New Directions in Multiple Sclerosis Therapy: Matching Therapy with Pathogenesis

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The characteristic clinical course of multiple sclerosis (MS) features an initial relapsing remitting course with evolution into a secondary progressive phenotype. As will be discussed in this presentation, the experience with therapies approved for treatment of MS over the past two decades indicates that therapeutic response varies with the clinical disease stage or phenotype. All of the currently approved agents for treatment of MS (interferon β (IFNβ), glatiramer acetate (GA), natalizumab) have the systemic immune system as their targets. Clinical trials with IFNβ and GA demonstrate their efficacy in reducing relapses of the disease whether early in the disease course or in well established disease that has entered the secondary progressive phase. These agents have failed to show efficacy for progressive components of the disease. More recent results using agents that are even more effective in reducing disease relapses such as alemtuzumab and rituximab have failed to prevent disease progression in patients with established disease\textsuperscript{1,2}. Even the most intense immunosuppressive regimens involving combinations of chemotherapies leading to complete ablation of the systemic immune system such that autologous stem cells can be reinfused have had only limited success in delaying or preventing disease progression and none appear to have impact on the progressive phases of disease.

The diagnosis of MS is based on clinical presentation and magnetic resonance imaging (MRI) findings, but the pathogenesis of the disease remains poorly understood. The clinical course of the disease is variable, and the disease can be episodic with periods of remission and relapse, or progressive. The disease can be categorized into four subtypes: relapsing-remitting, secondary progressive, primary progressive, and progressive-relapsing. The relapsing-remitting subtype is the most common, accounting for approximately 85% of MS cases. The secondary progressive subtype is the most common type of MS in older patients. This subtype is characterized by a slow, steady progression of disability over time, with occasional exacerbations of symptoms. The primary progressive subtype is characterized by a gradual progression of disability from the outset of the disease, with no periods of remission. The progressive-relapsing subtype is a combination of the two, with periods of relapse followed by progressive disability.

ABSTRACT: All currently approved therapies for multiple sclerosis (MS) modulate systemic immune components prior to their entry into the central nervous system (CNS). Available data indicate they lack impact on the progressive phases of disease; the more potent systemic immune-directed agents predispose to development of infectious or neoplastic disorders. Development of new agents that enhance disease stage related efficacy and limit systemic toxicity will need to consider the underlying mechanisms related to each phase of the clinical disorder, namely relapses, remission, and progression. This report focuses on disease related mechanisms ongoing within the CNS that contribute to the different phases of MS and how these may serve as potential therapeutic targets. Such mechanisms include CNS compartment specific immunologic properties especially as related to the innate immune system and neural cell-related properties that are determinants of the extent of actual tissue injury and repair (or lack thereof).

RÉSUMÉ: Nouvelles avenues dans le traitement de la sclérose en plaques appariant le traitement à la pathogenèse. Tous les médicaments approuvés actuellement pour le traitement de la sclérose en plaques (SP) modulent des composantes immunitaires systémiques avant leur entrée dans le système nerveux central (SNC). Selon certaines données, ils n’auraient pas d’impact sur les phases progressives de la maladie. Les agents systémiques les plus puissants dirigés contre la réponse immunitaire prédisposent à l’apparition de maladies infectieuses ou néoplasiques. Le développement de nouveaux agents thérapeutiques qui rehaussent l’efficacité en relation avec le stade de la maladie et limitent la toxicité systémique devra tenir compte des mécanismes sous-jacents à chaque phase de la maladie clinique dont les récidives, les rémissions et la progression. Cet article met l’emphase sur les mécanismes évolutifs reliés à la maladie dans le SNC, qui contribuent aux différentes phases de la SP, et comment ces mécanismes pourraient servir de cibles thérapeutiques potentielles. Ces mécanismes incluent les propriétés immunologiques spécifiques du compartiment du SNC surtout en relation avec le système immunitaire inné et avec les propriétés des cellules nerveuses qui sont des déterminants de l’étendue de la lésion tissulaire et de sa réparation.

hematogenous stem cell replacement is required, did not prevent disease progression in some patients even while completely preventing further clinical relapses and new MRI defined lesion formation. This dissociation between impact of such agents on relapses and disease progression does not exclude that there is a cause:effect relation between these events, as suggested by natural history studies that were conducted in the pre-therapeutic era at the University of Western Ontario. These latter studies showed that the frequency of initial relapses is a predictor of subsequent disease progression. The above observations have led to the paradigm that systemic immune directed therapy of MS should be instituted early to prevent further injury rather than waiting until the patient declares that (s)he has entered a progressive disease phase.

