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What is This?

'Gimme five': future challenges in multiple sclerosis. ECTRIMS Lecture 2009

Reinhard Hohlfeld

Abstract



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This article is based on the ECTRIMS lecture given at the 25th ECTRIMS meeting which was held in Düsseldorf, Germany, from 9 to 12 September 2009. Five challenges have been identified: (1) safeguarding the principles of medical ethics; (2) optimizing the risk/benefit ratio; (3) bridging the gap between multiple sclerosis and experimental autoimmune encephalitis; (4) promoting neuroprotection and repair; and (5) tailoring multiple sclerosis therapy to the individual patient. Each of these challenges will be discussed and placed in the context of current research into the pathogenesis and treatment of multiple sclerosis.

Keyword

multiple sclerosis, immunotherapy, biomarker, neuroprotection

Introduction: history of ECTRIMS

According to Otto Hommes and Richard Gonsette, pioneers and early witnesses of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), one of the first meetings in the series was held in the Dutch city of Nijmegen in 1982. The proceedings of that meeting, entitled 'Immunotherapies in Multiple Sclerosis', were edited by Hommes, Mertin and Tourtellotte (Figure 1). The first meeting that officially carried the name ECTRIMS in its title was held in Lyon, France, in 1987. Its proceedings were entitled 'Trends in European Multiple Sclerosis Research, edited by Confavreux, Aimard and Devic (Figure 1).

It is remarkable from today's perspective that these early meetings were attended by no more than 50 to 80 people. By comparison, the ECTRIMS meetings held in Madrid in 2006, and in Prague in 2007, were attended by between 4000 and 5000 participants (Figure 2) – almost 100 times as many as in the early days! This impressive growth reflects the growth of multiple sclerosis (MS) therapeutics, which accelerated after the first diseasemodifying agent, interferon beta 1-b, had been approved in 1993. MS has since become an important market, promising substantial profits for pharmaceutical companies. Financial support by industry has allowed the number of participants attending MS meetings to expand.

Future challenges in multiple sclerosis

For the ECTRIMS lecture presented at the 25th ECTRIMS meeting in Düsseldorf in September 2009,

I selected five challenges, which are all related either to MS therapeutics or MS pathogenesis (Figure 3). There are so many challenges ahead of us, that identifying 'future challenges in MS' presents a challenge in itself. Readers may come up with additional or altogether different challenges.

Challenge #1: safeguarding the principles of medical ethics

The sales of MS products have steadily increased during the past decade. Currently they amount to about 8 billion US dollars, equally divided between the USA and Europe (Figure 4). This is in a similar range as some of the blockbuster drugs used for treating more prevalent diseases, such as cardiovascular disorders. Compared with the more prevalent diseases, however, the number of patients with MS is relatively small, as is the number of neurologists specializing in MS (Figure 5). Therefore, in MS a single prescription is often worth thousands of dollars or Euros. Against this background it is sometimes

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Figure 1. Early ECTRIMS meetings held in Nijmegen, Netherlands (1982), and in Lyon, France (1987).



Figure 2. Rising number of ECTRIMS participants.



Figure 3. Five challenges related to MS therapeutics and pathogenesis.



Figure 4. Growing sales of multiple sclerosis therapeutic products. RoW: Rest of World, IMS: Intercontinental Marketing Services.

challenging to safeguard and maintain the principles of medical ethics. Several areas of concern can be identified (Box 1). For example, it is becoming more and more difficult to perform placebo-controlled trials in MS, although this trial format has produced information. Are invaluable placebo-controlled trials still feasible? Are they ethical at a time when more and more effective treatments are available? These are difficult questions which were recently addressed in an excellent review article by Polman et al.¹ A related ethical issue is the increasing tendency to move therapeutic trials to less affluent countries. Further, there is still a relative lack of head-to-head studies, mostly because industry sponsors are reluctant to support trials that could produce unwelcome results.

