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NOVEL APPROACHES AND CUTTING EDGE IMMUNOTHERAPIES IN MULTIPLE SCLEROSIS

Toni M. Bauman [1] and Lloyd H. Kasper [2]

[1] Department of Neurology, Dartmouth Hitchcock Medical Center, Lebanon, NH, [2] Department of Immunology, Dartmouth Medical School, Hanover, NH

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1. ABSTRACT

MS is a chronic inflammatory disease of the central nervous system (CNS). MS is a predominantly CD4+ T cell mediated autoimmune disorder. Recent studies have challenged this existing paradigm by supporting the role of other immune cells and factors (even non-immune) including CNS antigen-driven clonally expanded B cells, autoantibodies, complement and mediators of the innate immune responses in MS lesions. Further expansion of this global CNS dysfunction includes oligodendroglial cell (OGC) loss, attenuated remyelination,

axonopathy, and gliosis. The recognition of new "players" directing effector and regulatory functions and further insight into reparative mechanisms occurring at various stages of the disease within a given individual will influence ongoing and future therapeutic trials. The following discussion will encompass evolving concepts in the pathogenesis of MS with a focus on novel immunotherapies. These new approaches reflect targeting of a multifaceted spectrum of immune activity. The immunotherapies will be characterized by their intervening role of specific and/or multiple pathogenic steps including initiation, peripheral activation, molecular co-stimulation and immune effector responses during early, transitional and late phases of disease. Emerging strategies for the enhancement of neuroprotection and reparative mechanisms will also be reviewed. Classification of novel approaches will include the following main types of immunotherapies: (1) targeting of myelin specific T cells: antigen-specific therapies (2) targeting of B cell and autoantibody responses (3) targeting of immunologic steps of disease pathology (4) targeting of reparative stages of disease: neurotrophic and neuroprotective, (5) global therapies: broad-based polydirectional strategies.

2. GENERAL INTRODUCTION

2.1. Overview

The present review of current novel immune trials is intended to assist the reader in the understanding of evolving MS pathogenic paradigms and provide the reader with an update of cutting edge therapies in multiple sclerosis (MS), and potential future strategies toward the advancement of care of MS patients.

2.2. Introduction

Multiple sclerosis (MS) is a clinical neurological disease characterized by chronic inflammation, demyelination, variable axonopathy and gliosis of the central nervous system (CNS) (1-6). It is the principal (excluding trauma) neurological disease of individuals in early to middle adulthood and has been estimated to include 350,000 people in this country alone although the actual incidence including the undiagnosed may be considerably higher (7, 8). Immune mediated tissue injury in MS appears to develop in genetically susceptible individuals after exposure to a causal environmental agent that is yet undefined (9-12). The disease is clinically and histopathologically heterogenous. with several clinical types of MS. (8, 13). Four histopathologic subtypes of MS have been described with the demonstration of both CD4+ and CD8+ cells in MS lesions (3, 4, 14-17). The majority of MS cases present as a relapsing-remitting (RRMS) condition lasting approximately five to ten years that transitions into a secondary chronic progressive state. Here patients have less frequent relapses and radiographic evidence of blood brain barrier break down but continue to develop progressive neurologic deficits. Approximately 10-20% of patients begin with a primary progressive (PPMS) course characterized by a continuous progression without acute relapses (13, 14). Recent studies suggest the role of both regulatory and effector dysregulation in MS and have raised the possibility of an intrinsic perturbation of CNS factors and neurodengenerative mechanisms contributing to MS especially in the late chronic phases of disease. (18-20). Demonstration that both axonopathy and cerebral atrophy occur early in disease argue for early treatment which may limit long term disability (2-4, 6).

Recently reported CHAMPS and ETOMS trials have provided more direct evidence of the benefit of early initiation of therapy (21, 22). There are currently three FDA approved therapies for the treatment of RRMS and secondary progressive MS. This includes interferon-beta, glatiramer acetate and mitoxantrone. (23-26). These medications have modest effect on disease activity but do demonstrate the ability to reduce attack rate and frequency of active lesions as determined by gadolinium-enhanced magnetic resonance imaging (MRI) (24, 27). The limited efficacy, side effect profile of approved treatments for RRMS along with the failure to treat PPMS and chronic progressive phases of disease has highlighted the need for the development of new therapies to be utilized as mono or combination therapy addressing the heterogeneity of this disease (28, 29).

3. THE IMMUNOLOGICAL BASIS FOR DISEASE INTERVENTION AND RATIONALE FOR NOVEL IMMUNOTHERAPIES

3.1 Disease induction

MS pathogenesis involves a complex sequence of cascading immune events that culminates in chronic disease (Figure 1[step 1]) MS is presumed an autoimmune condition mediated at least in part by T cells (18, 30-34). The precise initiating trigger and induction of the acute phases of disease is unknown but occurs when immune tolerance is disrupted. This process may occur due to molecular mimicry whereby our immune system may attack "self" if a microbe and human share a common gene sequence encoding for a conserved structural peptide/protein(s) such as CNS immunogenic antigens such as, myelin basic protein (MBP), myelin oligodendrocyte glygoprotein (MOG), proteolipid protein (PLP) (5, 35-37). Initiation may occur from other mechanisms such as microbial superantigens, or a self protein. It is possible that the release of CNS antigens to the periphery following a CNS insult such as, the introduction of non-self proteins (CNS viral infection), or acute brain injury (trauma, stroke) may be a mechanism of initiation (19). This process may be perpetuated and expanded by "epitope spreading" that has been defined in EAE, the experimental model of human MS (38). Once initiated, neural antigens are processed by antigen-presenting cells (APC) for presentation to sensitized T cells (CD4+) with resultant activation and co-stimulation in the periphery (33, 39). B cells and CD8+ T responses primed and clonally expanded in the periphery may also be contributory to disease pathology (17, 40-43).

3.2. Peripheral co-stimulation

Paramount in MS immunopathogenesis is the activation, expansion, and differentiation of T cells that are dependent on a coordinated series of signals exchanged between the antigen-presenting cell (APC), the T cell, and the environment. (Figure 1[step 1])The maturity of the APC is