The theme of this paper is to consider mechanisms operative within the central nervous system (CNS) that contribute to each of the disease phases of MS and how defining these processes will direct inroads into new therapy. Such mechanisms include those related to the immune constituents found within the CNS and those related to the neural cell populations. For the former, I emphasize the role of the innate immune constituents in regulating and effecting immune responses within the CNS. For the neural elements, I consider properties which determine susceptibility to injury and that are involved in repair (or lack thereof).

**RELAPSING PHASES OF MS**

The pathologic hallmarks of the CNS lesions underlying the initial acute event or subsequent disease relapse in MS include active demyelination associated with inflammation both in the perivascular spaces and parenchyma. The observation from the time of Pasteur that systemic immunization with neural tissue containing vaccines CNS could induce an inflammatory demyelinating disorder of the CNS (usually referred to as post-vaccination or acute disseminated encephalomyelitis (ADEM)), established that systemic exposure to autoantigens could initiate a CNS directed disorder. The development of an animal model, termed experimental autoimmune encephalomyelitis (EAE), initiated by systemic immunization with autoantigens, usually myelin components, continues to be the most used model of MS including as a test bed to assess the potential efficacy of new therapeutic agents (see later discussion). To date there are no spontaneous onset inflammatory demyelinating diseases of the CNS in non-genetically engineered animals.

Although most current opinion favors that the initiating event in MS begins with systemic immune sensitization in a manner parallel to that of ADEM/EAE, the possibility exists that initial CNS insults (trauma, infection) could initiate release of autoantigens that result in subsequent immune sensitization. Transport of CNS released antigens to the systemic lymphoid organs is well demonstrated. Other factors to consider are that MS occurs in an out-bred population, living in a non-sterile environment.

Most studies of EAE are conducted in genetically-selected strains raised under well defined laboratory conditions. Some animal strains are completely resistant to EAE; there are also differences between strains as to which specific myelin antigens induce the disease. Even for a specific strain, the environmental conditions can determine whether disease will develop or not.

Almost always the immunization protocol to induce EAE involves antigen given in combination with adjuvant, a chemical means designed to boost immune reactivity. This experience from animals suggest that there may be multiple inducing antigens in the human disease and that susceptibility will be greatly influenced by environmental factors that can interface with the innate or adaptive immune system. Ongoing epidemiologic observations indicate the influence of environment on development and course of MS. There appears to be an increasing emergence of Western type of MS in the Orient where previously the optico-spinal form of the disease dominated. There have been consistent findings that the frequency of MS is increasing in women. Long recognized is that disease relapse frequency is linked with inter-current viral infections and has a seasonal variation.

The immune system can be divided into two overall but interactive components, namely the adaptive and the innate immune systems. The adaptive system is comprised of αβ receptor-expressing CD4 and CD8 T cells and B cells with immunoglobulin (Ig) serving as their receptors. These receptors have broad diversity, resulting in capacity to recognize a wide array of specific antigens. These adaptive immune cells retain immunologic memory. Attempts continue in the MS field to develop antigen specific therapies either by eliminating the specific T cells that recognize the putative disease-relevant antigen or altering their response (e.g., from a pro- to an anti-inflammatory profile) to such antigens. This type of therapy is referred to as tolerance induction. Pioneering studies on this approach using myelin basic protein as the candidate antigen have been conducted by Warren and colleagues to whom this supplement is dedicated. As mentioned, an ongoing challenge in MS is to establish that there is a crucial antigen (or antigens) that would serve as the target for antigen specific therapies. Such therapies would eliminate the risks associated with non-selective immune modulatory or ablative therapies.

The innate immune system is considered as the primitive form of the immune system, having much more limited diversity compared to the adaptive immune system; the constituents of the innate immune system using specialized types of receptors serve as the first responders when the host is challenged by stimuli from the environment. These can be stimuli derived from the external environment (eg infections) often referred to as “stranger signals” or from the internal environment (eg dead or injured tissues and cells) referred to as “danger signals”. The bi-directional interactions between the components of the adaptive and innate systems further result in each influencing the properties of the other.

