, turns out to contain a sequence of 7 amino acids that is nearly identical to a sequence in the core of the MOG molecule. So, one class of T cells that should only respond to MOG were also targeting neurofilaments. Wekerle's team have coined this “self-mimicry”.

The main thing you need to know to understand the significance of all of this is that the strain of mice used (C57BL/6) is notoriously resistant to most attempts to induce autoimmunity. So Wekerle speculates

## *More from Tiny TIM-3 and how to mimic oneself...*

### Is TIM-3 an important player in multiple sclerosis?

Vijay Kuchroo is a vet who holds a chair in Neurology at Harvard. His team are one of a handful of internationally competitive labs investigating the immunology of multiple sclerosis. And for the last few years, they have devoted quite a deal of effort to understanding the role of a curious little molecule, called TIM-3, in the pathogenesis of multiple sclerosis.

not global cytokine responses. Tregs were not involved. TIM-3 on these activated T cells bound to a ligand (such as Galectin-9) on other T cells to inhibit cytokine production. This negative feedback loop may be used to regulate Th1 / Th17 responses and may therefore be an important mediator in immunopathology.

Alternate studies have demonstrated dysregulation of TIM-3 in patients with autoimmune disease and in chronic viral exhaustion and TIM-3 has also been found constitutively expressed on human DCs and monocytes in other human models. The role

## Yet more evidence that vitamin D has something to do with multiple sclerosis...

that the mice's particular susceptibility to MOG-induced EAE is because one autoimmune response (against MOG) actually ends up targeting two self-antigens: a two-pronged attack. The other implication (which isn't mentioned and I thought up all by myself) is that an immune attack against myelin can also, of itself, induce an immune attack against nerves (for NF-M is a neuronal antigen). Hence perhaps, an explanation for the attrition of nerves in the predominantly demyelinating disease of multiple sclerosis.

It is hard to think of a clinical application for this discovery. But I think there is a good case for us to include this paper in ACNR because of that "wow" factor... just when we thought we knew everything, something quite unexpected comes along. Who'd have thought... – Alasdair Coles

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Krishnamoorthy G, Saxena A, Mars LT, Domingues HS, Mentele R, Ben-Nun A, Lassmann H, Dornmair K, Kurschus FC, Liblau RS, Wekerle H.

Myelin-specific T cells also recognize neuronal autoantigen in a transgenic mouse model of multiple sclerosis.

NATURE MEDICINE  
2009 Jun;15(6):626-32.

### Another vitamin D story

Lurking in the internet chat rooms for years, now attracting respectable attention, is the idea that people are prone to multiple sclerosis because their vitamin D levels are too low. The evidence to date is all circumstantial, but is nonetheless interesting... for instance, perhaps this explains why those who live in the sunless wastes get more MS than equatorial mad dogs and Englishmen?

Parallel with the epidemiology on multiple sclerosis and sunlight has been a concerted research effort in animal laboratories to find a mechanism. And there is now excellent basic immunology on the way in which

vitamin D, aided by corticosteroids, can promote the activity of regulatory T cells, the Policemen of the immune system.

Now, an Argentinian group has come up with a nice story which brings together this lab work and people with multiple sclerosis. They looked at 132 (Hispanic) people with multiple sclerosis and found, first off, that the serum of relapsing-remitting patients contained lower vitamin D than controls, and this dropped further when their disease was clinically active. (Primary progressive patients had normal vitamin D levels.) They then whipped out the T cells from their patients, and some healthy controls, and exposed them to various stimulants, and chucked in a range of different vitamin D molecules. They found that only 1,25 (OH)<sub>2</sub> vit D did the magic thing of suppressing the response of T cells. It also induced T cells to secrete lots of IL-10 ("a good cytokine" in the context of multiple sclerosis) and reduce IL-17 ("a very bad cytokine"). And vitamin D also boosted the number of regulatory T cells in the Petri dish...

The details, which I will spare you, are impressive. The immunological ins and outs of vitamin D and the T cell in multiple sclerosis have been widely explored. And one useful conclusion emerges: there is nothing fundamentally wrong with the capacity of T cells from people with multiple sclerosis to respond to vitamin D.

But the key question, asked in every multiple sclerosis clinic at the moment, is whether taking a vitamin D pill every day will keep the multiple sclerosis relapse away... and that this study does not address. Nice work nonetheless. – Alasdair Coles

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Correale J, Ysrraelit MC, Gaitán MI.  
Immunomodulatory effects of Vitamin D in multiple sclerosis.

BRAIN  
2009;132:1146-60.