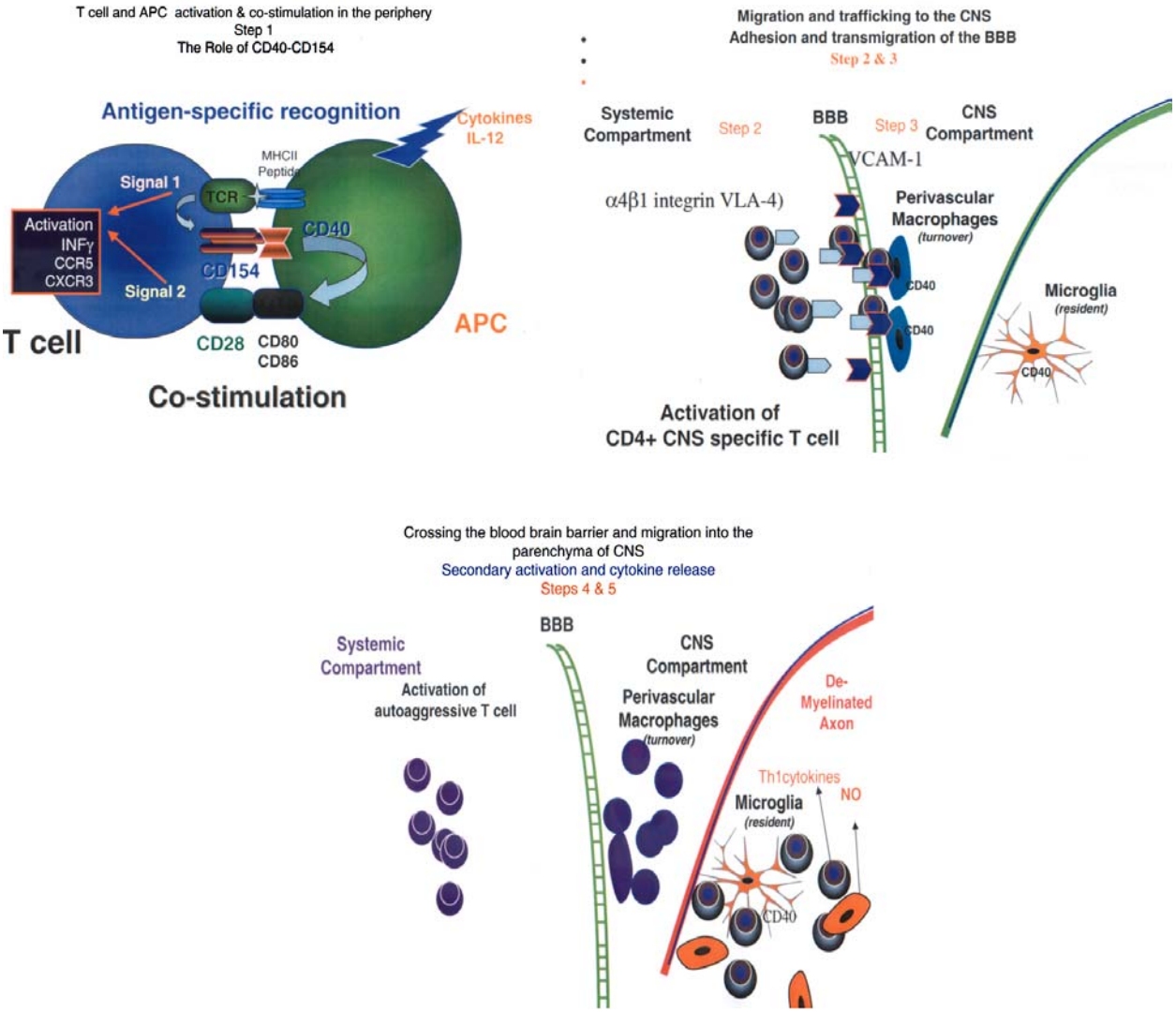

Figure 1. Schematic depiction of the pathogenic steps resulting in tissue injury in MS: (1-5) (1) Activation and co-stimulation of autoreactive T cells in the periphery (2) Migration and trafficking to the CNS (upregulation of homing chemokines & adhesion molecules) (3) Adhesion of proinflammatory autoreactive T cells to the endothelium, release of proteases and MMPs degrading ECM & affording transmigration of the BBB of T, B, moncytes/macrophages into the parenchyma of CNS (4) Secondary activation and amplication of the CNS intraparenchymal cellular and humoral responses with autoantibodies, induction of cytotoxic T lymphocyte (CTL) and cytokine/neurotoxin (NO) mediated CNS damage. (5) Effector stage of disease with injury to the myelin sheath.

controlled by the inflammatory milieu and expression of APC maturation factors (CD154, TNF, RANKL) by the cognate T cells. Initial recognition of peptide-MHC class II by the T cell leads to TCR stimulation delivering the "first signal" which then induces expression of CD154, which in turn binds to CD40 expressed on the APC. CD40 stimulation results in the upregulation of a series of co-stimulatory molecules, including CD80 and CD86. CD80 and CD86 engage the T-cell co-receptor CD28, thereby delivering a “second signal” that culminates in T-cell activation, proliferation, and proinflammatory cytokine production (INFγ, IL-2, IL12, TNFα) Evidence indicates that there are multiple interactions between the T cell and the APC leading to fulminant T-cell activation and expansion. Interruption of these co-stimulatory interactions (such as CD154-CD40) incapacitates APC maturation, thereby impairing the activation, proliferation, and differentiation of antigen-specific T cells becoming potential targets of novel therapies (39, 44, 45).

3.3. CNS migration and trafficking

After priming, highly coordinated sequential interactions involving cellular homing (most likely dependent on preferential expression of chemokine receptors, (CXCR3, CCR5) (46, 47), and trafficking into the CNS compartment proceed. Specifically a number of proinflammatory (Th1)-type cytokines are elicited that induce upregulation of adhesion molecules, and alter permeability of the blood-brain barrier (30, 48). This subsequently promotes the adhesion of proinflammatory autoreactive T cells (intergrins VLA-4 also known as α4β1, LFA-1 on T cells) to the endothelium (via corresponding VCAM, ICAM receptors) allowing T, B, monocytes, macrophages to the extravasate into the

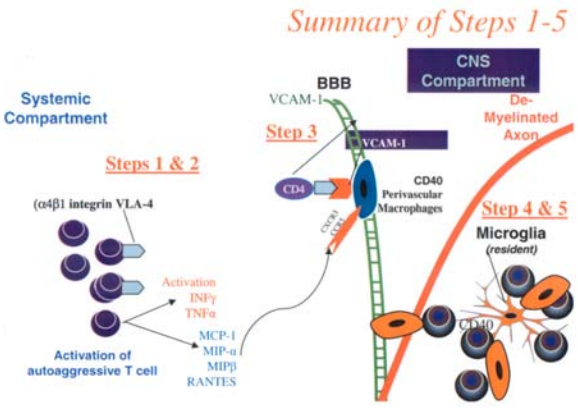

Figure 2. Schematic depiction of the pathogenic steps resulting in tissue injury in MS: (1-5) (1) Activation and co-stimulation of autoreactive T cells in the periphery (2) Migration and trafficking to the CNS (upregulation of homing chemokines & adhesion molecules) (3) Adhesion of proinflammatory autoreactive T cells to the endothelium, release of proteases and MMPs degrading ECM & affording transmigration of the BBB of T, B, moncytes/macrophages into the parenchyma of CNS (4) Secondary activation and amplication of the CNS intraparenchymal cellular and humoral responses with autoantibodies, induction of cytotoxic T lymphocyte (CTL) and cytokine/neurotoxin (NO) mediated CNS damage. (5) Effector stage of disease with injury to the myelin sheath, oligodendrocytes, and axons.

extracellular matrix (ECM) of the BBB (49, 50). In order to then effectively breach the BBB and traffic into the CNS compartment, activated cells must next pass through the ECM which is comprised of type IV collagen. Upon direct contact with collagen, T cells produce matrix melloproteineases (MMPs) 2 and 9 which specifically degrade collagen type IV, as well as contribute to the proteolysis of myelin components in MS (51-53). Alpha 1 integrin on activate T cells may play a role in the initial binding to collagen type IV (54). MMPs are inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs) (52). In MS brain tissue and spinal cord fluid patients MMP-2, MMP-7, MMP-9, MMP-12 and TIMP-1 have been reported (52, 55, 56). Adhesive molecules, chemokines and MMPs therefore represent another potential site of targeted intervention (Figure 1$^{step\ 2\&3}$).