B	Box 1. Some areas of ethical concern						
•	Placebo-controlled trials: still feasible/ethical?	•	Tendency to move trials to less affluent countries	•	Relative lack of head-to-head-studies		
•	Paucity of investigator-driven studies	•	Bias against publication of negative results	•	Marketing disguised as education		
•	Surveillance studies	•	Prescription-tracking	•	Ghost writing		



Figure 5. (Fictive) relationship between the number of patients (top), physicians (middle) and therapeutic market (bottom). MS (left) is compared with other, more prevalent diseases (right).

On the other hand, there is a notorious paucity of investigator-driven studies, and a strong bias against the publication of negative results. All these problems have long been identified, but are far from being solved.

Apart from such fundamental ethical issues of drug development, there are other problems that should be easier to overcome. These include the bad habit of 'ghost writing' of review articles or even original papers by sponsor-paid, anonymous authors; flimsy 'surveillance studies' (phase IV studies) which often have the purpose only of keeping patients on a particular drug, or of placing them on a new drug; 'prescription tracking', allowing sales representatives to monitor the prescription behaviour of individual doctors; and all sorts of marketing activities disguised as education. Despite these concerns, there is no question that drug development in MS depends on strong input and support from the pharmaceutical industry. Both the medical profession and industry equally share the responsibility for safeguarding the principles of medical ethics.



Figure 6. Optimizing the risk to benefit ratio of therapeutic agents used for multiple sclerosis. Realistically, increasing efficacy is accompanied by increasing risk. Ideally, risk should remain low despite increasing efficacy.

Challenge #2: optimizing the risk/benefit ratio

The first generation of 'disease-modifying drugs' (DMD), interferon beta and glatiramer acetate, combines moderate efficacy with an excellent safety record. A new generation of therapies, including new monoclonal antibodies, such as alemtuzumab and daclizumab, as well as oral agents such as fingolimod, cladribine, fumaric acid, teriflunomide, and laquinimod, seems to be somewhat more efficacious than the existing DMDs, but brings along new risks.² Ideally, drug development should achieve evermore increasing efficacy, while maintaining a benign risk profile (bent curve in Figure 6). Whether this lofty goal can be achieved remains to be seen. In the meantime, with the growing complexity of MS therapy, there is a growing need for common-sense guidelines which help to translate the results of therapeutic trials into treatment decisions for individual patients. Such consensus guidelines are regularly provided by professional societies such as the American Academy of Neurology, and national and international panels of independent experts such as



Figure 7. Schematic view of multiple sclerosis pathogenesis. See text for details. From Hohlfeld,⁴ with permission.

the MS therapy consensus group MSTKG, which started in the German-speaking countries, but has since developed into a concerted European effort.³

Challenge #3: bridging the gap between multiple sclerosis and experimental autoimmune encephalomyelitis

Figure 7 shows a typical schematic representation of experimental autoimmune encephalomyelitis (EAE) and MS pathogenesis (adopted from Hohlfeld,⁴ with permission). According to this widely held 'T-cell centric' view, potentially autoaggressive T cells exist even in the normal immune system. After initial activation (which occurs outside the central nervous system (CNS)), the autoreactive T cells acquire the capacity to traverse the blood-brain barrier and migrate into the CNS where they become locally re-activated. After reactivation, the T cells orchestrate a complex series of interactions culminating in the destruction of both myelin and axons. It is important to remember that our current thinking about the pathogenesis of MS is built to a large extent on results obtained in EAE. However, there is still a wide gap between research in human MS and its animal models.^{5,6} Bridging this gap remains a major challenge. Fortunately, progress in experimental technology is helping to achieve this. New techniques include cellular and molecular in vivo imaging; genetically engineered animal models; transcriptomics and proteomics approaches; and single-cell 'resurrection' from archival human tissues.

Cellular and molecular in vivo imaging: In what is now considered a classical experiment, Ben-Nun et al. demonstrated that purified myelin basic protein (MBP)-specific CD4 + T-cells are sufficient to induce EAE.⁷ They isolated MBP-specific T-cells from the lymph nodes of MBP-immunized rats, and cultured the cells in vitro in the presence of MBP. In this way, they could purify and expand long-term 'lines' of MBP-specific T cells. The T line cells were then injected into healthy recipients of the same rat strain from which the T cells had been originally isolated.⁷ After a lag phase of 4 to 5 days, the recipients of the purified MBP-specific T cells developed EAE. This experiment has become one of the pillars of EAE research, and especially of the 'T-cell centric' view of MS pathogenesis (Figure 8).

