Published in final edited form as:

Immunol Res. 2014 August ; 59(0): 254–265. doi:10.1007/s12026-014-8529-9.

Immune Regulation of Multiple Sclerosis by CD8+ T cells

Sushmita Sinha, Farah R. Itani, and Nitin J. Karandikar

Department of Pathology and the Interdisciplinary Graduate Program in Immunology University of Iowa, 200 Hawkins Dr., Iowa City, IA 52242, USA

Abstract

The role of CD8+ T cells in the process of autoimmune pathology has been both understudied and controversial. Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system (CNS) with an underlying T-cell-mediated immunopathology. CD8+ T cells are the predominant T cells in human MS lesions, showing oligoclonal expansion at the site of pathology. It is still unclear whether these cells represent pathogenic immune responses or disease-regulating elements. Through studies in human MS and its animal model, experimental autoimmune encephalomyelitis (EAE), we have discovered two novel CD8+ T cell populations that play an essential immunoregulatory role in disease: (1) MHC class Ia-restricted neuroantigen-specific “autoregulatory” CD8+ T cells and (2) glatiramer acetate (GA/Copaxone®) therapy-induced Qa-1/HLA-E-restricted GA-specific CD8+ T cells. These CD8 Tregs suppress proliferation of pathogenic CD4+CD25- T-cells when stimulated by their cognate antigens. Similarly, CD8+ Tregs significantly suppress EAE when transferred either pre-disease induction or during peak disease. The mechanism of disease inhibition depends, at least in part, on an antigen-specific, contact-dependent process and works through modulation of CD4+ T cell responses as well as antigen presenting cells (APC) through a combination of cytotoxicity and cytokine-mediated modulation. This review provides an overview of our understanding of CD8+ T cells in immune-mediated disease, focusing particularly on our findings about regulatory CD8+ T cells both in MS and EAE. Clinical relevance of these novel CD8-regulatory populations is discussed, providing insights into a potentially intriguing, novel therapeutic strategy for these diseases.

Introduction

The immune system has several endogenous checkpoints to curb “unwanted” immune reaction towards self-antigens. CD4+CD25+FoxP3+ T cells are the well-characterized regulatory cells that function primarily to keep inflammatory responses in check. The loss of the balance between effector and regulatory responses may lead to autoimmune diseases, where a sense of “self” vs. “foreign” by the immune system is compromised. Multiple sclerosis is one such inflammatory, demyelinating disorder of the CNS affecting more than 2.3 million people worldwide. Although the etiology of MS is unknown, it is thought to be

an immune mediated disease due to its characteristic histology, the presence of CNS-specific T cell responses, and the ability to modulate the disease using immune-based therapy [1–4].

For the most part, CD4+ T cell biology has dominated the research focus in MS and the disease is thought to be mediated by CNS-specific CD4+ Th1/Th17 responses and regulated by CD4+ Th2 or regulatory T cells. However, considerable evidence exists that points towards an important pathogenic and/or regulatory role of CD8+ T cells in MS including 1) CD8+ T cells outnumber CD4 T cells in MS lesions [5], 2) CD8+ T cells show oligoclonal expansion in the CNS of MS patients indicating an active role at the site of pathology [5], 3) MS patients have high prevalence of neuroantigen-specific CD8+ T cell responses in their circulation [6], and 4) these cells appear to have mixed functional phenotypes, in that, they express cytotoxic and regulatory effector molecules [6]. After some initial reports of immune suppressor function for CD8+ T cells in MS [7–13], such “suppressor cells” fell out of favor in the late 80’s/early 90’s in the entire field of immunology. However, evidence for a regulatory involvement by this subset of T cells has kept mounting and now there is a renewed interest in the suppressor/regulatory functions of CD8+ T cells in MS and other autoimmune diseases.

