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Research Focus

New directions in MS therapeutics: vehicles of hope

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Basic immunological research has greatly expanded our understanding of the suspected immunopathology of multiple sclerosis (MS) and, more importantly, spawned a new generation of clinical trials evaluating dozens of immune-based therapies. Adhesion molecules are the furthest into development, although patient acceptance and neutralizing antibodies both support the development of small, orally available molecules. Progressive MS probably has a significant neurodegenerative component, so progress with neuroprotective strategies will require appropriate animal models, as well as more advanced clinical imaging techniques, such as brain atrophy and diffusion tensor. Dozens of therapies are in various stages of clinical development and results from these clinical studies will provide important tests of immune and inflammatory mechanisms of MS disease.

Multiple sclerosis (MS) is a chronic disorder affecting the brain, spinal cord and optic nerve, which has traditionally evoked a nihilistic response by clinicians, as illustrated by the following quote:

> *'There is no known means of combating the [underlying] disease [of MS] nor of inducing a remission. There is therefore no justification whatsoever for subjecting patients to expensive and often unpleasant forms of therapy. It is better that they should husband their financial resources against the days of disablement which lie ahead. A placebo may be given as a vehicle of hope.'* [1].

Recent advances in understanding immunology and inflammation, accompanied by progress in MS clinical trial design, have animated a broad push to find new immune- and inflammation-based MS therapies. Clinical manifestations during the early, relapsing remitting stage of MS disease (RRMS) typically involve episodes of neurological dysfunction, which can range from numbness to weakness to double vision or blindness. Each clinical episode usually lasts 2–8 weeks, although residual symptoms can be permanent. Magnetic resonance imaging (MRI) scans of brain and spinal cord usually demonstrate multiple, discrete areas of increased T2 relaxation (hyperintensities on T2-weighted images), which can indicate a protean pathology, including varied combinations of demyelination, inflammation, gliosis, edema and axonal loss. Relapses recur over time and, after an average of 15–20 years, most patients develop gradually progressive and permanent disability instead of the episodic, temporary symptoms seen earlier in the disease. Pathological and radiological studies from both early and later stages of MS [the later stage is called secondary progressive MS (SPMS)] have shown significant axonal loss and atrophy [2–4]. These observations, coupled with the difficulty in slowing progressive disability, even with the most aggressive forms of immunosuppression, led to speculation that SPMS is predominantly a degenerative disorder, with relatively little contribution of immunological or inflammatory components [5]. Consequently, clinical therapeutic approaches using immunological manipulation have focused on the early stages of MS, in hopes that early therapeutic intervention will delay the later onset of progressive disability.

Is MS an immunopathological disease?

Pathological evidence for inflammation in demyelinating MS plaques led to the proposal that the immune system has an integral role in the pathogenesis of MS. The ensuing century of clinical investigation has neither confirmed nor falsified this hypothesis. It is clear that intrathecal inflammation occurs during MS (from analyzing both tissue sections and cerebrospinal fluid); immunosuppressive or immunomodulatory medications can affect disease course over the short term but have not provided convincing impact on long-term (10–20 years after onset) occurrence of disability; and unbiased genetic analysis of MS susceptibility confirmed earlier relationships between MS and the MHC. By contrast, recent pathological studies challenged whether or not MS is primarily an immunopathological process [6]. In one very early case of MS, these investigators reported oligodendrocytes with shrunken nuclei, condensed nuclear chromatin and nuclear fragmentation – all suggestive of apoptosis. Complement activation and ramified microglia were observed, whereas, T cells, early-activated macrophages and active demyelination were not seen in these lesions. It remains unclear whether such cases represent rare exceptions or the common, albeit infrequently observed, initial pathology of MS. Clearly, if MS plaques are initiated through non-inflammatory oligodendrocyte apoptosis, we will require a very different therapeutic strategy than currently contemplated.

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Lessons from EAE

Animal models, such as experimental autoimmune encephalomyelitis (EAE), act as platforms for evaluating the immune and inflammatory hypothesis of MS pathogenesis, and it is now evident that autoimmunity to myelin can produce inflammatory damage to the central nervous system (CNS) in animals, accompanied by episodic paralysis. Much subsequent research has aimed at dissecting the various components that contribute to this immune-mediated injury. EAE can be induced by either active immunization with brain-derived proteins or peptides or passively with activated lymphocytes.