Given the previous discussion regarding the influence of environment on development and course of MS, we will consider how the innate immune system can impact on the cascade of events that result in new lesion formation and tissue injury in MS, potentially providing further therapeutic targets. In the systemic compartment the innate immune system is comprised of elements of the myeloid lineage including monocytes, dendritic cells (DCs), and macrophages. The perivascular regions of the CNS contain myeloid cells sometimes referred to as “perivascular microglia” and are comprised of cells (monocyte, DCs) that are continually arriving from the systemic circulation. The resident myeloid population within the CNS...
IMMUNE-REGULATION

The peripheral innate immune cells fulfill the crucial role of being the antigen-presenting cells (APCs) required for allowing antigen recognition and response by the T cells of the adaptive immune system. T cell activation requires three signals namely antigen, major histocompatibility complex (MHC), and co-stimulatory (or inhibitory) molecules, all delivered via the APCs. The activation state of the peripheral APC is an important variable that will determine the degree of activation and phenotypic properties (Th1, Th2, Th17) of the T cells it engages. Studies in EAE that involve deletion of peripheral APCs such as with toxic liposomes or manipulating co-stimulatory molecule expression indicate the functional role of the APCs in the development of this autoimmune disorder8. Planned clinical trials for MS include use of myeloid cells whose activity is impaired by pharmacologic manipulation (phosphodiesterase inhibitors) or blocking or by-passing co-stimulatory signals8. Glaritamer acetate and IFNβ may both exert some of their actions by modulating properties of APCs10.

A potential further therapeutic approach would be to target the immune response ongoing selectively within the CNS compartment. Such an approach would have the advantage that one could manipulate disease activity without impairing required physiologic systemic immune responses. For a systemic immune compartment initiated response to lead to tissue-injury in the CNS, the relevant auto-reactive T cells must enter and persist within the CNS compartment and then, in concert with additional components of the immune system that enter the sites of inflammation, mediate the actual tissue injury. It has long been shown in animals that the antigen recognized by autoreactive T cells must be presented to these cells within the CNS for such cells to persist in this compartment11. Thus one identifies the important role for APCs within the CNS. Conversely one can consider whether specific CNS APCs may favor down regulating immune responses and contribute to the initial concept that the CNS was an immunologically privileged site.

An emerging series of studies in animals and humans have begun to address the relative role that different myeloid cells found in the CNS during an inflammatory process play in promoting or inhibiting T cell associated immune response in the CNS and how these regulatory properties are influenced by stimuli from the micro-environment. In the EAE model the perivascular myeloid cell population, specifically DCs, are shown to be crucial for development of the inflammatory response within the CNS12. In situ studies of post-mortem derived adult human CNS tissues suggest that the microglia may be more activated under “normal” conditions as judged by expression of MHC class II and co-stimulatory molecules compared to microglia in the CNS of rodents raised under clean laboratory conditions. Even in rodents there are apparent strain differences with regard to basal expression of these molecules. Systemic endotoxin administration has long been shown to activate these CNS cells13. Our own studies of microglia obtained from surgical resections of non malignant tissue (usually performed to alleviate intractable epilepsy) indicate donor-donor variability in expression of mRNAs encoding different toll like receptors (TLRs), crucial receptors involved in recognition of exogenous infectious agents (“stranger signals”)14. Microglia activation through engagement of different TLRs results in distinct patterns of regulatory cytokine production with significant impact as to whether T cells interacting with these microglia will demonstrate a pro- (Th1) or anti- (Th2) inflammatory phenotype. We further found that human microglia in vitro when exposed to conditions that generate DCs from monocytes, result in these cells producing anti-inflammatory cytokines and having an inhibitory influence of T cells when compared to effects produced by DCs15. These observations suggest that the different myeloid cell populations found in the CNS differ in their capacity to serve as immune-regulatory cells. In specific context of MS and response to tissue injury (“danger signals”), Boven and colleagues showed that myeloid cells that had ingested myelin, a characteristic feature of active MS lesions, expressed anti-inflammatory (IL-4, IL-10) rather than pro-inflammatory (IL-12) cytokines (i.e., M2 rather than M1 phenotype)16. Such cells could be derived from either microglia or infiltrating monocytes. Li et al observed that the IL-17 regulating cytokine IL-23 was expressed in activated microglia/macrophages including “foamy” cells in active MS lesions, as well as in DCs in perivascular cuffs17. Boven et al16 further showed that feeding myelin in vitro to human blood derived monocytes results in sequential down regulation of pro- (p40 subunit of IL-12 and IL-23) and subsequently anti- (IL-10) inflammatory cytokines. We had shown that feeding myelin with opsonized immunoglobulin (ie an immune complex as occurs in MS lesions which have both myelin and Ig) could actually induce pro-inflammatory cytokine production by myeloid cells18. Thus endogenous signals arising from injured tissue have important influences on the inflammatory milieu in the CNS.