## E. Clinical Management

### Breast is best

A frequent conversation in clinic is around the pros and cons of a woman with multiple sclerosis starting a family. One of the major worries is the increased risk of a relapse in the three months after pregnancy (nicely balanced by the reduced risk during of course). One of the current approaches is to try to introduce immunotherapy as soon as possible after delivery which, for nearly all the drugs, means that breast feeding is discouraged.

Now, a very small study of 29 pregnant women suggests that this may not be the best.... For those 14 who exclusively breastfed for at least 2 months post-partum had fewer relapses than the 15 who did not (unadjusted HR 5.0; 95% CI, 1.7-14.2; P=.003).

By no means is this a done deal; I happen to notice that the group who did not exclusively breast feed had had more relapses, and were more likely to have been on disease-modifying therapy before they became pregnant, than the "breast is best" lot.

But, given the importance of breast-feeding, this study seems like a good little starter for a more definitive investigation. – Alasdair Coles

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Langer-Gould A, Huang SM, Gupta R, Leimpeter AD, Greenwood E, Albers KB, Van Den Eeden SK, Nelson LM.

Exclusive breastfeeding and the risk of postpartum relapses in women with multiple sclerosis.

ARCHIVES OF NEUROLOGY  
2009 Aug;66(8):958-63.

### Do you know what to do with the bladder?

Urinary problems are common in multiple sclerosis with approximately 75% of patients having lower urinary tract symptoms which have a significant impact on quality of life. Professor Claire Fowler from the National Hospital for Neurology, together with a range of professionals in continence care, has developed a UK consensus on the management of the bladder in multiple sclerosis. It provides a clear, practical, evidence-based approach to symptom

management which is summarised below.

General advice should be given to avoid dehydration by drinking 1-2L per day and to reduce caffeine intake to below 100mg/day. Pelvic floor exercises can be tried for stress incontinence and can also have an inhibitory effect on the detrusor; they may be effective in patients with mild disability and intact neural pathways. All patients with new urinary symptoms should have infection excluded, initially with a urine dipstick. It is important to remember however that while this is useful for excluding urinary tract infection (UTI) the positive predictive value is only 50%.

Anti-muscarinics are the first-line treatment for detrusor overactivity, however some can cause deterioration in cognition or confusion. Anti-muscarinics that do not cross the blood-brain barrier, such as trospium chloride, should be used if cognitive impairment is a concern. Detrusor contractions will continue despite therapy if the post micturition residual volume is high so this should be measured by ultrasound prior to starting treatment (figure 1). If clean intermittent self-catheterisation is not possible then a suprapubic catheter should be considered.

Patients who fail to respond to this management algorithm should be investigated further with urodynamics. Desmopressin (100-400ug orally or 10-40ug

intrasally) is effective in the treatment of daytime frequency or nocturia in MS. However it should be used with caution due to the risk of hyponatraemia and not given more than once in 24 hours or in the presence of renal failure. Botulinum toxin given via a cystoscope is an effective, unlicensed treatment for severe detrusor overactivity. It can be useful for patients who have failed antimuscarinic therapy however local approval is needed and almost all patients need to do clean intermittent self-catheterisation afterwards.

Recurrent UTI should be investigated with cystoscopy and ultrasound scan to exclude underlying abnormalities such as bladder stones. If no cause is found than prophylactic antibiotics may be appropriate. Bladder symptoms should be routinely screened for at clinic visits and if present the patient should be assessed by appropriately trained health care professionals.

– Claire McCarthy

Fowler CJ, Panicker JN, Drake M, Harris C, Harrison SC, Kirby M, Lucas M, Macleod N, Mangnall J, North A, Porter B, Reid S, Russell N, Watkiss K, Wells M.  
A UK consensus on the management of the bladder in multiple sclerosis.  
J NEUROL.NEUROSURG.PSYCHIATRY  
2009;80(5):470-7.

## F. Disease-modifying Treatments

### Treating clinically isolated syndromes not worth the BENEFIT

I told you so...

This is the third time I have commented on this trial, the BENEFIT study of interferon beta-1b treatment of people with a "clinically isolated syndrome". In a 2006 Neurology article, we got to see data on time to conversion of multiple sclerosis over two years with interferon beta-1b versus placebo. None of us were surprised by the outcome, which replicated the CHAMPS and ETOMS studies: beta-interferon reduces the conversion rate to multiple sclerosis by about one third (placebo arm conversion rates: CHAMPS 0.50, ETOMS 0.45, BENEFIT 0.45 versus treated arms: CHAMPS 0.35, ETOMS 0.34, BENEFIT 0.28). I wrote a snooty editorial which said, pretty much, "it is all very well, but where is the data on disability, which is what really matters?" I may even have implied that there was no treatment effect on disability, or else they would have published it.