Subsequently, the T-cell transfer system could be further refined, allowing spectacular insights into crucial steps of EAE pathogenesis. For example, Flügel and Wekerle developed a technique for permanently labelling MBP-specific T cells in vitro with a fluorescent dye, green fluorescent protein (GFP).⁸ For this purpose, the T cells are transduced in vitro with a GFP-carrying viral vector. As a consequence, they become fluorescent. The GFP-labelled T cells are then injected into a healthy recipient rat, according to the classical T cell transfer approach (Figure 8). The spinal



Figure 8. Different types of experimental autoimmune encephalomyelitis (EAE). EAE can be induced by active immunization with autoantigen (e.g. MBP, myelin basic protein; left), or by transfer of purified, MBP-specific CD4+ T cells (T-cell transfer EAE, right). Green animals = healthy; red animals = EAE.



Figure 9. Four phases of T-cell migration across the blood-brain barrier (BBB). See text for details.

cord of the recipient animals is surgically exposed, so that the in vivo behaviour of the transferred GFP-labelled autoreactive T cells can be directly observed under a 2-photon microscope. This allows, for example, real-time imaging of the transmigration of autoreactive T cells from the blood into the surrounding tissue. By imaging T-cell traffic in and around meningeal spinal blood vessels, Bartholomäus et al. recently identified several new features of this crucial process.⁹

The first striking observation was that, contrary to current dogma, T cells do not just 'roll' with the blood stream along the endothelium, but rather they actively crawl, oftentimes in a direction against the blood stream (phase 1; Figure 9). Next, the crawling T cells stop and squeeze through the vessel wall (diapedesis; phase 2). Subsequently, the T cells crawl along the outside of the blood vessel (phase 3). During the course of its extravascular crawling, the T cell encounters a perivascular antigen-presenting cell, e.g. a dendritic cell. If the antigen-specific receptor of the T cell recognizes 'its' antigen on the surface of the dendritic cell, the T cell is re-activated, and thereby becomes 'licensed' to leave the area of the vessel and penetrate into the surrounding tissue (phase 4).

These observations help to dissect the crucial process of T cell transmigration with new precision. Obviously the findings are also relevant for therapy, as is exemplified by the already approved monoclonal antibody against $\alpha 4\beta 1$ integrin called natalizumab. The effect of anti- $\alpha 4\beta 1$ integrin antibody on T-cell crawling has been nicely captured in a movie.¹⁰ After injection of the antibody, intravascular crawling of T cells is completely abolished. The movie shows how the blood vessels are literally flushed free of adhering T cells.

Genetically engineered animal models: Modern EAE research relies on genetically engineered animals. State-of-the-art methods make it possible to switch on or switch off specific genes in specific tissues, enabling investigators to perform extremely sophisticated experiments. If, for example, a particular fluorescent dye is inserted into certain cells of the nervous system, and another, different, dye is used for labelling a certain subtype of immune cells, in vivo imaging technology can be applied elegantly to visualize the interaction between the distinctly labelled cells.¹¹

'Spontaneous' EAE models represent another advance made possible with genetically engineered animals. Whereas classical EAE is induced by active immunization with autoantigens such as MBP, or by transfer of MBP-specific T-cells, in spontaneous EAE the animals develop clinical symptoms without any external manipulation. This is illustrated with a new relapsing mouse model of spontaneous EAE, stunningly mimicking the clinical course of human MS: SJL/J mice carrying a T-cell receptor specific for myelin oligodendrocyte glycoprotein (MOG) develop relapsing EAE with spontaneous symptoms often alternating between different CNS locations such as the cerebellum, optic nerve and spinal cord.¹² A movie demonstrates the spontaneous relapsing-remitting course of such a transgenic mouse.¹³ This model is an excellent test system for novel immunotherapies. Further, these animals are ideally suited for studying the influence of environmental factors on the course and development of disease.