3.4. Effector mechanisms in the CNS compartment

Immune cells including B cells and sensitized T cells (CD4+ helper and CD8+ cytotoxic) that successfully traffic into the CNS are reactivated by antigen-presenting macrophages and resident microglial cells with a subsequent amplification of CNS cellular, humoral and complement effector responses leading to the production of antibodies, complement, toxic cytokines, apoptosis-mediating molecules, and release of other neurotoxic mediators such as the oxygen and nitrogen free radicals, glutamate and osteopontin (57-59). This complex and inter-related process reflects both the innate and acquired immune systems which target and remove the antigenic source from the CNS tissue (19). For instance, nitric oxide (NO) secreted by activated microglia is a potent mediator of oligodenroglial cell loss. Expression of induced nitric oxide synthetase (iNOS) catalyzes NO and transcription of iNOS is upregulated by macrophage/ migroglial elaborated (TNFα) and T cell elaborated (IFNγ). In the setting of this proinflammatory milieu, NO and TNFα along with autoantibodies and complement produce demyelination. Macrophages and migroglia not only present antigen but in this setting phagocytose myelin debris, and capture antibody-antigen complexes by their Fc receptors. Th1 cytokines (INFγ, IL-12) are further perpetuated by macrophage/T cell elaborated osteopontin while downregulating Th2 cytokines (53). This concerted attack of varied immune cells and their relative effector mechanisms results in damage to oligodendrocytes, myelin sheaths and even invariable degrees of axons and neurons as well as, cerebral atrophy during early acute inflammatory phases of disease. (Figure 1$^{step\ 3\&4}$) stepIf these effector mechanisms are of significant severity or persistence resultant irreversible axonopathy and neuronopathy may occur (60). The contribution of axonopathy appears to correlate to irreversible disability (2, 6, 61). Clinically this stage is reflected early by exacerbations during the relapsing of disease which may be antagonized by immunoregulatory and reparative mechanisms (20, 62-70).

3.5. Protective and reparative mechanisms operative in MS

Neuroprotective and homeostatic regulatory processes appear concurrent with the immune attack down-regulating inflammation such as upregulation of regulatory cell populations. Even proinflammatory mediators such as TNFα may be involved in a dual role of tissue repair (71). Oligodendrocyte progenitor cells (OCPs) are recruited to areas of demyelination, expand and differentiate into remyelinating cells that repair local tissue damage (72, 73).

Remyelination appears most efficient in the early inflammatory phases of disease and within acute MS lesions. In chronic lesions and progressive phases of disease oligodendroglial cells may still be observed but with little remyelination present (74, 75).

The debate regarding the cause of inadequate repair is ongoing and may be due to a neurodegenerative processes such as intrinsic abnormalities in recruitment and/ or differentiation of OCPs or possibly secondary to chronic severe demyelination (74, 75). Likewise axonal damage may be a consequence of direct effector mechanisms of the immune attack (macrophages, CD8 cytotoxic cells) (15, 17), or may arise secondarily to chronic severe demyelination and membrane destabilization. This may lead to increased Ca+2 influx and disrupted axonal transport, and/or primary axonal dysfunction (76). Enhancement of neuroprotective and regenerative factors have become attractive targets of novel therapeutic strategies (62-65, 67, 77).

4. EMERGING NOVEL THERAPEUTICS

(Figure 2, Tables 1).

4.1. Immunotherapies targeting myelin specific T cells: antigen-specific therapies

Induction of tolerance by targeting immunoregulation and the modification of autoreactive T cells or antigenic epitopes is currently being explored.

Table 1

Target BBB	**Agent**	**Human Study**	**Animal Study**	I.	II 1.	II 2.	II 3.	II 4.	II 5.	III	IV
	Anti-α_4 integrin (VLA-4) mAb-natalizub Antegren	Phase III	x			x					
	*MMPs inhibitors		x			x					
	*TIMPs		x								
	Chemokine blockade		x		x	x					
MHC-Class II	Anti-MHC mABs		x	x							
	iNOS inhibitor		x						x		
	Hypervariable Peptide vaccines		x	x							
Antigen	Altered peptide ligands	Phase I/II topped	x	x							
	Myelin specific T cell Immunization MBP298	Multiple		x							
	DNA encoding Autoantigen vaccine			x	x						
TCR	TCR Vβ immuni-zation/NeuroVax	Multiple		x							
	TCR peptides		x								
Co-Stim Ulatory Ligands	Anti-CD154 mAB IDEC-131	Phase I/II DB, PC	x		x						
	Anti-IL12 (p40) mAb CNTO 1275	PhaseI/II DB, PC	x		x						
	Anti-IL2rα mAb Daclizumab	Phase I/II OL,CO,R	x		x						
	Anti-CTLA4 mAb	Phase II DB, PC			x						
	Recombinant IL-1 receptor Antagonist Anakinra	Phase I/II DB, PC	x				x				
	IL-10		x		x						
PPARγ receptor	Agonist Rosiglitazone maleate Avandia	PhaseII DB,PC	x								x
Cell Recruitment	Anti-cytokines Chemokines (i.e MIP-1)		x		x	x					
Macrophage Astrocyte	Inhibitors of exocyotoxin Products (,free radicals, PAF,		x						x		
	Cytokines TNFα inhibitors		x				x	x			
	Treosulfan Apoptosis APC and T cell		x					x			
	Protein kinase inhibitors		x								
	Caspase signaling inhibitors		x					x			
CD8 cell	Chemotherapy Anti-CD8+ mAbs		x					x			
B cell	Chemotherapy Anti-CD20+ mAbs/Rituxamab	Phase II/III	x					x			
Complement	Anticomplement mAbs		x					x			
	Inhibitors of complement MAC Membrance attack Complex interactions		x					x			
Transplantation	Autologous bone marrow/peripheral stem cell	Multiple OL	x								
	Oligodendrotocytes (fetal, adult)		x							x	
	Schwann cells		x							x	
Hormone	Estriol	Phase I/II	x								x
	AndroGel Testosterone	Phase II CO	x								x
Regenerartion	Riluzole Inhibitor of glutamate receptor (AMPA/kainite)	OL	x						x	x	
	iNOS2 inhibitor in glial cells/Ansamysins		x						x		
Neuroprotection	Ginko Biloba	Phase I								x	
	A-lipoic acid, VitamenE/Selenium	Phase II								x	
	Inosine/precursor of Antioxidant uric acid	Phase II								x	
Neurotrophic Growith factors	CNTF, NGF, IGF-1	IGF-1 PhaseI/II								x	

Table modified from the National Sclerosis Society at http://www.nationalmssociety.org/pdf/research/clinicaltrials.pdf Subtypes of immune therapy; (**I**) Antigen Specific Therapy, (**II**) Directed Immunotherapy see figure 1 and 2, [1. Activation and co-stimulation of autoreactive T cells in the periphery 2. Migration and trafficking to the CNS 3. Adhesion of proinflammatory cells to the endothelium 4.Secondary activation and amplification of the CNS] (**III)** Repair Therapy, (**IV**) Global-polydirectional Therapy DB, double blinded; OL, open labeled; PC, placebo controlled, CO, crossover

Antigen-specific therapies target the autoreactive myelin-specific T cell, a major player of MS pathology. The therapeutic goal is to enhance homeostasis and restore immune tolerance. Suppression of clonally expanded effector or helper T-cells and/or enhancement of regulatory populations are potential therapeutic strategies. These strategies include (1) induction of T cell anergy (2) activation induced T cell apoptosis whereby an activated T cell upon exposure to an antigen undergoes cell death and deletion (3) induction of bystander suppression whereby an

activated T cell upon exposure to the same or modified version of the autoantigen induces T cells with immunoregulatory function.