A year later, a tortuously complicated paper in the Lancet reported on the "three-year follow-up" of these cohorts. Anyone on placebo who had developed clinically definite multiple sclerosis, or who had got to two years of follow-up without it, had been put on interferon beta-1b. So this was really a comparison between "early" and "delayed" treatment of the clinically isolated syndrome. This time, we got the disability data and, at first glance, it seemed to support the authors' conclusion that "early initiation of treatment with interferon beta-1b prevents the development of confirmed disability, supporting its use after the first manifestation of relapsing-remitting MS". I had to eat my words. But, in an editorial, I queried the rigour of the analysis and suggested that the conclusion was not sufficiently robust to alter practice, given the enormous hole in healthcare budgets that would follow.

It seems that caution was the correct response. For now we have the final result, with follow-up at five years from 235 (80%) of

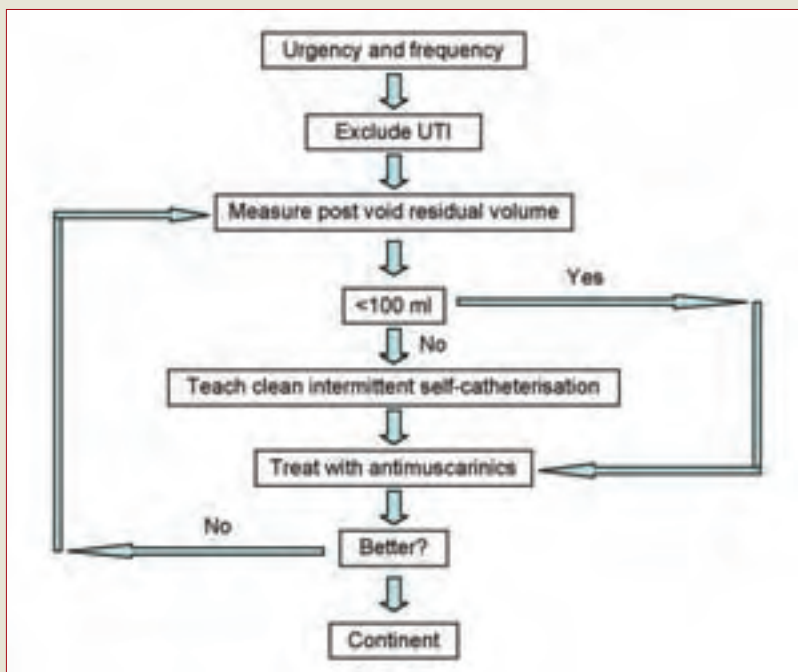


Figure 1. Management of multiple sclerosis patients presenting with urinary tract symptoms, adapted from 'A UK consensus on the management of the bladder in multiple sclerosis'.

## *The evidence that beta-interferon has a long term impact on clinically isolated syndromes, or regular relapsing-remitting MS, has taken a hit in 2009*

the "early" and 123 (70%) of the "delayed" treatment groups. As before, early treatment reduced the risk of having a second attack, and thus converting to multiple sclerosis, by 37% (hazard ratio [HR] 0.63, 95% CI 0.48-0.83;  $p=0.003$ ). But the risk of acquiring fixed disability whilst on interferon was now no longer significantly different between the early and delayed group (HR for early v delayed, 0.76, 95% CI 0.52-1.11;  $p=0.177$ ).

So, if early interferon beta treatment of the clinically-isolated syndrome does have a long-term effect on disability, as its advocates continue to claim, it is too small for a trial of several hundred people to detect confidently. That is not going to convince many hard-pressed healthcare funders, who are already struggling to pay for widely accepted and proven treatments.

The Association of British Neurologist's report on the use of beta-interferon treatment of multiple sclerosis (published in November 2009, [http://www.theabn.org/abn/userfiles/file/ABN\\_MS\\_Guidelines\\_2009\\_Final.pdf](http://www.theabn.org/abn/userfiles/file/ABN_MS_Guidelines_2009_Final.pdf)) does not refer to this 5-year follow-up of BENEFIT. But it does allow neurologists to consider beta-interferon treatment for "patients within 12 months of a clinically significant clinically isolated syndrome when MRI evidence predicts a high likelihood of recurrent episodes". Ummmmm. At the risk of stirring up another hornet's nest (see ACNR, vol 7(3) p 17 and 7(5) p 14), I wonder just how helpful that advice will turn out to be. I very much doubt that NICE would agree.

In passing, we should note that the PreCISe study was published this year, showing that glatiramer acetate reduces the risk of converting from a clinically isolated syndrome to multiple sclerosis by 45% over three years. As with the BENEFIT trials' first report, there is no disability data. So we are back to square one. – Alasdair Coles

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Kappos L, Freedman MS, Polman CH, Edan G, Hartung HP, Miller DH, Montalban X, Barkhof F, Radü EW, Metz C, Bauer L, Lanius V, Sandbrink R, Pohl C; BENEFIT Study Group. Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial. *LANCET NEUROLOGY* 2009 Nov;8(11):987-97.