Figure 10. Growing complexity of CD4+ T cells. Th1: T helper 1 cells, Th2: T helper 2 cells, Th17: Interleukin-17-producing T cells, Treg: regulatory T cells. Thumbs up = protective cell type; Thumbs down = pathogenic cell type. See text.

Genetically engineered mice are not only essential tools for unravelling the molecular details of EAE pathogenesis, but also for investigating fundamental principles of immunology in general. One of the most prominent advances in this regard is the discovery of several new types of T cells. According to the traditional scheme, CD4+ T cells come in two varieties, called TH1 and TH2. More recently, other types of T cells have entered the limelight. So-called regulatory T cells (T_{reg}) suppress and control the naturally existing autoreactive immune cells. Another new subtype of T cell, called TH17 cells, plays a significant role as pathogenic effector T-cells in EAE, and possibly MS. There is a complex developmental relationship between the T_{reg} and the TH17 cells, and it is difficult to ascribe a purely beneficial (or detrimental) function to any one subset.14

With the discovery of Th17 cells and T_{reg} , the complexity of the CD4 T-cell population has considerably extended (Figure 10). Nevertheless, the new scheme is, of course, still a gross oversimplification. Many additional types of immune cells, including CD8 + T cells, B cells, macrophages, different types of dendritic cells, natural killer (NK) cells, NKT cells, and many others, likely contribute to the immunopathogenesis of EAE and MS.

Transcriptomics and proteomics techniques: 'Unbiased' ('omics') approaches have identified many novel molecular targets and pathways in many areas of biology and medicine. MS is no exception: pioneered by Steinman and colleagues, transcriptomic¹⁵ and proteomic analyses¹⁶ of MS tissue have revealed important insights into the pathogenesis of MS, leading to



Figure 11. Combination of transcriptomics and proteomics analyses of human multiple sclerosis tissue with functional experiments in experimental autoimmune encephalomyelitis.

identification of novel therapeutic targets, such as osteopontin¹⁷ and angiotensin converting enzyme¹⁸. The essence of this approach, which is geared to bridging the gap between EAE and MS, is illustrated in Figure 11. Starting with an unbiased 'omics' analysis of MS tissue, candidate mRNA or protein molecules are identified by comparing the expression profiles of MS and control tissues. Next, the potential functional role of the candidate molecules is tested in EAE models. If the EAE experiments support the hypothesis, it makes sense to further explore the newly identified molecule or mechanism in human MS.

Single-cell 'resurrection' from human tissue: Another approach for bridging the gap between EAE and MS is the 'resurrection' of particular immune cells from autopsy or biopsy tissue of MS patients.¹⁹ Most immunological investigations in human MS are done with living immune cells isolated from the blood or cerebrospinal fluid (CSF). One disadvantage is that these cells are captured far away from the crime scene, the MS lesion in the CNS. It is therefore only logical to try to analyse immune cells directly in the CNS. One problem of this more direct approach is that CNS samples from MS patients are scarce and, if they are available, they are usually frozen or fixed, containing only dead cells.

Each B cell or T cell carries one type of 'clonotypic', antigen-specific surface receptor. Clonotypic refers to the fact that the existing billions of different antigen-specific receptors are distributed over billions of different 'clones' of immune cells. Each receptor molecule is composed of a pair of two different chains, called TCR alpha and beta in the case of T-cell



Figure 12. Laser-assisted microdissection of single T cells from MS brain tissue. Green stain = T-cell receptor variable region 5 (TCR V-beta 5); Red stain = CD8 (marker for cytotoxic T cell). The four numbered, dissected T cells are double-positive for both markers (right panel). From Junker et al.²⁰ with permission of the publisher.

receptors (TCR). Using laser capture micro-dissection to isolate individual tissue-infiltrating T cells (Figure 12)²⁰, and polymerase chain reaction (PCR) to amplify the TCR transcripts contained in each microdissected cell, it is possible to identify the complete cDNA (and by inference, amino acid) sequence of the paired alpha and beta chains in each dissected T cell.²¹ This strategy involves sophisticated multiplex PCR. Further, the PCR need to be miniaturized down to the level of single cells. This is necessary because PCR analysis of bigger pieces of tissue (containing many different clones of T cells) would yield myriad different TCR alpha and beta chains, making it impossible to identify the correct pairings of chains.