Studies using EAE have been strikingly successful at clarifying mechanisms by which tolerance is broken and through which tissue damage occurs. However, translation of this information to the human disease has been problematic and it is uncertain why. For example, one standard therapy for MS, glatirimer acetate, was originally developed to exacerbate EAE but was instead observed to ameliorate disease. This observation in EAE led to successful clinical trials in MS and subsequent clinical application. The other main therapy for RRMS is interferon-β (IFN-β). The efficacy of IFN-β was discovered almost serendipitously, through small open-label trials that were undertaken with the intent of treating what was believed to be a chronic viral process. After its approval for treating MS, IFN-β was also found to be modestly efficacious in the EAE model.

Hurdles in translating EAE results to MS treatment extend beyond these examples. In particular, several immunological manipulations that reduced or abrogated EAE were inert in MS or caused unexpected clinical and/or radiological worsening. These interventions included injections of IFN-γ, oral tolerization with bovine myelin, the tumor necrosis factor-α (TNF-α) sequestrants RO-452081 (Lenercept) and cA2 (Infliximab), as well as the altered peptide ligand CGP77116. Other drugs that were safe in rodents but were toxic to humans include the IFN-γ and TNF-α inhibitor roquinimex. Therefore, EAE can be regarded as an incisive model for evaluating scientific hypotheses, however, it appears less helpful than anticipated for direct screening of potential clinical therapies.

MS clinical trial methodology: the impact of imaging

Clinical trials in MS have taught us how to study inflammation-directed putative therapies for RRMS. Anti-inflammatory therapies can be studied best using frequent MRI scans over a short period of time, using gadolinium-enhancing lesions as a primary outcome. If these initial, MRI-based trials are successful, then larger licensing studies can be executed using primary clinical outcomes, such as clinical relapses.

However, assessment of therapies that do not affect inflammation require more complex clinical and MRI assessments, making small screening studies more difficult. Studying therapies for the SPMS stage is similarly challenging because clinical relapses and enhancing lesions become less common in SPMS and natural history studies found that clinical progression in SPMS proceeds irrespective of concomitant inflammatory disease [7]. Larger and longer clinical studies will be needed in SPMS, and advanced MRI techniques measuring tissue injury, such as brain atrophy, and diffusion tensor imaging, might provide radiological insight. These techniques are under development for application in clinical trials.

Current therapeutic strategies for MS

Table 1 outlines different immune targets and a sampling of proposed therapies for these targets. Some therapies are at the very beginning of development, others are in mid- and late-stage clinical development and yet others have been abandoned along the way. The breadth of these therapies is a testament to our increased understanding of immunological mechanisms and their potential implications for human disease. Clinical success with these therapies will help expand our understanding of the role of these mechanisms in MS disease pathogenesis. However, the paradoxical activation of MS disease activity with some of these therapies not only illustrates the complexity of immune dysfunction in MS but also emphasizes the importance of small safety studies when evaluating all of these therapies. As a holy grail therapeutic objective, individualized, antigen-specific targets might maximize therapeutic efficacy while minimizing adverse effects [8].

A new generation of anti-inflammatory MS treatments?

Perhaps the most exciting therapeutic strategy currently in clinical development targets lymphocyte adhesion molecules. Natalizumab is a humanized IgG4 monoclonal antibody with specificity for the α-chain of α4β1 integrin [also named very late antigen 4 (VLA4)]. The integrins are essential receptors for cellular interactions between immune cells and endothelial surfaces. α4β1 integrin is expressed on activated monocytes and lymphocytes and mediates transendothelial migration and immune activation, in particular during CNS inflammation. Natalizumab also blocks interactions of α4β7 integrin with its ligands.