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Comi G, PreCISe study group. Effect of glatiramer acetate on conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome (PreCISe study): a randomised, double-blind, placebo-controlled trial. *LANCET* 2009 Oct 31;374(9700):1503-11.

### **Oops! The risk sharing scheme**

Given the claims made for treating the clinically isolated syndrome with interferon-beta and glatiramer, you might have got the impression that the drugs were firmly in the formulary for other stages of the disease. Oh no.

The saga of the Department of Health multiple sclerosis risk-sharing scheme continues. You will remember that in 2002, NICE did not approve interferon-beta drugs and glatiramer acetate for use in the NHS. The problem was that the Sheffield School of Health and Related Research, having created an economic model of the impact of these drugs on quality of life compared to the natural history dataset from London, Ontario, concluded that they were not cost effective over 15 years but might become so over 20 years.

There was a huge fuss, out of which came the Risk-Sharing Scheme: a deal was struck with the companies that these drugs would be prescribed on the NHS, provided their

efficacy was monitored over 10 years. These data would then be compared to the natural history of multiple sclerosis and the long-term cost-utility of the interferons calculated. If any of the drugs failed to hit the benchmark of £36,000 per quality adjusted life year (QALY), the price of that agent to the NHS would be reduced.

Mike Boggild and Jackie Palace, representing the neurology community, have been bravely running the show since then, cajoling us all to fill in forms and corraling the interested parties. And now they publish the first set of results, on all patients treated in the scheme from May 2002 to April 2005. The result is, despite everyone's best intentions, a bit of a muddle.

First off, having recruited 5583 patients, two years of data was only available on 1479 (34.5%) relapsing-remitting patients. All the others had done the awkward things that people do: switch to other drugs, become secondary progressive, wander off into the blue.... Secondly, and nihilistically, it turns out that the London, Ontario, database was designed so that it is not possible for someone to have improved disability! To overcome these wrinkles, statistical jiggery-pokery was used ("sensitivity analyses"). But, pretty much however the cake is cut, the outcome is the same: those on disease-modifying treatment do worse than would be expected if left untreated. In absolute terms, the EDSS score was 0.10 worse than the control data and 0.28 worse than predicted if the disease modifying treatments really did delay progression of the disease. The authors are quick to point out the methodological difficulties and slow to make any conclusions about the efficacy of the interferons and glatiramer.

Oh dear.

There is much internecine wrangling about what all this means, as you might expect given the cocktail of government, companies, patient bodies and with lots of money at stake. In the eye of this particular storm, Mike Boggild and Jackie Palace have maintained a cool diplomacy for which they should be congratulated.

– Alasdair Coles

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Boggild M, Palace J, Barton P, Ben-Shlomo Y, Bregenzer T, Dobson C, Gray R. Multiple sclerosis risk sharing scheme: two year results of clinical cohort study with historical comparator. *BRITISH MEDICAL JOURNAL* 2009 Dec 2;339:b4677. doi: 10.1136/bmj.b4677

## *Glatiramer acetate is as effective (or ineffective) as any of the interferon-beta drugs*

### **Why not just give steroids as well as interferon-beta?**

The short-term benefit of pulsed corticosteroids in the treatment of the acute relapse of multiple sclerosis is well known, and well-proven. There has always been this lingering question: would regular steroids treatment have a long-term effect on multiple sclerosis? Personally, I have always found the Zivadinov trial (Neurology.2001 Oct 9;57(7):1239-47) intriguing: regular pulses of steroids seemed to have no effect on relapse rate, but did reduce the accumulation of disability and the rate of cerebral atrophy over five years. I wondered why no-one had tried to replicate the study...

Enter, the Nordic Trial of Oral Methylprednisolone as Add-on Therapy to Interferon Beta-1a for Treatment of Relapsing-Remitting Multiple Sclerosis (NORMIMS) study! 110 patients were given interferon beta and add-on therapy of either 200 mg methylprednisolone or matching placebo, orally for 5 days every 4 weeks, for at least 96 weeks. The first thing to say is that 26% of those taking steroids withdrew from the study (compared to 17% on interferon beta alone), due to sleep disturbance and neurological or psychiatric symptoms. There was a significant effect on annualised relapse rate: 0.22 for methylprednisolone compared with 0.59 for placebo (62% reduction, 95% CI 39—77%;  $p < 0.0001$ ). Bone mineral density had not changed after 96 weeks.

At the late-breaking session at the American Academy of Neurology 61st Annual Meeting, Dr Mads Ravnborg from the Danish Multiple Sclerosis Research Centre,

reported on a similar study: relapses were reduced by steroid add-on to interferon-beta therapy. However, most importantly, the risk of acquiring fixed disability was unaffected by steroids.