In our laboratory this technology has now advanced to the stage where several TCR alpha and beta chain pairs could be identified from CNS tissue-infiltrating T cells. Once the matching alpha/beta chain pairs have been identified from individual tissue-infiltrating T cells, the corresponding TCR proteins can be functionally expressed in a living (mouse) cell line which can be co-transfected with appropriate additional human molecules and then be used to search for the target antigen(s) using in vitro assays (Figure 13). In this way, it should eventually be possible to track down the target antigens recognized by the T cells in MS tissue. In parallel, it might be possible to insert the human TCR identified from human tissue into genetically engineered mice. This would allow investigation of the potentially pathogenic properties of such human TCR in vivo. In principle, humanized, TCR-transgenic mice can indeed be constructed, as was elegantly shown by Fugger and



Figure 13. Resurrection of tissue-infiltrating T cells from multiple sclerosis brain (autopsy or biopsy). First, both chains of the antigen-specific T cell receptor (TCR) of the dissected human T cell (green) are identified as described in the text. Next, the TCR is expressed in a living mouse cell line (brown). The TCR transfectants can then be used for identification of the unknown target antigen.

colleagues.²² They provided proof-of-principle that such models can be most valuable for studying antigen-specific human TCR in vivo.

Challenge #4: promoting neuroprotection and repair

The observation that irreversible neuronal and axonal damage occur early during the course of MS has gained great importance for MS therapeutics. However, presently only the inflammatory component of the pathological process can be effectively targeted with existing drugs. Although it is likely that immunomodulatory agents reduce demyelination, and thus indirectly also axonal loss, it is unclear whether the existing therapies have a primary neuroprotective component. Inflammation is usually considered detrimental in MS pathogenesis. However, there is no doubt that inflammatory reactions may be beneficial, as can be illustrated with the example of wound healing. By analogy, CNS inflammation also likely has a beneficial component.

Neuroprotective role of inflammation: On the one hand, there is a close correlation between inflammation and neurodegeneration.^{23–26} On the other hand, inflammation may contribute to protection and repair.²⁷ The 'net effect' of inflammation – destruction or protection – is obviously determined by the relative weight of these processes in any specific inflammatory condition or situation.²⁸ Some years ago, we observed that brain-infiltrating inflammatory cells in MS and other inflammatory CNS disorders produce abundant amounts of brain-derived neurotrophic factor (BDNF)²⁹ (Figure 14).



Figure 14. Immunohistological localization of brain-derived neurotrophic factor (left: BDNF stain; right: negative control) in inflammatory cells surrounding a blood vessel in human brain. From Kerschensteiner et al.²⁹ © Kerschensteiner *et al.*, 1999. Originally published in *J. Exp. Med.* 189:865–870.

The cognate receptor for BDNF, TrkB, is expressed on neurons and astrocytes in the direct vicinity of brain-infiltrating immune cells.³⁰ These observations led us to speculate that inflammatory cells may 'import' neurotrophic factors such as BDNF into the brain, and that local secretion of BDNF by inflammatory cells can mediate neuroprotective effects. Recent experiments by Linker and Gold provide functional support for this concept (personal communication). Conditional knock-out mice lacking BDNF in macrophages or T cells show a more progressive and severe course of chronic EAE. This is consistent with the notion that BDNF imported by immune cells has a beneficial role in the CNS milieu. It seems reasonable to assume that it depends on the stage and type of lesion whether the destructive or protective role of inflammation prevails. This concept is relevant for all types of immunosuppressive therapy.