In a 6-month randomized, placebo-controlled trial, monthly infusions of natalizumab reduced gadolinium enhancing lesions on MRI by $>90\%$ and clinical relapses by $>50\%$ [9]. During the subsequent six months of wash-out, enhancing lesion and clinical relapse activity returned to baseline levels, suggesting that natalizumab therapy alters the effector stage of disease and does not induce permanent alterations in immune function. Two large controlled trials of natalizumab are close to completion and, if successful, will probably merit marketing license throughout the world. Efficacy of natalizumab in these studies will provide strong evidence in support of inflammation as an important pathogenic mechanism and therapeutic target in controlling RRMS relapses. Post-release longitudinal analyses are required to determine the impact of anti-inflammatory therapy for delaying, ameliorating or preventing SPMS. Resolving this issue constitutes the 'gold standard' test of the hypothesis that MS is primarily an immunopathogenic, rather than neurodegenerative, disorder.

The search for small molecules

All standard MS therapies for RRMS require either injection or intravenous infusion. This needle-based

Table 1. Potential therapeutic mechanisms of action in treating MS[a]

Mechanism	Example agents[b]	Implications, if found to be effective
Adhesion molecule blockade	CDP 323 (oral VLA-4 antagonist, CellTech); ISIS-107248 (antisense oligonucleotide against α-4/β-1 integrin, Isis Pharm); natalizumab (Antegren, Elan/Biogen Idec); 683699 (α-4/β-1 and α-4/β-7 integrin antagonist, Tanabe Seiyaku/GlaxoSmithKline)	Would support the importance of hematogenous leukocytes in pathogenesis
Altering Th1:Th2 cytokine ratio	Anakinra (IL-1 receptor antagonist, Kineret); daclizumab (IL-2 receptor antagonist, Zenapax); CNTO-1275 (IL-12/23 antagonist, Centocor); infliximab (TNF-α inhibitor, Remicade); IL-10; pirfenidone (TGF-β and PDGF antagonist, Intermune); RO-452081 (TNF-α inhibitor, Lenercept, Genentech); roquinimex (IFN-γ and TNF-α inhibitor, Linomide); salbutamol (oral albuterol, β2-adrenoceptor agononist, which inhibits IL-12); fumarate (Biogen Idec/Fumapharm)	Would support a pathogenic role of $CD4^+$ lymphocytes and their secreted products. Previously, anti-cytokine therapies produced paradoxical disease worsening, suggesting the additional challenge of regulatory feedback loops
Antigen-specific bystander suppression	Altered peptide ligands (GP77116, and NBI-5788 Neurocrine Biosciences); glatirimer acetate (Copaxone)[c]; MBP8298 (BioMS Medical); oral myelin (Myloral)	Would support a myelin-based autoimmune pathogenesis; supports the feasibility of bystander suppression
B-cell depletion or reduction	Azathioprine (Imuran); rituximab (anti-CD20 antibody, Rituxan)	Would support role of B cells (antibody production; antigen presentation; cytokine production)
Chemokine receptor blockade	AZD-4750 (CCR inhibitor, AstraZeneca); BX-471 (CCR1 inhibitor, Berlex); L-0124467 (CCR2 inhibitor, Merck); 1d9 (anti-CCR2 antibody)	Would support the importance of lymphocyte communication
Clearance of humoral components	Plasma exchange	Would support importance of circulating humoral component
Co-stimulation inhibition	BMS-188667 (CTLA4-Ig, Bristol-Myers Squibb); RG2077 (CTLA4-IgG4m, Repligen)	Would indicate the importance of T-cell function in disease pathogenesis. The lack of clinical efficacy of CTLA4-Ig despite positive mechanistic immunologic studies suggests that co-stimulation might be a challenging target
Depletion or inactivation of pathogenic T cells	AG284 (DR2:MBP84–102 complex, TCR blockade, Anergen); ATM-027 (Vβ5.2/5.3 receptor peptide vaccine); TCR vaccine (three TCR CDR2 peptides, Immune Response Corporation)	Would support an autoimmune, T-cell-mediated, myelin-targeted pathogenesis. Application might require tailored analysis of patient's immune reactivity to target antigens
Fc-mediated tissue injury; antibody production	Intravenous immune globulin (various preparations and manufacturers)	Would support B-cell-dependent pathogenesis
General immunosuppressants	Alemtuzumab (anti-CD52 antibody, Campath-1H); chlorodeozyadenosine (adenosindeaminase-resistant purine nucleoside, Cladribine); cyclophosphamide (Cytoxan); laquinimod (SAIK-MS, Active Biotech); leflunomide (purine synthesis inhibitor, Arava); methotrexate (Rheumatrex and Trexall); mitoxantrone (Novantrone)[c]; mycophenolate mofetil (Cellcept); parsepil (PARP inhibitor, Inotek); sulfasalazine (Azulfadine); teriflunomide (dihydroorotate dehydrogenase and protein tyrosine kinase inhibitor, Aventis)	Would support the importance of inflammatory damage in disease pathogenesis, although the broad immunosuppressant effects of these preparations limit further insights
Immune ablation with autologous stem-cell rescue	Hematopoietic stem cell transplant	Would support the concept that 'resetting the immune system' can circumvent the need for continuous immune modulation
Increase pregnancy-associated hormone signalling	Estriol (various); MM-093 (α fetoprotein, a TGF-β carrier, Merrimack)	Would support the concept that gender disparity in MS incidence is hormonal and suggests that reduced disease activity during pregnancy is also hormonal and cytokine regulated
Inhibit MMPs	Minocycline (Minocin and Dynacin)	Would support the pertinence of MMP activity, although this agent is pleiotropic. Several other agents also inhibit MMP as a secondary effect
MHC complex	MS-AnergiX (Corixa)	Supports the importance of antigen presentation to T cells