In other words, we now have the complete opposite result to the Zivadinov trial. Oh dear.

Once again, I fear, we have to say that all these trials are just too small and two of them are too short. Someone, somewhere, will have to bite the bullet and do a definitive study. – Alasdair Coles

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Sorensen PS, Mellgren SI, Svenningsson A, Elovaara I, Frederiksen JL, Beiske AG, Myhr KM, Søgaard LV, Olsen IC, Sandberg-Wollheim M. NORdic trial of oral Methylprednisolone as add-on therapy to Interferon beta-1a for treatment of relapsing-remitting Multiple Sclerosis (NORMIMS study): a randomised, placebo-controlled trial. LANCET NEUROLOGY 2009 Jun;8(6):519-29.

### **Which is better: interferon-beta or glatiramer?**

Given the results of the risk-sharing scheme, you might think this question was a bit redundant. However it is a very real one, if only for the warring drug companies. They have invested big time in trials to establish a pecking-order amongst the licensed therapies, with the hope of capturing larger proportions of the shrinking "ABCR" market share. To little avail.

In the REGARD study, published by Lancet Neurology at the end of 2008, interferon beta-1a tiw (Rebif) was trialled head-to-head against glatiramer acetate (Copaxone). There were no differences in

the primary outcome measure of time to first relapse or in the safety profile. The BEYOND study, published, again by Lancet Neurology, in 2009, is very similar: interferon beta-1b (Betaferon) was put up against glatiramer acetate for 2 years. Again there was no difference in efficacy. An additional question tested in this study was whether double the normal dose of IFNB-1b would offer improved efficacy: it does not.

In both REGARD and BEYOND, there was some evidence for superior efficacy of the interferon-beta over glatiramer in MRI outcome measures: significantly fewer gadolinium-enhancing lesions with interferon-beta in both trials and also fewer new T2-hyperintense lesions in the latter. For some, this will indicate authentic superior efficacy of interferon over glatiramer that was just not detected by crude clinical measures. For others, it will be yet more evidence for the disappointing relevance of MRI measures to what matters for patients. Yet others will propose complex mechanistic differences.

Interestingly, both REGARD and BEYOND were compromised by unexpectedly low relapse rates on the trial, reducing statistical power for their primary endpoint. This has been a long-term trend. The annualised relapse rate of patients on interferon-beta in the licensing studies lay between 0.6 and 0.8; in recent trials, patients on interferon-treated arms typically have relapse rates of 0.2-0.3 / year. This may reflect increased willingness of physicians to expose people with milder multiple sclerosis to treatments, as confidence in their safety profile grows. In BEYOND, the investigators faced relapse rates of 0.18-0.19. Their imaginative response was to introduce a "filling of the triangle" design and have a variable follow-up of between 2 and 3.5 years. This is perfectly valid but difficult to follow.

The bottom line here has to be that (a) interferon-beta and glatiramer are equivalently effective (or ineffective) (b) no more money should be spent on these sorts of trials when so many exciting new agents, with promise of far greater efficacy, await testing. – Alasdair Coles

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O'Connor P, BEYOND Study Group. 250 microg or 500 microg interferon beta-1b versus 20 mg glatiramer acetate in relapsing-remitting multiple sclerosis: a prospective, randomised, multicentre study. LANCET NEUROLOGY 2009 Oct;8(10):889-97.

Mikol DD, REGARD study group.

Comparison of subcutaneous interferon beta-1a with glatiramer acetate in patients with relapsing multiple sclerosis (the REbif vs Glatiramer Acetate in Relapsing MS Disease [REGARD] study): a multicentre, randomised, parallel, open-label trial. LANCET NEUROLOGY 2008 Oct;7(10):903-14

## What is the risk of PML from Tysabri?

The main gossip and concern, at multiple sclerosis conferences this year, has been the risk of the progressive multifocal leukoencephalopathy with natalizumab. In September 2009, the FDA reported that there had been 13 confirmed cases. By October, the EMEA reported that there were now 23. And, in November, in an article for the on-line magazine Xconomy, Dr Sandrock (senior vice president of neurology R&D at Biogen) said there had been 27 cases amongst the 63,000 patients prescribed the drug. Much is made of the fact that only 5 of these have died; however the state of the survivors is not clear.

A key question is whether the risk of PML accumulates with duration of exposure to natalizumab. Data I have seen presented would suggest that it is. But it would be good to see the facts and figures in print.