The infiltrating adaptive immune cells (T cells) that are subject to regulation by the myeloid cells in MS also provide feedback signals to the myeloid cells. Th1 polarized T cells favor induction of the M1 phenotype in myeloid cells, Th2 T cells favor induction of M2 myeloid cells19,20. Neither of the major currently approved therapeutic agents for MS (IFNβ, GA) readily access the CNS; thus their effects on innate immune cells within the CNS would likely be indirect, i.e., act on systemic T cells or myeloid cells which subsequently access the CNS. A future therapeutic direction would be development of therapeutic molecules that can directly access the CNS. The sphingosine-1 phosphate receptor (S1PR) agonist fingolomid (FTY720) represents an example of an agent that accesses the CNS21. We can show differential expression of individual S1PRs on the different myeloid cell types that are present in the CNS22. The advantage of selectively manipulating myeloid cells within the CNS would be to suppress immune activity in a single compartment while sparing overall immune competence.
**Immune effector mechanisms**

The actual mechanisms by which myelin and/or its cells of origin, oligodendrocytes (OGCs), and axons/neurons are injured in MS remain to be defined.

**Adaptive immune effector mechanisms**

The relative selective target injury seen in MS has focused the search on specific mediators of the adaptive immune system as these cells (antigen specific T cells) and molecules (Ig) have the receptor diversity to be target selective. The CD8 rather than CD4 T cells isolated from MS lesions have restricted clonality implicating antigen specificity but to date the putative antigen is not identified. Both neurons and OGCs can express MHC class I molecules making them potential targets of such cytotoxic T cells; these neural cells do not express MHC class II molecules, the recognition element of CD4 T cells. The search for disease-specific antibodies in MS continues with candidates including those than recognize constituents of myelin and axons, especially those expressed at the para-nodal regions, a site that may have most exposure to the immune system. Less explored mechanisms relate to the contribution of innate immune system constituents as direct mediators of injury or the acquisition of innate immune effector properties by adaptive immune cells in response to an inflammatory environment. The corollary as mentioned is that neural cell susceptibility to such injury would also be markedly influenced by signals from the inflammatory microenvironment.

**Innate immune effector cells**

Potential innate immune cells contributing to tissue injury in MS include the myeloid cells (microglia, macrophages) and the specialized lymphoid cells, NK cells and γδ T cells (discussed later in context of innate immune properties of CD8 T cells). The myeloid cells, especially when activated, are an established source of an array of effector cytokines (TNF, IL-1, IL-6), death receptor ligands (fas ligand, TRAIL) neurotransmitters (glutamate), and proteases that are implicated as mediators of tissue injury. Many of these molecules mediate their effects via engagement of specific receptors that initiate a cascade of intracellular signaling networks leading to cell death or dysfunction. Receptor expression such as for fas ligand and TRAIL can be induced by pro-inflammatory cytokines as found in active MS lesions. Such expression can also be induced by a range of sub-lethal insults (ischemia, trauma), pre-disposing the specific neural cells to subsequent immune-mediated injury, a paradigm, which would fit with speculation that the initiating event in MS need not be a primary immune attack but rather one that evolves in response to an initial insult. This raises the possibility that neural cell type-specific injury as seen in MS could be determined by the properties of the target cell rather than the effector. Under this scenario, a therapeutic strategy would be to down regulate or block access to receptors for the putative injury mediating molecules. Related to this paradigm is that neural cells can up-regulate a range of molecules that contribute to the cell’s resistance to injury such as stress (heat shock) molecules; induction of such molecules is considered to underlie the phenomenon of tissue conditioning in ischemia models.