In type 1 diabetes, low avidity CD8 T cells specific for IGRP₂₀₆₋₂₁₄ (islet-specific glucose-6-phosphatase catalytic subunit-related protein) were shown to inhibit the development of diabetes and could also cause reversal of established disease. These findings were later confirmed through TCR transgenic mice where low avidity CD8 T cells were able to prevent diabetes induction in NOD.*scid* mice [14]. CD8+ T cells from the lamina propria of patients with inflammatory bowel disease (IBD) lack regulatory activity otherwise present in healthy donors [15]. Synovial fluids of rheumatoid arthritis patients are enriched in suppressor CD8+ T cells [16]. Regulatory CD8+ T cell biology is more complex than appreciated earlier due to the heterogeneity in the phenotype of cells, as characterized by the surface markers.

We have demonstrated an unexpected and novel immune regulatory role for both CNS-specific autoreactive CD8+ T cells as well as therapeutically induced CD8+ T cell responses in the context of MS and its animal model, EAE [6, 9–13, 17–24]. The mechanisms, biology and potential therapeutic benefit of these CD8+ T cells is a major focus of studies in our laboratory and the predominant focus of this review.

Regulatory CD8+ T cells in autoimmune diseases: emphasis on multiple sclerosis

Recent advances in the field of CD8+ T cells biology have greatly improved our fundamental understanding of diverse roles of CD8+ T cells in regulating autoimmunity. Classically, CD8+ T cells are recognized as pathogenic mediators due to their cytotoxic killing potential. Not surprisingly, CD8+ T cells in MS and EAE have traditionally been considered to be the damage causing cells in the CNS, as suggested by studies shown in Tables 1 and 2. However, direct evidence for the “causal” association of CD8+ T cells with inflammation in MS has been lacking so far and many of the animal studies have been based on transgenically modified models, rather than dissection of their role in a wild-type setting.

At the very least, these studies certainly show that specific CD8⁺ T cells may have a pathogenic role in demyelinating disease.

As mentioned above, a regulatory role for CD8⁺ T cells is now appreciated in several autoimmune diseases including, inflammatory bowel disease [15], Type I diabetes (T1D) [25], rheumatoid arthritis (RA) [26], and multiple sclerosis [7–9, 11, 27]. These CD8⁺ T cells come in a variety of flavors and can potentially target both pathogenic CD4⁺ T cells and APCs. Low avidity autoreactive clones of CD8⁺ T cells possessing regulatory properties are critical in maintaining tolerance in the pancreatic islets in T1D [25]. CD8⁺ T cells specific for Hsp60sp bound to HLA-E from recent-onset T1D individuals have defects in suppressing CD4⁺ T cells in vitro [25]. CD8⁺CD28⁺CD56⁺ T cell clones from synovial tissues of RA patients have an anti-inflammatory and immunosuppressive activity in NOD-SCID mice engrafted with synovial tissue from RA patients [28].

The regulatory role of CD8⁺ T cells is also evident in several animal models of autoimmune diseases such as collagen induced arthritis [29], autoimmune myocarditis [30], herpes stromal keratitis [31] and EAE [32, 13]. EAE is characterized by autoimmune CD4⁺ T cells gaining encephalitogenic potential and crossing the blood brain barrier (BBB) to cause inflammation in CNS, culminating in paralysis. It is the most extensively studied animal model for MS and has been instrumental in MS drug discovery. Mice deficient in CD8⁺ T cells exhibit more severe EAE with increased relapses, suggesting a regulatory role for these cells [32]. Further evidence stems from studies showing that highly enriched MOG₃₅₋₅₅-specific CD8⁺ T cells were unable to adoptively transfer disease into naive mice and, further, transfer of such neuroantigen-specific CD8⁺ T cells was able to suppress the induction of EAE as well as suppress ongoing EAE [13, 18]. These findings are well translated into human studies, where we demonstrated that neuroantigen-specific, but not foreign-antigen-specific, CD8⁺ T cells exhibit immune regulatory ability, suppressing proliferation of CD4⁺CD25⁺ T cells when stimulated by their cognate antigen [9]. This finding is particularly relevant for the pathogenesis of MS, as MS patients have a high prevalence of neuroantigen-specific CD8⁺ T cell responses that can potentially suppress pathogenic myelin-specific CD4⁺ T cells. Moreover, CNS lesions in MS patients shows a predominance of CD8⁺ T cells undergoing clonal expansion, so it is tempting to speculate that these CD8⁺ T cells present in the lesion might have an important function of curbing CNS-specific CD4⁺ T cell mediated inflammation. Several other reports have presented increasing evidence for a role of CD8⁺ T cells in the downmodulation of autoimmune demyelination, both, in human MS and its EAE model (Tables 1 and 2). CD122^{−/−} mice are prone to autoimmune diseases due to high numbers of abnormally activated T cells. The adoptive transfer of CD8⁺CD122⁺ T cells into CD122^{−/−} mice restores tolerance in these mice [33]. Concomitantly, transfer of CD8⁺CD122⁺ T cells into EAE mice, leads to attenuation of EAE [34]. While MS patients have normal levels of total CD8⁺ T cells and CD28⁺ cytotoxic CD8⁺ T cells, they had a specific deficit of the CD28[−] suppressor subset of CD8⁺ T cells [35]. Evidence for the regulatory functions of CD8⁺CD28[−] T cells also comes from the EAE model, where CD8⁺ T cell depletion renders CD28[−] mice susceptible to EAE [36]. Recently, another phenotype of CD8⁺ T cells, defined by CD25 and FoxP3 expression, with regulatory potential has been detected in MS patients [27]. These cells inhibited CD4⁺ T cell proliferation and IL-17 secretion and also downregulated