The Tysabri information centre on the Biogen website (<http://www.biogenidec.com/site/tysabri-information-center.html>, accessed 31 December 2009) does not mention PML at all. How responsible is that? – Alasdair Coles

## A convenient pill for multiple sclerosis?

Time was when people with multiple sclerosis would have been content with anything that was effective as a treatment for their disease, no matter how unpalatable. Over the years, people ingested snake venom, stung themselves with bees, drunk bottles of coke laced with venlafaxine, immersed themselves in hyperbaric chambers.....But now the concept of convenience has crept into the lexicon of multiple sclerosis therapeutics. And what could be more convenient than a pill? No more needles in the bathroom, or monthly visits to the hospital to spend all day waiting for the Tysabri infusion. And there is no shortage of contenders to be the first pill to be licensed

for multiple sclerosis: fingolimod, cladribine, and now fumarate.

What is fumarate? Nothing to do with smoking. It activates the nuclear-factor-E2-related factor-2 (Nrf2) transcriptional pathway, which may mean that the drug could be both anti-inflammatory and neuro-protective, which would be handy. 257 patients with relapsing-remitting multiple sclerosis were given fumarate in three different doses, and compared to a placebo, for 24 weeks then there was a "safety extension study" where everyone got active drug. The principal outcome measure was the total number of gadolinium-enhancing lesions added up from four scans done throughout the first 24 weeks. On the highest dose of fumarate (240mg tds) there was a statistical difference from placebo: a reduction in new lesion total by 70%. Before getting too excited, let me remind you that this is about the same as interferon-beta's effect on MRI scans. The lower doses did not make the statistical cut. There was no effect on any clinical outcome, although to be fair the group taking the highest fumarate dose trended towards the lowest relapse rate. Taking the drug did not seem to cause much bother: some flushing and GI upset only.

My bottom line is that fumarate, for all its Nrf2-thingummy, shows no sign of being any more efficacious than the interferons, so far at least. So, a lot of time and money is being put into phase 3 trials, plans to get the drug licensed and all the rest.... for convenience. But it is not exactly convenient to have inadequately treated multiple sclerosis. – Alasdair Coles

Kappos L, Gold R, Miller DH, Macmanus DG, Havrdova E, Limmroth V, Polman CH, Schmierer K, Yousry TA, Yang M, Eraksoy M, Meluzinova E, Rektor I, Dawson KT, Sandrock AW, O'Neill GN;

BG-12 Phase IIb Study Investigators.

Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. LANCET 2008 Oct 25;372(9648):1463-72.

## Rebooting the immune system

The idea behind autologous haemopoietic stem cell transplantation (AHSCT) in MS is to "reset" the immune system; that is, to generate new, self-tolerant lymphocytes from the stem cell transplant, after chemotherapy-induced elimination of autoreactive cells. Like other immunotherapies, AHSCT has no impact on disability if given to patients in the progressive phase of the disease. In contrast, here Burt and colleagues demonstrate that if given early in the inflammatory, relapsing-remitting phase, AHSCT improves disability.

Although encouraging, the study conclusions must be viewed with some caution. In particular, 17/21 patients treated received alemtuzumab (Campath-1H) as part of their conditioning regime (4 were given antithymocyte globulin when it became apparent that alemtuzumab can lead to immune thrombocytopenia). Alemtuzumab is highly effective in the treatment of relapsing-remitting MS, reducing the risk of relapse by >70% and improving disability. Given that most patients in this study received alemtuzumab, differentiating between the effects of alemtuzumab and AHSCT is difficult, if not impossible. Burt and colleagues state that the results for those receiving antithymocyte globulin were just as encouraging, however, it is difficult to see

*The big worry of 2009 has been:  
just what is the risk of PML  
from Tysabri?*

how a comparison between groups of 4 and 17 patients had enough statistical power for reliable conclusions to be made.

– Joanne Jones

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Burt RK, Loh Y, Cohen B, Stefosky D, Balabanov R, Katsamakos G, Oyama Y, Russell EJ, Stern J, Muraro P, Rose J, Testori A, Bucha J, Jovanovic B, Milanetti F, Storek J, Voltarelli JC, Burns WH. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. LANCET NEUROLOGY 2009 Mar;8(3):244-53.

## Antibodies that get to places you never even knew existed

The cerebral perivascular spaces are the main CNS compartment where brain antigen is presented to T cells, so initiating the critical phase of T cell reactivation within the brain which leads to inflammatory plaques.

So, do drugs get into these perivascular spaces? Olav Stuve, from Dallas, has been on the hunt for patients who can answer the question for him: which means people who have died. In both these case reports, the cause of death was progressive multifocal leukoencephalopathy due to a monoclonal antibody. In the first, the patient had multiple sclerosis and was treated with natalizumab. In the second, the patient had gastrointestinal mantle-cell lymphoma and was treated with rituximab. Their post-mortem pathology was compared with that from people with multiple sclerosis and PML.