**Acquisition of innate immune properties by adaptive T cells**

Although, both CD4 and CD8 T cells are prominent feature of acute MS lesions and T cells are required to initiate the EAE disease process, the basis for actual tissue injury remains to be defined. In some EAE models, prominent inflammation can be found without significant demyelination. In early studies we showed that both NK cells and γδ T cells could induce cytotoxicity of human adult CNS-derived OGCs in vitro. Subsequent studies showed that cell contact-dependent injury was related in part to interactions between NKG2D receptors expressed on the immune cells and the corresponding ligands (MIC A and B, UCB) expressed on the target cells. Expression on the latter was up-regulated by pro-inflammatory cytokines. Ligand expression could also be shown in situ on OGCs in active MS lesions. No in vivo data yet exists regarding blocking access to these ligands in a neuro-inflammatory model.

Both CD4 and CD8 T cells if maintained in vitro for prolonged periods in presence of pro-inflammatory cytokines can become “promiscuous” cytotoxic cells, i.e., bypass antigen and MHC restrictions. Observations in celiac disease indicate that non-antigen restricted CD8 T cells can be mediators of tissue injury, which is dependent on NKG2D expression by such cells and with IL-15 being the key cytokine up-regulating such expression as well as the expression of cytotoxicity mediating molecules (perforin/granzymes). Within the MS CNS, IL-15 is prominently expressed by astrocytes and by myeloid cells. Our in vitro studies show that surface bound IL-15 expressed by astrocytes can also induce such changes in CD8 T cells.

Neuronal injury and loss is increasingly recognized even as an early feature of MS. Neurons are reported to be susceptible to injury mediated by perforin/granzymes, which are the expected effector molecules of cytotoxic lymphocytes. When we added NK cells to dissociated cultures of fetal human CNS we added NK cells to dissociated cultures of fetal human CNS-derived neurons grown on a bed of astrocytes we observed destruction of the cultures over several hours. Time-lapse imaging demonstrated that initial injury was directed at the underlying astrocytes and involved NKG2D receptor-ligand interactions. In such in vitro systems, one can also induce sub-lethal astrocyte injury with CD8 T cells as measured by fragmentation of GFAP, an occurrence also seen in MS lesions. Loss or impaired trophic function of astrocytes or reduced capacity of these cells to buffer the environment represent further potential mechanisms contributing to impaired neuronal function in MS.

**Combined innate-adaptive immune effector mechanisms**

Immune effector mechanisms can also involve the interplay between the innate and adaptive immune constituents. Myeloid, NK, and γδ T cells all express Fc receptors that can bind the Fc portion of Ig molecules. Such engagement can activate the effector functions of these cells, termed antibody-dependent cell cytotoxicity (ADCC). If the Ig recognizes an epitope on a neural cell, this would promote specificity of immune-neural interactions and specificity of the injury response. We used serum from patients with neuromyelitis optica (NMO) to show that specific antibody binding to aquaporin-4 expressed by astrocytes, supported an ADCC response by NK cells. Thus antibody deleting therapies can impact immune effector...
mechanisms beyond isolated direct antibody mediated toxicity with or without complement activation.