Table 1 (*continued*)

Mechanism	Example agents[b]	Implications, if found to be effective
Neuroprotection (numerous potential mechanisms)	Corticosteroids (e.g. methylprednisolone); E-2007 (AMPA receptor antagonist, Eisai); neurotrophic cytokines (CNTF, LIF); erythropoietin (Aranesp, Eprex, Epogen, Procrit); phenytoin (Dilantin); proprionic acid	Would support the relevance of any clinical trial methodology that could provide convincing evidence for efficacy
Phosphodiesterase-4 inhibition	Mesopram (Schering AG); rolipram (Schering AG)	Would support the pathogenetic importance of phosphodiesterase action either at the blood–brain barrier or in cytokine modulation
Statins	Atorvastatin (Lipitor); simvastatin (Zocor)	Supports the relevance of Rho signaling for pathogenesis. These are approved oral agents that could rapidly be integrated into current treatment
Type I IFNs	IFN-β-1a (Avonex[c], Rebif[c]); IFN-β-1b (Betaseron/Betaferon)[c]; IFN tau (IFN tau agonist, Pepgen); pegylated IFN-β-1 (a longer-acting IFN-β, Serono)	Type I IFNs exert unexplained benefits for MS. Greater success with higher doses or improved pharmacokinetics would suggest that 'more is better'

[a]Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4 isoxazole proprionic acid; CD, cluster designation; CDR, complementarity determining region; CNTF, human ciliary neurotrophic factor; CTLA, cytotoxic; EPO, erythropoeintin; PDGF, platelet-derived growth factor; IFN, interferon; IgG, immunoglobulin G; IL, interleukin; IVIg, intravenous immune globulin; LIF, leukemia inhibitory factor; MBP, myelin basic protein; MMP, matrix metalloproteinase; MS, multiple sclerosis; PARP, poly-ADP ribos polymerase; TCR, T-cell receptor; TGF, transforming growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.
[b]An outline of different immunological mechanisms and selected agents that could test these mechanisms. Many agents have multiple potential mechanisms, but are listed with their main hypothesized or targeted mechanism. ( ) indicates the mechanism, commercial name, and/or development company name for non-licensed therapies.
[c]Indicates US FDA-approved treatment for MS.

administration has obvious drawbacks and orally available therapies are greatly sought after by MS patients. Statins are oral lipid-lowering drugs that block prenylation of intracellular signaling components, such as Rho. Consequently, statins are potently anti-inflammatory and exhibit immunomodulatory properties in EAE (including reduction of leukocyte–endothelial interactions) and reduce MRI-detectable inflammation in MS [10,11]. Several large studies of statins for the treatment of MS are underway. One such study, involving patients with a single inflammatory attack, will use the extensive core immunology laboratories of the Immune Tolerance Network (ITN, http://www.immunetolerance.org) and should provide broad mechanistic data regarding the effect of statins on immune function in patients at the early stages of MS. Studies of other oral agents are planned or underway, including SAIK-MS (Laquinimod), which is a derivative of roquinimex, the adenosine receptor agonist cladribine and the guanosine nucleotide synthesis inhibitor mycophenolate mofetil. The potential impact of oral therapies on patient acceptance cannot be over appreciated.