To accomplish the above goal of immune tolerance several novel therapies for the reinduction of peripheral tolerance through immunization of the putative pathogenic T cell, TCR receptor, and autoantigens have been explored in animal and human studies. Efficacy of T-cell immunizations may be less beneficial than those reported in animal models due to the diversity of potential antigenic epitopes, pathogenic T cells and TCR repertoire in the outbred human population. Additionally, intact immunoregulatory mechanisms present in experimental allergic encephalomyelitis (EAE) an animal model for MS may be dysregulated in the MS population. The challenge is to identify the "key" autoantigens or encephalopathic T cell populations and TCR repertoire that contribute to disease and maybe overcome to provide a safe and viable therapy in MS. The following are few examples of these currently pursued approaches.

4.1.1. Immunoregulatory mechanisms

Induction of regulatory cell populations such as CD4+CD25+CD45RB^{lo} and invariant Cd1 NKT cells have been shown to restore peripheral tolerance and attenuate in EAE (20, 78-80). These regulatory cells may delete and/or suppress pathogenic T cells by a variety of mechanisms including the induction of anergy or a shift in polarity from a Th1 to a Th2 cytokine milieu. Analogous human regulatory populations have been identified and appear to play a critical role in balancing the need for autoimmunity for protection and risk for non-controlled autoimmunity offering potential future strategies (69, 81, 82). Although the specific neural peptides involved in the induction of these regulartory cells have not yet been characterized, this would represent a novel approach for the induction of antigen-specific regulatory response in active MS.

4.1.2. T-Cell and T-Cell receptor immunization

One novel strategy has utilized immunization with putative pathogenic T cells (MBP-specific T cells) reporting effectiveness in MBP induced EAE model (83).In EAE and several small pilot T cell vaccination trials in MS short term depletion of T cells reactive against different myelin antigens was demonstrated. Peripheral tolerance appeared to be mediated through deletion of myelin specifc cells by $CD8^+$ lysis in a MHC Class I restricted manner, or by $CD4^+$ lysis MHC Class II restricted cytolytic activity (84-88). A more recent phase II open label trial of MBP-specific T cell immunization of RRMS and SPMS demonstrated depletion of MBP specific T cells following vaccination (43). Another T cell mediated approach is to utilize T cell receptor Vβ (TCR) peptides derived from the complementarity-determining region 2 and 3 (CDR2) and (CDR3) of autoreactive T cells. These T cells have been shown to ameliorate disease in EAE models (89, 90). MS patients may have an overrepresentation of TCR Vβ subsets. Early pilot TCR Vβ peptide vaccination trials in MS patients reported safety, induction of Th2 IL-10 cytokine and some depletion of the complimentary targeted T cells (91). A later phase II trial did not show a clinically significant reduction gadolinium-enhanced MRI lesion, their primary outcome (92). These modalities require larger, randomized, double blind, controls studies to demonstrate clinical efficacy in MS patients.

4.1.3. Modification and targeting of antigenic epitopes

Other antigen-specific therapies underway have attempted to downregulate autoreactive T cells by the development of auto-antigenic peptides designed to resemble the antigenic epitopes such as, altered peptide ligands (APL), new modified copolymers, and vaccination with DNA-encoding auto-antigen. Altered peptide ligand (APL) is an auto-antigenic peptide with modifications (amino acid substitution) in TCR contact positions. Promising animal studies demonstrated APL could alter pathogenic T cell responses to native peptide by T cell anergy, TCR antagonism, partial agonism, or bystander suppression (93-95). An APL of the human immunodominant MBP (83-99) peptide was utilized in two separate phase II clinical trials in MS but stopped due to adverse events (96, 97). These preliminary trials however highlighted important immunologic findings. APL induce hypersensitivity reactions in several patients (97) induced APL-specific Th1 cells (98). Recently, a study confirmed that myelin antigens induce classic anaphylactic responses but can be easily treated with antihistamine prophylaxis (99). This trial although clinically disappointing linked disease exacerbation with expansion of activated MBP (83-99). This gave support that disease induction and tissue injury in MS is driven by myelin specific CD4+ Th1 cell population against CNS myelin components. Interestingly lower doses of APL showed a trend towards clinical benefit associated with a reduction of inflammation demonstrated by MRI and a polarity skewing of APL-specific cells with a Th2 immunoregulatory phenotype, suggesting APL bystander suppression (96, 100). To establish optimal dose, timing and administration for safe and efficacious therapy larger, randomized, double blind, controls studies are planned to address these issues.

Glatiramer acetate (GA) (Copaxone®, Teva Industries) and FDA approved drug appears to employ several mechanisms including bystander suppression with the induction of GA-specific T cells that cross react with native MBP autoantigen (101). New copolymers and peptides are presently in investigational development to find molecules with optimal benefit. One recent report has shown that a four amino acid copolymer based on MBP (85-99) but distinct from GA (aa size and content) had greater affinity for the HLA-Dr2- restricted T cell clones and more effectively attenuated EAE (102).

4.1.4. Modification of gene DNA encoding autoantigen

Another novel antigen-specific approach being explored is DNA vaccination. This approach exploits recent advances in gene therapy with the goal of preventing the generation of encephalogenic T cells (103-106). A recent study of DNA vaccine under development (106) involves covaccination with DNA encoded myelin autoantigen(s) alone or given with DNA encoded imummoregulatory cytokines such as IL-4 in EAE. This model of proteolipid

protein (PLP) induced EAE was attenuated after the covaccination with IL-4 DNA and naked DNA encoding (PLP139-151). PLP-specific T cells demonstrated a polarity skewing towards a Th2 phenotype (106) DNA vaccination in the presence of statins may also be explored. Although preclinical methods offer potential site-directed efficacy further investigation are essential to establish safety and efficacy in humans.