Secondary axonal damage: At least part of the axonal damage observed in MS is likely to be secondary to demyelination (Figure 15). Several mechanisms might account for secondary axonal damage. First, axons might suffer collateral damage when myelin is acutely attacked by immune cells.³¹ Second, chronic demyelination might lead to a state of chronic metabolic deficiency of axons.³² It appears that the increased energy demand of impaired conduction along demyelinated axons and reduced axonal ATP production



Figure 15. Axonal injury secondary to demyelination.

induce a chronic state of 'virtual hypoxia. This leads to disturbance of mitochondrial function, sodium influx through voltage-gated Na+ channels and axonal AMPA receptors, calcium release from intracellular stores, overactivation of axonal glutamate receptors, and activation of voltage-gated calcium channels.³² This cascade culminates in the activation of calcium-dependent toxic pathways. The concept of virtual hypoxia forms the basis for several therapeutic neuroprotective strategies, some of which target common pathways shared between inflammatory and degenerative CNS diseases (Box 2).³³

Box 2. Candidate neuroprotective therapies

- Na channel blockers
- Immunomodulators: IFN-β, GLAT, Fingolimod, Dimethyl fumarate?
- Calpaine inhibitors
- Minocycline
- Cell therapy

- Glutamate receptor antagonists
- Reactive oxygen and nitrogen scavengers
- Cannabinoids
- Growth factors
- Remyelination



Figure 16. Primary axonal damage (occurring prior to demyelination), mediated by T cells or specific antibodies. CTL: cytotoxic T cell.

Primary axonal damage: In addition to secondary axonal damage, axons may also be the primary targets of immune-mediated damage, in the absence of demye-lination. In principle, such primary axonal damage could be mediated by the so-called adaptive immune system (that is, antigen-specific T cells or antibodies), or by the innate (antigen-nonspecific) immune system (Figure 16). Indeed, there is evidence to support both of these possibilities. The recently discovered autoantibodies against neurofascin may serve as an example of the first possibility, damage inflicted by the adaptive immune system.

Neurofascin exists in two isoforms, NF155 and NF186. NF155 is expressed in the paranodal loops of myelin, whereas NF186 is expressed on the nodal surface of axons. Starting with a proteomics-based approach, Mathey et al. detected neurofascin-specific autoantibodies in serum samples of patients with MS.³⁴ The antibodies cross-reacted with both isoforms of neurofascin. To evaluate whether these antibodies are pathogenic in vivo, the investigators co-transferred a neurofascin-specific monoclonal antibody together with MOG-specific T-cells. In this co-transfer model,



Figure 17. Primary axonal injury mediated by antibodies against neurofascin at the node of Ranvier.³⁴



Figure 18. Primary axonal damage mediated by diffusible toxic mediators, e.g. reactive oxygen or nitrogen species produced by macrophages in experimental autoimmune encephalomyelitis lesions.

the anti-neurofascin antibodies bound to the nodes of Ranvier, resulting in local deposition of complement, axonal injury and disease exacerbation (Figure 17).³⁴

A recent example of the second mechanism of primary axonal injury – damage inflicted by the innate immune system – is a newly discovered process of axonal degeneration observed by in vivo imaging of EAE lesions (Kerschensteiner and Misgeld, personal communication). In the vicinity of infiltrating immune cells axons undergo a series of changes, starting with focal swellings and culminating in multi-focal fragmentation. The changes appear to be mediated by macrophage-derived reactive oxygen species (Figure 18). Importantly, there is an intermediate stage of axon damage that is reversible, offering encouraging perspectives for neuroprotective therapy.



Figure 19. Tailored (personalized) therapy depends on the availability of biomarkers to differentiate between different types of disease.

Challenge #5: 'tailoring' MS therapy to the individual patient

With an increasing number of available therapies, the 'one-size-fits-all' treatment model becomes outdated. Ideally, therapy should be tailored to the specific demands of each individual patient. Obviously, we are still far from the goal of 'personalized medicine'. Customized therapy will only become possible when biomarkers become available allowing the reliable identification of therapeutically relevant subgroups of patients. Therefore, 'tailored therapy' and 'disease markers' are really two sides of the same coin (Figure 19).