costimulatory molecule expression on DCs. Importantly, both CD8+CD25+FoxP3+ and neuroantigen-specific CD8+ T cells discovered by us, show greatly diminished regulatory potential during an acute MS relapse, showing a clear clinical relevance for this regulatory role.

A unique subset of CD8+ T cells with prominent regulatory functions in EAE and MS are Qa-1 (or HLA-E)-restricted CD8+ T cells. Qa-1 deficient mice have exaggerated secondary CD4+ T cell responses to foreign and self-antigens, which is associated with resistance of Qa-1-deficient CD4+ T cells to Qa-1-restricted CD8+ T cell suppressor activity [37]. Qa-1 restricted CD8+ T cells induced by antigen-triggered CD4+ T cells during T cell vaccination can protect mice from MBP-induced EAE [38]. CD8+ T cells capable of lysing myelin-reactive CD4+ T cell in an HLA-restricted manner have been isolated from MS patients [39]. Higher levels of NKG2A expression, corresponding to less cytotoxic potential of CD8+ T cells, are present on HLA-E-restricted CD8+ T cells during MS relapse as compared to remission or healthy donors [39]. A recent study [40] demonstrated that HLA-E-interacting CD8+ T cell subsets are altered phenotypically in MS patients. This study identified reduced expression of FoxP3 and CD122 on NKG2C+CD8+ T cells of MS patients, suggestive of reduced regulatory potential.

Chronic progressive MS, characterized by ongoing neuronal degeneration with paucity of inflammation, is still an enigma to researchers and has not been associated much with immune system irregularities. Chronic progressive MS is difficult to manage since the patients show continuous clinical progression without any remission. In this context, it is worth appreciating that defective CD8 regulatory functions have been reported in chronic progressive MS patients [7, 8]. Taken together, considerable evidence exists to suggest that CD8+ T cell regulatory pathways comprise an indispensable immunological checkpoint to prevent the development of autoimmunity and these pathways are compromised in MS patients.

Different subsets of regulatory CD8 T cells

Unlike conventional thymically derived or peripherally induced CD4+CD25+ regulatory T cells, which are identified by the transcription factor *foxP3*, regulatory CD8+ T cells have been difficult to characterize based on phenotypic markers, therefore limiting their study. Several subtypes of CD8+ T cells with regulatory functions have been identified in multiple disease models. Work from our group demonstrated that the most potent neuroantigen-specific CD8+ Tregs were harbored in the terminally differentiated subset (CD27-CD28-CD45RO-CD62L-CD57+) of CD8+ T cells [19]. This difference in the suppressive potential between CD8-subsets was masked when suppression cultures were polyclonally activated with anti-CD3 antibody. The findings were corroborated in MS patients where reduction in the frequency of terminally differentiated CD8+ T cells underlies the loss in suppressive ability of CD8+ T cells during disease relapse [19]. Other subsets of CD8+ T cells with regulatory functions in MS include CD8+CD25+FoxP3+ [27] and CD8+CD28- T cells [35]. CD8+CXCR3+ T cells (human counterpart of murine CD8+CD122+ T cells) are a naturally existing subset in humans with regulatory functions attributed to IL-10 secretion [41]. Qa-1

restricted CD8⁺ T cells comprise a distinct class of CD8⁺ T cells with prominent regulatory functions in autoimmune several diseases. These cells have been extensively reviewed [42].