4.2. Targeting of B cells

Autoreactive B cells and humoral responses appear to contribute to EAE and MS pathology. The functions of these B cells is not yet fully understood and the functions may be quite diverse (107). Autoreactive B cells can produce antibody that can directly mediate effector mechanisms responsible for some of the pathologies associated with autoimmune disease. Immune complex deposition in the kidney and the resulting tissue damage is a hallmark of systemic lupus erythematosis (SLE). Antibody-mediated demyelination in the CNS in primates is also characteristic of MS and MOG induced EAE induced in rats, mice and marmosets (5, 108-110). Secretion of effector antibodies may provoke direct CNS damage or may also play less obvious roles in redirecting T cell activities. This may be through mechanisms which generate inflammatory mediators and chemokines that alter the recruitment of T cells into an inflammatory site, as has been shown in a model of oophoritis. In addition, antibodies may facilitate the cross-presentation of antigens via immune complex binding to FcR on dendritic cells and facilitate the generation of self-reactive $CD8^+$ T cells. Beyond the pathological effects of autoantibodies, B cells themselves can also influence the development of autoimmunity through their antigen presentation functions and regulatory capacities. B cells, through their membrane immunoglobulin can capture autoantigens and present these antigens to self-reactive T cells. Under such circumstances B cells can also function in the context of antigen uptake, processing and presentation resulting in the expansion of self-reactive $CD4^+$ T cells. In model systems where B cells cannot secrete autoantibody but can express autoreactive surface immunoglobulin, these B cells have been shown to be able to mediate a systemic autoimmune syndrome, clearly showing that the secretion of autoantibody is not critical for autoimmunity to develop. In summary increasing evidence supports the role of B cell and humoral responses in MS (3, 12, 42, 111-116).

4.2.1. Anti-human CD20 mab (Rituximab) B cell deletion

Rituximab is a chimeric anti-human CD20 mab used in the treatment of B cell lymphoma that maybe of potential importance in the treatment of both RRMS and primary progressive disease. The anti-CD20 monoclonal antibody (mAb) is a genetically engineered chimeric murine human monoclonal antibody that depletes circulating and tissue based B-cells. CD20 Rituximab has been granted FDA approval for relapsed or refractory B cell lymphoma with delineated safety and toxicity guidelines. Rituximab (anti-CD20) has additionally been administered in a number of B cell mediated autoimmune diseases such as rheumatoid arthritis (RA) (117-122), cold agglutinin, disease (123, 124), warm antibody hemolytic anemia (125), idiopathic thrombocytopenic purpura (126), paraproteinemic polyneuropathy, (127) and myasthenia gravis (128). Studies in RA suggest that the immunologic benefit of rituximab is related more closely to decreases in circulating autoantibodies than to B-cells (121). Like MS, both B and T cell activation and proliferation may mediate the pathogenesis of progressive RA, and several trials support the targeting of–B-cell immunomodulation in autoimmune diseases such as MS (129). Both a Phase II randomized trial in RRMS as
well as a Phase II/III randomized, multicenter, double blind, parallel group, placebo controlled study of Rituximab are currently under development.

4.3. Immunotherapies targeting directed immunologic steps of disease pathology

4.3.1. Targeting peripheral co-stimulatory and proinflammatory cytokines

4.3.1.1. CD40L-CD40 co-stimulatory blockade

Treatment with a humanized monoclonal antibody CD154 is a potential new immunomodulatory treatment for RRMS. Preclinical studies have suggested the importance of CD40L-CD40 costimulatory interaction in the pathogenesis of MS. In specific the engagement of CD40 and it's ligand CD154 are critical in eliciting the activation of T cells and cell-mediated immunity (CMI) responses (130-133). Blockade of CD154/CD40 interactions prevents the development of CMI and a variety of autoimmune disease models in mice. Importantly it has been shown to prevent the progression of both monophasic and relapsing remitting EAE models (39). Animal studies also support that CD40-CD154 interactions are ongoing in human MS plaques (132, 134). It is hypothesized that the effects of CD154 blockade on CMI are due to a central impairment of APC maturation (130, 132, 135, 136). It is clear that CD40 signaling is critical for the "maturation" of APC via the induction of a wide spectrum of APC activities and that blockade in this pathway may lead to T-cell tolerance or T-cell skewing. A phase I clinical trial of the treatment of RRMS with anti-CD154 showed no evidence of either systemic or neurological toxicity, which were the primary outcomes for the study. Although the number of subjects was insufficient to draw significance, several of the secondary outcomes including MRI and EDSS suggest that therapy may stabilize the disease process. These results provide a rationale for a larger scale study and a phase II masked, placebo-controlled, randomized partial crossover study in 46 subjects with relapsing-remitting multiple sclerosis (RRMS) is underway to test safety and efficacy (Figure 2, Table 1).

4.3.1.2. Interleukin - 12p40 (IL-12p40) blockade

Another novel therapeutic approach involves the inhibition of a proinflammatory IL-12, a heterodimeric predominant cytokine in immune mediated inflammatory disorders (137-140). Accumulating evidence indicates that IL12 plays a pivotal role in the pathogenesis of EAE mediating both cellular (Th1) and humoral responses. (141). IL-12 antagonists and neutralization of IL-12 can prevent EAE in both rodent and marmoset models, as well as, being implicated in the pathogenesis of MS (142-150).

The IL-12p40 subunit appears most important and other monokines such as IL-23 that express this subunit may also participate in the development of EAE (151). Local expression of IL-12 within the CNS has been demonstrated in EAE animal models (149, 152). Furthermore local expression of IL-12 within the CNS of MS patients as well as increased levels of IL-12 in CSF, plasma, serum and PBMCs during active disease has been demonstrated (153-156). A Phase I/II clinical trial for the treatment of psoriasis another Th1 mediated disorder with a humanized monoclonal anti-IL-12p40 antibody evidenced statistical clinical benefit. These studies provided theoretical evidence that therapy directed against IL-12p40 may be another new effective MS treatment. A multi-center Phase I double blind, placebo-controlled trial in patients with relapsing forms of MS, evaluating the safety of a single administration of monoclonal antibody to IL-12p40 (CNTO1275) is presently in progress. A phase II trial is planned later this year to examine drug efficacy in RRMS patients (Figure 2, Table 1).

4.3.1.3. IL-2/IL-2 receptor α blockade

Daclizumab (Zenapax) is a humanized monoclonal antibody against the IL-2 receptor alpha subunit. IL-2 is a autocrine growth factor necessary for T cell growth. The rationale for this novel therapy is that the interaction of of IL-2 and its receptor IL-2 on the surface on T cells leads to the activation and expansion of autoreactive T cells and blockade may result in the downregulation of encephalogenic T cells. This agent has been FDA approved in renal transplant recipients to induce immunosuppression for optimization of graft acceptance (157) patients who had responded incompletely to interferon beta therapy was pursued.

They reported during the past three years the tolerability and safety of monthly administration of daclizumab, as well as a reduction of disease activity by MRI imaging. A further amendment of this study demonstrated persistent efficacy of daclizumab after the interferon beta therapy was discontinued. A current Phase II (ZAP) open-label, baseline to treatment cross over trial is testing the efficacy of daclizumab alone in RRMS patients.