The absolute number of perivascular spaces in the patient with MS treated with natalizumab was reduced compared to controls, due to the extensive pathology and, within the remaining spaces, there were fewer dendritic cells and no CD4+ T cells at all. In the second case, no B cells could be found in the cerebral perivascular spaces 8 months after rituximab.

It is not possible to know from these studies if the lack of T cells after natalizumab, or B cells after rituximab, is secondary to their depletion in the periphery, or due to direct elimination from the perivascular spaces. Nevertheless, this is a useful finding, as it shows how a critical phase of the aberrant immune response in multiple sclerosis can be disengaged.

– Sara Thompson

## Starvation seems a good treatment of autoimmunity

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del Pilar Martin M, Cravens PD, Winger R, Kieseier BC, Cepok S, Eagar TN, Zamvil SS, Weber MS, Frohman EM, Kleinschmidt-Demasters BK, Montine TJ, Hemmer B, Marra CM, Stüve O. Depletion of B Lymphocytes From Cerebral Perivascular Spaces by Rituximab. ARCHIVES OF NEUROLOGY 2009;66(8):1016-20.

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del Pilar Martin M, Cravens PD, Winger R, Frohman EM, Racke MK, Eagar TN, Zamvil SS, Weber MS, Hemmer B, Karandikar NJ, Kleinschmidt-DeMasters BK, Stüve O. Decrease in the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces due to natalizumab. ARCHIVES NEUROLOGY 2008 Dec;65(12):1596-603.

## For the future: a tolerant police force, and hold the doughnuts

Tweaking and nudging of the immune system, rather than heavy-handed immunosuppression, will probably form the basis of future treatments of early multiple sclerosis, other autoimmune diseases and organ transplants. There is a need for increasingly specific therapies with minimal side effects that can be offered to mild as well as severe disease – all to be considered in the weighing up of benefits and risks in outpatients. And ideally in tablet form. Of two fruitful approaches, recruitment of the policing cells of the immune system (regulatory T cells, Tregs), and elimination of the 'worst-offenders' (Th17) may come to clinical trials and the clinic sooner (and may already explain in part the efficacy of drugs such as sirolimus). But who would have thought that starvation had anything to do with helping the police with their enquiries?

The paper from Whitman, Keller and Rao at Harvard (Sundrud et al. Science 2009) takes a small molecule, halofuginone, derived from the active ingredient of a medicinal Chinese herb, and show that it can selectively intimidate those Th17 cells, leaving Th1, Th2 and inducible Treg cells well alone. The key experiments were in vitro, with mouse and human lymphocytes. The dose appears to be critical, with too much in the petri-dish inhibiting all T lymphocytes. The group then compared the genes up-regulated in Th17 cells by this treatment, in comparison with an inactive derivative related to the drug. The surprise finding was that the genes are mostly related to the 'amino acid starvation response', a protective signalling pathway, in times of low essential amino acid supplies, that cuts off translation of unnecessary RNAs. Manipulating this pathway directly in the absence of the drug had the same Th17 reduction effect. The drug reduced clinical scores in an antigen driven model of experimental allergic encephalomyelitis (EAE), sensitive to Th17, but not in a Th1-driven passive EAE model. The findings certainly deserve further exploration.

Two related papers come from Oxford and Zurich. Steve Cobbold and Herman Waldmann (originator of the policing metaphor) and others in Oxford, Georgia and New York show that antigen-specific Tregs, purveyors of 'infectious' tolerance, can induce consumption of essential amino acids and therefore reduce T cell proliferation. von Allmen et al (PNAS 2009) take this whole selective therapeutic approach further and show efficacy of a novel secreted molecule derived from Tregs can work in a similar way and also suppress EAE. Roll on the pilot studies and trials.

– Mike Zandi

Sundrud MS, Korolov SB, Feuerer M, Calado DP, Kozhaya AE, Rhule-Smith A, Lefebvre RE, Unutmaz D, Mazitschek R, Waldner H, Whitman M, Keller T, Rao A.  
Halofuginone inhibits Th17 cell differentiation by activating the amino acid starvation response.  
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The mechanism of action is discussed further in a commentary by Blander and Amsen.  
SCIENCE  
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Secretory phospholipase A2-IIID is an effector molecule of CD4+CD25+ regulatory T cells.  
PNAS  
2009;14;106:11673-8.