**Recovery from Relapses in MS**

Multiple mechanisms could contribute to the recovery process including resolution of the inflammation due to active or passive mechanisms, neural reorganization involving recovery of the electrical conduction properties of individual axons or of cortical circuits, and actual tissue repair. Immunohistochemical-based studies indicate the potential for remyelination to occur in recent MS lesions. Current experimental data indicate that such CNS remyelination is mediated by recruitment of progenitor cells rather than by previously myelinating OGCs. Progenitor cells committed to the myelin lineage can be detected in the normal adult human CNS and in vicinity of active MS lesions. Windrem et al showed that such progenitors selected on the basis of expression of the ganglioside A2B5, could myelinate axons when transplanted into the CNS of dysmyelinating mice (shiverer). The experimental studies in rodents demonstrate that the capacity for progenitor cells from both adult and fetal sources to proliferate and differentiate can be boosted by combinations of selected growth factors. Studies of human progenitors, largely from fetal sources, indicate that similar potential may exist in the human situation. Our in vitro studies show that a number of pharmacologic agents that can access the CNS, including lipophylic statins and the SIPR agonist, FTY720, can interact with human fetal CNS derived progenitor cells; their effects on cell proliferation and differentiation are both time- and dose-dependent. These same statins, when given in vivo, were found to actually impair the remyelination process in the cuprizone toxicity model. We found that FTY720 boosted remyelination in rodent cerebellar slice cultures exposed to the demyelinating toxin lysolecithin. Gregg and colleagues reported that prolactin boosts the extent of remyelination in the spinal cord of mice that had a local demyelinating lesion induced by lysolecithin. The challenge for clinical trials with such agents is how to document ongoing remyelination with physiologic or imaging techniques. Relying on clinical outcomes may be difficult in small scale short term studies.

**Progression of MS**

The failure of systemic immuno-therapy in cases of primary and secondary progressive MS emphasizes the need to define the biology accounting for this clinical phenotype as a basis for finding new therapeutic directions. To be considered are the contributions of continuing tissue injury and failure of repair mechanisms. The more chronic but still active lesions are dominated by myeloid cells although some lymphoid cells are present. There is recognition of more organized germinal type follicles in the meninges consistent with chronic immune reactivity especially chronic antibody production. The mechanisms whereby innate immune mediators acting alone or in concert with antibody (ADCC) can effect injury have been described in a previous section. One postulate is that recurrent or chronic insults mediated by individual or combinations of immune effector molecules lead to the progression of tissue loss in white or grey matter. Related to this concept would be that the initial insults may be sub-lethal but enhance susceptibility of the target cells to a subsequent lethal insult. Such scenarios would particularly apply to post-mitotic cell populations, namely neurons and OGCs. As also mentioned earlier, immuno-therapies that directly access the CNS compartment would be suitable candidates to test these hypotheses.

Continued disease progression may also be linked to the effectiveness of the repair processes that occur in response to initial injury or to failure of these processes to continue to be sustained. Redistribution of specific sodium channels along the entire length of demyelinated axon segments may initially enhance nerve conduction but then lead to excess calcium influx and ultimate axonal transaction. Similarly impaired energy production (ATP production) by damaged neurons can lead to progressive axonal failure. Further neuronal-related pathology could involve loss of dendritic spines and of synaptic connections. Recent neuropathologic studies of very late MS cases indicate that at this time there may be little evidence of immune activity and active neuron/myelin destruction presumably even in face of continued clinical decline and MRI based evidence of ongoing tissue loss, further raising the question of what pathologic process should be targeted. As regards remyelination, immunohistochemical studies further suggest that although there are at least some remaining progenitor cells, there appears to be a “block” in their capacity to further differentiate and become involved in the remyelination process.

**Conclusion**

The initial therapeutic era in MS has demonstrated that systemic therapies that modulate or destroy some or all components of the immune system or alter their trafficking patterns can have significant impact on reducing disease relapses with the anticipated consequence that subsequent development of the progressive disease phase will be delayed or avoided. Emerging systemic therapies have increased efficacy in reducing disease relapses but introduce the risk of immune-deficiency related toxicities. As discussed elsewhere in this supplement, one approach to avoid this problem would be to selectively target only disease-specific immune mediators, such as myelin-reactive T cells and antibodies. This presentation focuses on the events ongoing within the CNS during each phase. Selectively modulating immune reactivity within this compartment during the inflammatory phase of the disease, blocking interactions of immune effector molecules with neural cells, and/or increasing neural cell resistance to such injury mediators are all potential means to reduce immune-mediated injury within the CNS, while sparing the systemic immune system. Such therapies may also be applicable to the more chronic and progressive phases of the disease. Optimal therapy will need to incorporate means to augment repair after initial acute injury, maintain integrity of damaged tissues, and overcome failure of the repair and compensatory mechanisms. Furthering our understanding of the complex interplay of immunologic and neurobiologic aspects of MS will form the foundation for development of therapies that link to actual disease-related mechanisms. Let’s make the punishment fit the crime.
REFERENCES