4.3.1.4. Targeting B7-CD28-CTLA-4 co-stimulatory pathway with CTLA-4Ig agonist

Cytotoxic T lymphocyte-associated antigen 4 Ig [CTLA4Ig]) is a soluble chimeric protein. Costimulatory blockade using CTLA-4Ig has recently been explored as a novel therapeutic in human studies. A phase I clinical trial of the treatment of psoriasis vulgaris, with CTLA-4Ig improved clinical outcomes and was associated with reduced cellular activation of lesional T cells, keratinocytes, dendritic cells (DCs), and vascular endothelium (158). Another pilot clinical trial of the treatment rheumatoid arthritis evaluating CTLA-4Ig also demonstrated safety, tolerance and dose-dependent effectiveness (159). A current phase I clinical trial is testing the safety of CTLA4Ig (BMS-188667) and CTLA4Ig (Repligen-RG2077) in MS patients.

4.3.2. Targeting adhesion molecules and cell trafficking across the blood brain barrier (BBB)

Cellular homing, adhesion and transmigration of a number of activated immune cells including autoreactive encephalopathic T cells across the BBB into the CNS compartment is an early and critical step involved in the immune pathology of MS. This is supported by *in vitro* and *in vivo* animal and human studies (160). Targeting these steps are intergral in a number of current and novel MS therapies.The following are a few examples of these novel strategies.

4.3.2.1. Inhibition of α4β1 integrin-VCAM-1 mediated adhesion

Natalizumab (Antegren) is a humanized monoclonal antibody against the α4 chain of the α4β1 intergin (VLA 4) expressed on the surface of activated lymphocytes and monocytes. α4β1 intergin (VLA 4) and it's associated receptor VCAM-1 on endothelial cells are important prerequisites of adhesion, transendothelial migration and enhanced cellular activation within inflamed tissue. In an EAE model natalizumab evidenced inhibition of T cells trafficking across the BBB with subsequent amelioration of disease (160). In a Phase II clinical trial, two doses of natalizumab were administered over a 8 week course. The results of this novel study showed a reduction of gadolinium-enhancing MRI lesions at 12 weeks but not at the follow-up second 12 weeks which were the primary outcomes for the study. Secondary outcome measures showed a significant increase in relapse rate in the follow up period (161). This suggested a rebound effect with drug cessation and the possible need for long term drug therapy. (161). In a multicenter, randomized, double-blind, placebo controlled phase II trial, 213 patients with RRMS or relapsing SPMS were assigned 3mg/kg, 6mg/kg or placebo every 28 days for 6 months. This extended both treatment and follow-up period (162). In both treatment arms a 90% reduction in the number of new gadolinium lesions, the primary outcome. A secondary outcome showed a significant reduction of clinical relapses compared to placebo but was not powered for clinical outcomes (162). Patients returned to pre-treatment relapse rate following discontinuation of therapy. Currently two larger multi-center Phase III trials are in progress in RRMS. One trial is a monotherapy trial of 1200 patients (AFFIRM) and the other is a combined trial of 900 patients with interferon beta-1a (Avonex) (SENTINAL). A smaller phase III trial of 110 patients has combined natalizumab with glatiramer acetate. Another Phase III trial utilizing small-molecule antagonists has been initiated. These agents may offer advantages over monoclonal antibodies such as oral availability and lack antigenicity in the future. (163).

4.3.2.2. Inhibition of matrix metalloproteinases (MMPs): treatment of minocycline

Preclinical evidence supports the pathologic role in MS for several of 23 MMP family members. In normal CNS low to non detectable levels of MMPs are found whereas upregulation and high levels are found in several neurologic diseases including MS (55, 56, 164, 165). A recent study extended the identified members of the MMP family in MS and showed a distinctive pattern of expression in T, B, and monocytes (166). Several EAE animal studies support the promotion of disease activity and course (52, 165). Synthetic inhibitors of MMPS (TIMP-1 like) have been shown to ameliorate or prevent

EAE and genetically deficient MMP-9 mice are more resistant to EAE induction compared with wild-types (167, 168). A pivotal role of MMPs in MS and EAE is the facilitation of leukocyte transmigration across the BBB, reversed by inhibitors of MMP activity (169-171). However many other effector functions of MMPs may contribute to MS pathology including breakdown of the BBB, promotion of CNS inflammation, and direct neurotoxicity (172-174). Based on the above data, identification and targeting of MMP members is a promising area of future treatments. Interferon beta IFN-β has been shown to inhibit the activity of MMPs. Minocycline, an antibiotic has been shown to attenuate EAE (175) and inhibit MMP activity and production (MMP-9). A phase II open label cross over trial of minocycline in RRMS is underway.

4.3.3. Targeting secondary activation and amplification within the CNS

As previously discussed immune cells including B cells and sensitized T cells (CD4+ helper and CD8+ cytotoxic) that successfully traffic into the CNS are reactivated by interactions with resident APCs with the subsequent amplification of CNS cellular and humoral effector responses. These complex molecular interactions in the CNS may lead to inflammation, demyelination, oligodentrocyte loss and axonal and neuronal dysfunction. Several animal and human studies are currently exploring this class of immumodulators and immune independent neurodegenerative mechanisms operative in the CNS compartment which may provide effective future MS therapeutic interventions. The following are a few examples of preliminary animal studies elucidating data that support pivotal roles of several novel CNS targets but which require further research to determine therapeutic possibilities in MS patients.

4.3.3.1. Targeting of a brain proinflammatory mediator: osteopontin

In recent animal studies performing genetic sequencing of MS brain libraries revealed a role of a pleiotrophic brain binding protein osteopontin produced by glial cells may have a role in MS progression (59). In transgenic mice deficient for osteopontin (OPN) the progression of EAE was inhibited and severity of disease was reduced. (59, 169, 176, 177). Furthermore, inhibition of CD44, a ligand of OPN prevented EAE and elevated a non-inflammatory Th2 cytokine IL-10 (169).This study suggests that the proinflammatory effect of OPN may be mediated by CD44 and provide a new CNS target. (169).Genetic osteopontin polymorphisms appear to correlate with disease course in MS. (178, 179). In addition, elevated osteopontin levels have recently been shown to be associated with disease activity in relapsing remitting MS patients (10, 180) supporting their future role in the arsenal of novel strategies.

4.3.3.2. Suppressive effects of ansamycins on inducible nitric oxide synthetase 2 (iNOS2)

Another central target of immune effector mechanisms in the CNS is the suppression of inducible nitric oxide synthetase (181). The inducible form of nitric oxide synthetase (iNOS2) by brain glial cells is thought to contribute to the production of neurotoxic mediators and expression of proinflammatory cytokines such diseases as Alzheimer and MS (181). Reduction of iNOS2 by a heat shock response (HSR) attenuated the histologic and clinical symptoms of EAE (182). Recently ansamycins, a class of antibiotics demonstrated a suppressive effect on iNOS2 exerting a potent anti-inflammatory response on brain glial cells in EAE (181). Other novel nitric oxide scavengers such as (NOX-100) have been shown to reduce the severity or ameliorate EAE progression in mice (183).