There are different types of biomarkers for different aspects of disease (Box 3A, 3B) (reviewed by Bielekova and Martin).³⁵ Among the most impressive examples of а successful immunological biomarker are the anti-aquaporin-4 antibodies now serving as diagnostic markers of neuromyelitis optica.³⁶ Initially these antibodies were identified as a diagnostic marker of a particular clinical syndrome that is now separable from MS. Using a co-transfer model similar to that described above for anti-neurofascin antibodies, several groups of investigators have now shown that anti-aquaporin-4 antibodies are pathogenic in vivo when co-injected with myelin-specific T cells.^{37–39} This observation serves to illustrate an important more general point: potentially pathogenic antibodies can reach the CNS only after the blood-brain barrier has been breached. This principle was established in the early 1980s with anti-MOG antibodies. When anti-MOG antibodies are injected into healthy animals, nothing happens. If, however, they are co-injected with MBP-specific T cells, transfer-EAE is exacerbated.^{40,41} In this model, the MBP-specific T cells breach the blood-brain barrier

Box 3A.	Multiple	sclerosis	features	for	which	biomarkers	are
needed							

•	(Differential)	Demyelination •	Pathogenetic
	diagnosis of MS	and remyelination	heterogeneity
	Disease activity	Course of MS •	Predisposition
	Prognosis	Therapeutic response •	Axonal loss

Box 3B. Types of biomarkers

,,		
Imaging techniques (MRI, MRS, OCT, etc)	Electr testing	ophysiological g
Immunological assays Others	Genet	ic markers

and make it permeable to antibodies. A parallel approach was used to demonstrate the pathogenic potential of anti-neurofascin and anti-aquaporin-4 antibodies.34,37-39

Fascinating new prospects for biomarker development come from recent advances in genetics. Genome-wide association studies and pathway analyses are powerful tools which have added new candidate genes to the list of genetic risk factors for MS. Although each individual gene makes only a minute contribution to the overall genetic risk of MS, certain combinations of factors may confer clinically relevant risk.42

An intriguing recently discovered risk factor for MS is the R92Q variant of the TNFRSF₁A gene.^{43,44} This locus is of special interest because the R92O substitution was previously detected in a special subgroup of MS patients.^{45,46} MS patients with this mutation have additional symptoms compatible with the autoinflammatory syndrome TRAPS (TNF receptor 1-associated periodic syndrome). TRAPS is an autoinflammatory disorder which typically manifests in childhood, and is usually characterized by unprovoked episodes of systemic inflammation with fever, abdominal pain, myalgia, cutaneous inflammation, arthralgia and ocular involvement. Interestingly, most patients with MS and TRAPS have a late-onset form of TRAPS with arthralgia, myalgia, skin involvement, severe fatigue and headache, but without the otherwise typical fever episodes.45,46

Initially, it was unclear whether the occurrence of MS and TRAPS in individual patients represented a pure coincidence, or whether the two distinct disorders might have a pathogenetic relationship (Figure 20). In some (but not all) families, TRAPS and MS seem to be inherited together (Figure 21). De Jager et al. subsequently demonstrated that indeed there is an association between MS susceptibility and the R92Q



Figure 20. Multiple sclerosis and TRAPS: coincidence or pathogenetic relationship? TRAPS: TNF receptor 1-associated periodic syndrome. Prevalence of multiple sclerosis is around 1:1000; prevalence of the TRAPS-associated R92Q mutation around 1:25.



Figure 21. Apparent concordance of multiple sclerosis and the TRAPS-related mutation R92Q in one generation. (see Kümpfel et al.,^{45,46} and Dr Tania Kümpfel, unpublished observation).

amino acid substitution.^{43,44} Taken together, these separate lines of evidence shed a new light on the relationship between MS and the TNF pathways, demonstrating that the results of large genome-wide studies and clinical bedside observations may converge to uncover fascinating clinical and pathophysiological relationships. The TRAPS-related mutation R92Q may be also be relevant for the therapy of MS: in pilot studies we observed that patients with this mutation are more prone to side effects of various immunotherapies. Perhaps this and other related mutations might eventually serve as therapeutic biomarkers.

Conclusion

It is impossible for mere mortals to look into the future. The challenges discussed here in the 2009 ECTRIMS lecture may be outdated in 5 or 10 years from now. Whatever the fate of the 'five future challenges', ECTRIMS is likely to survive well into the future. However, we should not be aiming primarily at breaking the 10,000 participants sonic barrier, but rather get on with our research into the pathogenesis and treatment of MS. For discussing progress and exchanging ideas, ECTRIMS is and will remain an ideal forum.

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