Studies in animal models have been instrumental in recognizing different cellular subtypes of CD8⁺ T cells with regulatory functions, as summarized in Table 3. With the ever-increasing list of regulatory CD8-subtypes, one can appreciate the complexity and heterogeneity of the CD8⁺ T cell pool. This also stresses the need for better understanding of the molecular markers and pathways associated with the CD8⁺ T cell effector vs. regulatory functions.

Molecular signatures of regulatory CD8⁺ T cells

Major recent advances have been made to characterize molecular requirements for the regulatory functions of CD8⁺ T cells. For the purpose of this review, we will focus on the neuroantigen-specific regulatory CD8⁺ T cells in MS and EAE. Unlike Qa-1 restricted CD8 Tregs important for regulating diabetes, neuroantigen-specific CD8⁺ T cells are MHC class I restricted. Our EAE studies have shown that, in vivo, MHC class I presentation is absolutely required for CD8⁺ T cell mediated EAE regulation [18]. The clinical course of MOG induced EAE was unaffected in MHC class I deficient $\beta 2M^{-/-}$ mice that received MOG₃₅₋₅₅-specific CD8⁺ T cells. Similarly, WT MOG₃₅₋₅₅-specific CD8⁺ T-cells are incapable of suppressing disease when transferred into $Tap^{-/-}$ (MHC Class I deficient) mice, further reinforcing the requirement for the presentation of cognate antigen on MHC class I [18]. Like effectors CD8⁺ T cells, neuroantigen-specific regulatory CD8⁺ T cells need to see their antigen in context with classical MHC class Ia molecules as these autoreactive CD8⁺ T cells are ineffective in ameliorating EAE in $K^bD^b^{-/-}$ mice, which are MHC Class Ia deficient and Class Ib competent. The requirement of classical MHC class Ia by these regulatory CD8⁺ T cells is corroborated by our human studies. Inclusion of anti MHC class I antibodies abolished CD8⁺ Treg mediated suppression of neuroantigen-specific CD4⁺ T cells [19]. This suggests that the targets for neuroantigen-specific CD8⁺ cells appear to be the cells that can present the peptides in the context of their class Ia molecules. CD8⁺ T cells with similar regulatory potential are also induced in response to T cell vaccination [38] and during glatiramer acetate (GA) therapy of EAE and MS [10, 12]. However, these cells are different since, instead of classical MHC molecules, they identify mature Th1 cells in Qa-1-restricted manner.

Besides MHC class I, we have identified two other key molecular mediators that are required by these CD8⁺ Tregs. In suppression assays with human PBMC, blocking IFN- γ and perforin completely abolished the suppressive property of neuroantigen-specific CD8⁺ T cells [19]. Direct evidence for the absolute requirement of IFN- γ and perforin by CD8⁺ Tregs comes from EAE studies where transferring IFN- γ and perforin-deficient MOG₃₅₋₅₅-specific CD8⁺ T-cells into WT mice had no suppressive effect on MOG induced EAE [18]. IFN- γ from CD8-Tregs is particularly interesting since the pleiotropic effects of this cytokine can potentially explain the regulatory phenotype of CD8⁺ T cells. IFN- γ produced from CD8 Tregs can induce indoleamine 2,3-dioxygenase (IDO) expression in dendritic cells (DCs) [43], which can potentially induce regulatory activity in T cells. By modulating MHC class I expression on APCs [44], CD8⁺ T cell-derived IFN- γ can inhibit myelin