4.3.3.3. Targeting of intracellular cyclic amino monophosphate (cAMP) in the CNS

Human and animal studies examining phosphodiesterases (PDE) which critical enzymes expressed in the immune system and brain have been shown to be responsible for the degradation of cAMP and/or cGMP (184). PDE enzymes exist as 11 distinct families (135, 185, 186). Recent data has shown that inflammatory cells predominantly express PDE4 followed by PDE3 and, to a lesser extent, PDE7 with isotypes of PDE4 being preferentially expressed in the brain (184). Inhibitors of cAMP-specific PDE4 have been shown to inhibit T cell proliferation, proinflammatory mediator release with modulation of T cell-polarization (Th1 toTh2 skewing) contributing to the cytokine milieu and influencing the upregulation of distinct costimulatory signals.They recently have been shown to alter DC capacity and cytokine production (187-190). PDE4-specific inhibitors, such as Rolipram, Cilomilast and Mesopram, (191), have been demonstrated to elevate cAMP levels, and inhibit proliferation, cytokine production and mediator release of several cells, including T cells, monocytes and eosinophils (96, 192). Other animal studies have shown PDE4 inhibitors demonstrate the ability to abrogate acute and chronic-relapsing EAE in mice, rats and marmosets (188, 193, 194). Human *in vitro* studies have demonstrated similar immunmodulating effects of PDE4 inhibitors with a preferential inhibition of Th1 type cytokines elaborated by autoreactive T cells in MS patients versus healthy controls (188, 193-195). These studies lead to the support of PDE inhibitors as candidate therapies for T_h1-mediated human diseases such as MS. Presently a phase II, open label, crossover trial with the treatment of Rolipram (PDE4 inhibitor) for RRMS and SPMS patients is in progress. The trial will use MRI measures as the primary outcome and the treatment phase will be for 8 months.

Salbutamol also inhibits cAMP albeit by distinct mechanisms versus PDE inhibitors and demonstrated disease suppression in EAE models (196). The administration of oral salbutamol evidence a shift in T cell polarity toward a Th2 phenotype in peripheral blood moncyte cells (PBMCs) of MS patients (196, 197). Presently phase II trials of this agent are underway to examine drug efficacy in MS patients.

4.4. Immunotherapies targeting reparative stages of disease

Recent evidence supports that axonal and neuronal degeneration occur as the disease progresses (2-6,

60). These MS lesions predominantly involve the white matter however, recently demyelination and neuronal pathology have also been demonstrated in the (gray matter) cerebral cortex of MS patients. Novel approaches inhibiting brain neurotoxicity and/or promotion of repair and recovery affording ODG, axonal and neuronal protection represent a rapidly evolving area of research potentially offering protective and beneficial treatment for MS patients at various stages of disease. Interestingly, even immune cells associated with inflammatory responses in the CNS can produce a variety of neurotrophic factors of different molecular families supporting their potential for not only detrimental but beneficial effects in MS. Indeed some of the most potent members of the neurotrophin family such as nerve growth factor, (NGF), brain-derived neurotrophic factor (BDNF) act on or are endogenously produced by immune cells in MS lesions (62-65, 77). Resident CNS cells appear also to have immuno-regulatory properties (66). The following are only a few examples of the investigations underway exploring the use of neuroprotective-reparative agents.

4.4.1. Neurotrophic factor

Neutotrophic factors are proteins that direct differentiation and survival/apoptosis acting through specific neurotophin receptors (65, 77, 198, 199) that have been shown to shift Th1-Th2 and may promote neuroprotection (65, 200). Potential neurotrophic therapeutic candidates include brain-derived neurotrophic factor (BDNF), glial growth factor, ciliary neurotrophic factor (CNTF), neuroimmunophilin ligand (FK506) and NGF (77, 201-203). CNTF elaborated by activated astrocytes induced growth and trophic factors such as FGF-1 and IGF-1 which indirectly protected neurons from cell death and promoted oligodendrocyte generation. CNTF was also shown to inhibit neuronal and glial degeneration resulting from microglial cytotoxins (202). A phase I/II trial examining insulin-like growth factor is currently being explored. There is evidence from other neurodegenerative systems to support the use of gene delivery of growth factors for the promotion of remyelination (204).

4.4.2. Inhibition of the glutamate receptor α-amino-3-5-methyl-4-isoxazolepropionic acid/kainite (AMPA/kainite) receptor

Preliminary studies suggest the glutamate neurotoxicity is an important contributing factor in MS pathogenesis (69, 70, 205). In EAE and MS lymphocytes, brain microglia and macrophages release excessive levels of glutamate which activate AMPA (α-amino-3-5methyl-4-isoxazolepropionic acid)/kainate receptors on oligodendrocytes (OGCs) and neurons. OGCs are especially vulnerable to glutamate-AMPA/kainate excitotoxicity (206, 207). Blockade with AMPA/kainate antagonists have been shown to ameliorate EAE (58, 76, 205). AMPA/kainate antagonists also appear to protect OGCs and axons from immune-mediated damage. A recent study investigated riluzole affect in MOG-induced EAE a chronic model and demonstrated this agent attenuated the clinical severity of disease and reduced inflammation, demyelination and axonal damage in the CNS (205) of MS patients versus controls. MS patients also demonstrated increased CSF glutamate levels correlating with disease severity (70). An anti-glutaminergic agent riluzole (2-amino-6-triflouromethoxy benzothiazole) found to be protective in several models of neurodegenerative disease including ALS (208), Parkinson's (209) and ischemia (210). Based on riluzole's neuroprotective properties, safety and tolerability the FDA approved this agent for the treatment of ALS (211). Riluzole is presently being explored as a potential neuroprotector in MS. An open label clinical trial in primary progressive MS patients to test the neuroprotection of riluzole (Rilutek®) is currently in progress.

4.4.3. Targeting of neurotoxic and nitrogen free radical mediators

Other studies suggest a role of oxygen and nitrogen free radicals in the immunopathogenesis of EAE and MS. In animal models of MS, these chemical reactions have been associated with break-down of the BBB and CNS tissue injury. Additionally, increased levels of iNOS have been evidenced in active demyelinating lesions, as well as, showing increased CNS and CSF levels of reactive nitrogen oxide species (RNOS) in MS patients versus matched controls (164, 212). Uric acid (UA) is a RNOS scavenger and natural inhibitor of chemical interactions associated with peroxynitrite (213). In several mouse EAE models uric acid administration attenuated disease severity and was associated with alterations in BBB permeability, inhibition of CNS inflammation, and tissue injury (213-215). The above data and observations that MS patients have serum uric acid levels that are lower than age and sex matched healthy controls (216-218). This data provided the rationale for a novel treatment of MS aimed at raising levels of the natural antioxidant UA or it's precursor inosine (219).

A small preliminary clinical trial of oral administration of inosine in 11 MS patients showed clinical stability in 9 patients and improvement in 3 patients. Further in two patients who had notable pretreatment gadolinium-enhanced lesions none were detectable following inosine treatment. Currently a phase II, double blind, placebo controlled trial is underway to determine whether oral treatment with inosine has an effect on cumulative number of newly active lesions on MRI and to evaluate safety and tolerability of inosine in 30 RRMS and SPMS patients. Upcoming phase I/II trials examining three other natural anti-oxidants (ginko biloba, α-lipoic/essential fatty acids and selenium) for MS treatment is planned and sponsored by National Center for Complimentary and Alternative Medicine (NCCAM).

4.5. Global Therapies: broad based polygenic mechanisms

The following class of novel therapies have diverse properties but share the characteristics of exhibiting polygenic mechanisms and broad based immunomodulatory targets. Below are a few examples of this category of agents.