## G. Repair & Rehabilitation

**'A little less conversation, a little more myelination, please.'**  
(With apologies to Elvis)

The patient with advanced multiple sclerosis and established damage has little to be cheery about with each announcement of a novel anti-inflammatory drug. Reparative therapy has been slow to come to the clinic, but new candidate drugs are showing promise in animal models. There is evidence that repair and production of myelin protects axons from further damage. Failure of remyelination in the CNS is related to disease-independent factors (age, genetic background) and disease-specific factors – inactivation of the reparative machinery. The cells that are called in for this job, oligodendrocyte precursor cells (OPCs), are already found within MS lesions, and so drugs that can encourage their action may

represent a first step. OPCs also may have some 'stem cell'-like properties, which are the basis of an exciting area of research (see Franklin and French-Constant [NAT REV NEUROSCI. 2008 Nov;9(11):839-55] for an introduction).

It is a curious business, but there are also mechanisms that inhibit remyelination even in the context of demyelination, at least in animal models. So, in this paper from Biogen Idec, Cambridge, Massachusetts, an antagonist monoclonal antibody is used against one of the chief offenders: Leucine rich repeat and Ig domain containing NOGO receptor interacting protein 1 (conveniently, LINGO-1). Neutralisation of this molecule releases inhibition of oligodendrocyte differentiation and myelination in vitro, with evidence of functional repair in three different animal models of demyelination. Evidence of remyelination in co-culture and brain slice preparations was first demonstrated. A rat MOG-induced optic neuritis experimental allergic encephalomyelitis (EAE) model was then used with LINGO-1 antibody or control given by injection into an eye at the start of disease, with marked remyelination observed on optic nerve histology. Similar results were seen in a rat lysophosphatidylcholine (LPC)-induced spinal cord demyelination model (including observation of increased motor evoked potentials after LPC injection into the ventrolateral funiculus), and a mouse cuprizone induced corpus callosum demyelination model. The efficacy in three different models is promising, but the problems of drug delivery and adverse

effects are yet to be assessed. – Mike Zandi

Mi S, Miller RH, Tang W, Lee X, Hu B, Wu W, Zhang Y, Shields CB, Zhang Y, Miklasz S, Shea D, Mason J, Franklin RJ, Ji B, Shao Z, Chédotal A, Bernard F, Roulois A, Xu J, Jung V, Pepinsky B.  
Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells.  
ANNALS OF NEUROLOGY  
2009;65:304-15.

## Physios are better than electricity

The National Clinical Functional electrical stimulation Centre in Salisbury has done a very decent thing: test the product it advocates.

48 people with secondary progressive multiple sclerosis and unilateral foot drop were randomised to receive either a functional electrical stimulator or a physiotherapy home exercise program. 18 weeks later, the exercise group had an increase in their walking speed and distance; whereas those who had been electrified were unchanged.

Inevitably, the authors concluded with that old favourite "more research is required...". But, you would have to, wouldn't you? – Alasdair Coles

Barrett CL, Mann GE, Taylor PN, Strike P.  
A randomized trial to investigate the effects of functional electrical stimulation and therapeutic exercise on walking performance for people with multiple sclerosis.  
MULTIPLE SCLEROSIS  
2009 Apr;15(4):493-504.

*It is a curious business, but there are mechanisms that inhibit remyelination even in the context of demyelination*

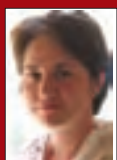
# Contributors



**Alasdair Coles** is co-editor of ACNR. He is a University Lecturer in Neuroimmunology at Cambridge University. He works on experimental immunological therapies in multiple sclerosis.



**Allison Curry** is a Senior Research Associate in the research team of Dr Alasdair Coles, the Department of Clinical Neurosciences at the University of Cambridge. Allison is a cellular immunologist and her research is focused on the role of Alemtuzumab in Multiple Sclerosis.



**Joanne Jones** is a Clinical Lecturer at the University of Cambridge. Her research interest is the use of experimental immunological therapies in Multiple Sclerosis.



**Claire McCarthy** (née Helliwell) is a Neurology trainee doing a PhD in the Department of Clinical Neurosciences at the University of Cambridge. She is based at Addenbrooke's Hospital and is involved in the phase III trials of Alemtuzumab in Multiple Sclerosis. Her research projects focus on the recently described Th17 lymphocyte and also immune responses to vaccination following Alemtuzumab treatment.



**Sara AJ Thomson** is a Research Associate who has worked in the Department of Clinical Neurosciences for 9 years with Dr Alasdair Coles at Addenbrookes Hospital. She has been involved in Phase II and III clinical trials of Alemtuzumab in MS. Recent work has been on B cell reconstitution and BAFF after Alemtuzumab published in Journal of Clinical Immunology 2009.



**Mike Zandi** is co-editor of ACNR. He is an Honorary Specialist Registrar in Neurology at Addenbrooke's Hospital, Cambridge and a Research Fellow at Cambridge University. His research interests are in neuroimmunology, biomarkers and therapeutics in particular.



Cover image courtesy of Mike Zandi showing microtubule-associated protein staining of cultured neurons.