Another motivation for finding oral small molecule agents comes from understanding the impact of neutralizing antibodies on protein-based therapeutics. Several MS therapies under development use humanized monoclonal antibodies, which can elicit anti-idiotypic responses. Numerous studies have shown that neutralizing antibodies to the commercially available IFN-β preparations abrogate efficacy, as measured by gene induction and on MRI, with probable impact clinically [12–14]. Strategies to minimize the development of neutralizing antibodies will be an important aspect of MS therapeutics that use humanized monoclonals and other protein therapeutics [15].

The challenge of neuroprotection

Progressive forms of MS, which include SPMS, currently have no proven effective therapy. In part, this deficit arose because previous MS research focused on anti-inflammatory and immunomodulating strategies. Clearly, prevention of inflammatory tissue damage is a form of neuroprotection. However, protecting neural tissue from secondary degeneration following inflammatory injury is another facet of neuroprotection. This type of neuroprotection will probably require very different therapeutic strategies and basic immunological animal models for secondary progression following inflammatory damage are greatly needed. Intermittent corticosteroids appear to have potential efficacy in later-stage disease, although further study is needed [16,17].

The autoimmune basis for MS remains an unproven hypothesis, however, immune-targeted therapeutic interventions will help to validate or discard the role of inflammation and autoimmunity, as well as to understand the role of individual immune mediators in disease pathogenesis. We clearly already have some therapies to decrease MS clinical relapses and MRI evidence of inflammation. Considering the number of therapeutic approaches under study, better therapies will surely be available in the near future. Application of mechanism-based research through new clinical trial techniques will help to avoid our patients' days of disablement and should give them a true vehicle of hope.

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25 years since the eradication of smallpox: why poxvirus research is still relevant

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The World Health Organization (WHO) announced the eradication of smallpox twenty-five years ago this month. This conquest of an infectious disease, which has been the bane of humankind for centuries, still stands as the WHO's greatest achievement. The anniversary of such a scientific and medical landmark provides an appropriate occasion to reflect on this feat and to assess the significance and necessity of the poxvirus research that has followed this.

Members of the Poxviridae family (comprising six genera) are the largest known DNA viruses, which can replicate in the cytoplasm of vertebrate and invertebrate cells. The genus orthopoxviruses comprise of variola, monkeypox, vaccinia and cowpox viruses, which result in febrile illnesses associated with vesicular rash in humans and animals. The most notorious member is variola virus, which resulted in the disease called smallpox that killed ~500 million people during the 1900s. It has claimed hundreds of millions of lives between its first recorded outbreak (Ancient Egypt) and its eradication in 1979 [1,2] (Table 1).

Smallpox, a specifically human disease with a characteristic clinical picture, was the most successful of all human infectious diseases to eradicate. Both biological and sociopolitical factors contributed to the eradication of smallpox [1,3–5] (Box 1). The concept of the global eradication of smallpox was first proposed in 1953 by the first Director-General of the World Health Organization (WHO) (http://www.who.int), Canadian Brock Chisholm, however, after two years of debate, it was rejected as unrealistic by the World Health Assembly. In 1958, the representative of the USSR (Viktor Zhdanov) proposed that the WHO should support a program of global eradication of smallpox by vaccination of 80% of all inhabitants of the 59 countries in which the disease was then endemic; the World Health Assembly accepted this. By 1965, it was clear that, although smallpox had been eradicated from 12 small countries, global eradication could not be achieved by vaccination alone. In September 1965, D.A. Henderson, of the US Centers for Disease Control (CDC; http://www.cdc.gov), was asked to join three WHO officials to prepare a plan that could be discussed by the World Health Assembly in May 1966. With a modest budget of $2.4 million (worth approximately $14 million in today's dollars) annually for ten years, it was passed by a margin of only two votes [1,3–5].

Unlike the USSR program, the Intensified Smallpox Eradication Program was administered by a Smallpox Eradication Unit based in Geneva, with Henderson as its Chief, Isao Arita as medical officer and four other staff. Routine vaccination remained a basic requirement, however, several other strategies were introduced. Arita

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