4.5.1. Depletion of multiple immune cellular subsets: treatment with anti-CD52, alemtuzumab

Alemtuzumab (Campath) directed at CD52 appears to have polygenic mechanisms including the

depletion of leukocytes (T & B) as well as, monocytes and macrophages. This agent was initially approved by the FDA (2001) to treat patients with B-cell chronic lymphocytic leukemia and revealed immune suppressing actions. A phase II clinical trial in SPMS demonstrated that alemtuzumab had pronounced effects the immune system reducing relapse rate and brain inflammation as shown by serial MRI. Specifically, during the 18 month follow phase gadolinium enhanced lesions were significantly reduced (220). Safety concerns were raised however, due to the observation that about 30% of patients in these early studies developed Grave's autoimmune thyroiditis (221). Currently there are two clinical studies extending the investigation of Campath® affect on immune function including a retrospective study of 58 RRMS and SPMS patients and an open labeled multicentered clinical trial of Campath®/MABCAMPATH® versus Rebif (interferon-beta-1a) in early active RRMS.

4.5.2. Statins

A class of orally administered cholesterol (lipid) lowering drugs, the statins or (HMG-CoA) reductase inhibitors are safe and appear to have biological effects independent of their cholesterol reducing properties (222). Neuhaus and collegues reported that cells (PBMCs) of RRMS exposed *in vitro* to several forms of statins including mevastatin, simvastatin (Zocar®) and lovastatin (Mevastatin®) inhibited several different immune responses involved in MS. This study along with other early laboratory studies demonstrated that statins have several immunomodulatory effects by varied polygenic mechanisms including; (i) suppression of T and B cell proliferation,(ii)reduced expression of activation -induced adhesion molecules on T cells,(iii) skewed the polarity of Th1 to Th2 cytokines,(iv) downregulated chemokine receptors of T and B cells and (v) reduced the secretion of MMP-9 protease, inhibition of potential neurotoxins such as TNFα and inducible nitric oxide synthetase (iNOS) (223-229). Furthermore, statins have been shown to ameliorate in several EAE models modifying the balance of Th1 and Th2 cells to a proinflammatory Th2 phenotype (230, 231). A small pilot open labeled proof of concept/Phase II was the first human study of simvastatin (Zocar®) in 30 RRMS. The preliminary analysis of the study results were recently reported demonstrating that Zocar® safely reduced the number of new lesions. Analysis of immune responses suggested a shift away from inflammation however no differences were observed in neurologic disability in this short study (232) controlled trials of simvastatin and atorvastatin (Lipitor®) are in the planning and will begin this year.

4.5.3. Pregnancy-induced hormonal therapy: treatment with oral estriol (E_3) hormone

Pregnancy induced hormones and their potential as therapeutic agents has been entertained. This has evolved from longstanding clinical observations that sex-specific differences and hormonal influences exist in MS patients. Patients with MS have fewer relapses during pregnancy especially during the third trimester and disease exacerbations during first few months after birth (233). In several EAE models exogenous estrogens (estriol/estradiol) ameliorated disease by altering multiple immune responses (234-236). These polygenic mechanisms included; (i) suppression of Th1 proinflammatory cytokines,(ii) reduced the secretion of metalloproteases, (iii) downregulated of chemokines and chemokine receptors of T and B cells with inhibition of T cell, B and macrophage recruitment (234-236). A small open label, cross-over, proof of concept/Phase II trial with the treatment of estriol in nonpregnant women with RRMS and SPMS was performed. Compared with pretreatment baseline, patients with RRMS but not SPMS showed a significant reduction of number and volume of gadolinium enhancing lesions. Furthermore, when the estriol was stopped during the posttreament phase the number and volume of gadolinium enhancing lesions returned to pretreatment values and then decreased when treatment was reinstated (237). Correlating with these clinical findings were decreases in proinflammatory cytokines including INF-γ (237). The above studies are the rationale for a larger placebo-controlled trials to examine long term efficacy with an acceptable side effect profile. One such trial is currently in the planning. The potential long term benefits must be weighed against side effects and toxicities including carcinogenesis and thrombosis.

4.5.4. Targeting with oral peroxisome proliferator-activated receptor gamma ((PPARγ) agonists: treatment with avandia

Potential targeting of EAE and MS with oral peroxisome proliferator-activated receptor gamma (PPARγ) agonists are currently being investigated. The ligand agonists of the peroxisome proliferator-activated receptor (PPARγ) exert anti-inflammatory effects on a number of inflammatory cells including glial cells. This molecular interaction results in reduced proliferation and activation of T cells, and induction of myelin gene expression (238-241). Several of these oral agents have been FDA approved to treat diabetes. PPARγ-deficient heterozygous mice evidence myelin oligodendrocyte glycoprotein (MOG) induced EAE exacerbation and Th1 response (239). Several other models of EAE have shown that orally administered PPAR gamma ligands ameliorate the severity of monophasic, chronic disease and of relapsing disease in mice immunized with myelin oligodendrocyte glycoprotein (MOG) and/or myelin basic protein (MBP). Attenuation of clinical signs appear to correlate with decreased CNS inflammation and a reported reduction in lymphocyte infiltration, inflammatory chemokine and cytokine expression, and increased inhibitor of kappa B (IkB) expression in the brain (238-240). A clinical phase II double blinded placebo controlled trial of an oral diabetic agent (Avandia ®) is underway to examine safety and effectiveness in RRMS patients.

5. SUMMARY

Advancing knowledge of disease mechanisms and a deeper understanding of the immune pathogenesis in MS will refine current drug-specific therapies and shape the rationale for future immunotherapeutic strategies. Exploding technologies in areas such as neuroimaging, genomic cDNA microarray and proteomic analysis of MS

will further transform and delineate the heterogeneity and mechanisms underlying MS, yielding new targets for therapeutic strategies. Currently a more complex and intriguing picture of MS immune dysregulation is evolving. For example, immune cells and CNS inflammatory reactions may have dual roles inducing both proinflammation and neurotoxicity as well as, anti-inflammatory and protective immunity. Insight into this interrelated and aberrant neuroimmune imbalance, reflecting variable components such as encephalogenic effector cells, dysregulated regulatory populations and possible neurodegenerative processes will yield more specific and complimentary therapies. These mechanisms of disease pathology may impact differently upon subpopulations of patients and even vary within different stages of MS in a given individual. Future treatment options therefore will most likely utilize a combination of synergistic therapies with distinct mechanisms of action to address the treatment of a diverse MS patient population in all stages of disease offering greater efficacy and long term benefit. Tommorow's arsenal of novel immune agents will reflect a patient-tailored treatment regimen of immune modulatory, suppressive and protective strategies for the treatment of MS shaped by different disease types and stages.

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Send Correspondence to: Toni M. Bauman, M.D. Department of Neurology, One Medical Drive, Lebanon, New Hampshire 03756 or Immunotherapeutics Program at Dartmouth Medical School, Department of Immunology & Microbiology, Reuben Research Building, Lebanon, New Hampshire 03756, Tel: 603-653-9948, Fax: 603-653-9900, E-mail: Toni.M.Bauman@hitchcock.org