antigen presentation to CD4⁺ T cells. More recently IFN- γ has been shown to induce FoxP3 expression in T cells causing the conversion of CD4⁺ T cells to CD4⁺ Tregs[45]. Another potentially interesting molecular marker with relevance to conferring regulatory properties on CD8⁺ T cell is NKG2D, which when blocked in suppression cultures results in the abrogation of CD8⁺ T cell mediated suppression of CD4⁺ T cells[19]. NKG2D is an activating receptor on CD8⁺ T cells that when engaged can potentially induce killing of its ligand expressing cells. Although future studies are needed to delineate its role in the CD8⁺ T cell mediated neuroantigen-specific suppression, blocking studies suggest that this receptor is an important component of the interplay between CD8⁺ T cells, CD4⁺ T cells and APCs in suppression cultures.

Mechanisms of CD8 T cells mediated immune regulation

CD8⁺ regulatory T cells are a heterogeneous population that can modulate immune responses through diverse mechanisms of action. The known immunoregulatory effects can be mediated by cytotoxicity, involving the direct lysis or apoptosis of specific targets. Immune regulation can also be mediated by the secretion of cytokines including IL-4, IL-6, IL-10, TGF- β , and IFN- γ . These cytokines can suppress or modulate the differentiation patterns of CD4⁺ T cells. Furthermore, regulatory CD8⁺ T cells can also modulate immune responses indirectly by modifying APCs, which in turn influences the development and function of other cells.

The mechanisms used by neuroantigen-specific regulatory CD8⁺ T cells to cause immunomodulation in MS and EAE have provided important insights into the biology of these cells. These autoregulatory CD8⁺ T cells suppressed CD4⁺ T cell-mediated adoptive EAE in an MHC class Ia-dependent manner. Interestingly, these regulatory cells can directly target MHC Class I-replete CD4⁺ T-cells, as neuroantigen-specific CD8⁺ Tregs were capable of suppressing adoptive EAE induced by transferring MHC class Ia-sufficient CD4⁺ T cells into an MHC class Ia devoid host [18]. Using in vivo killing assays, we have demonstrated that CNS-specific autoregulatory CD8⁺ T cells kill antigen-loaded immune targets including CD4⁺ T cells and APCs [13]. These findings were confirmed in disease setting, where reduction in MOG specific CD4⁺ T cell numbers were observed both in the periphery and CNS in EAE mice harboring autoregulatory CD8⁺ T cells. Human in vitro cultures also demonstrated apoptosis induction in CD4⁺ T cells cultured with CD8⁺ T cells in the presence of neuroantigens[19]. Interestingly, in mouse models, transferred MOG-specific CD8⁺ T cells are undetectable in the CNS of naïve mice and access the CNS only during active EAE [18], further reducing the possibility that they could be independently pathogenic and strongly suggesting that they may potentially have their regulatory effect at the site of pathology.

The regulatory function of CD8⁺ T cells is mediated through a combination of IFN- γ -mediated regulation and perforin mediated cytotoxicity and is independent of IL-10 and IL-4[18]. IL-12 can potentiate suppressive function of CD8⁺ T cells by boosting their IFN- γ production [18, 19]. Immunomodulatory effects of these CD8⁺ T cells on APC function is being actively addressed in our ongoing studies. We have shown that APCs derived from protected mice that received MOG₃₅₋₅₅ specific CD8⁺ T cells produced more IL-10 and less

IL-12[13], suggesting an anti-inflammatory phenotype with an inefficient antigen presenting function [13]. Regulatory CD8⁺ T cell mediated modulation of APC functions have also been reported in T1D. Autoregulatory CD8⁺ T cells engage cognate pMHC on the surface of APCs leading to suppression and killing of auto-antigen loaded APCs by IFN- γ , IDO, and perforin dependent mechanisms, therefore, inhibiting the activation of naïve and pathogenic autoreactive-specific T cells. Defects in CD8⁺ T cell recognition of HLA-E/Hsp60sp, which is expressed on target cells, have been documented in human T1D patients. Interestingly, the functional defect in CD8⁺ T cells can be corrected by in vitro exposure to Hsp60sp-loaded DCs[25].

Qa-1 restricted regulatory CD8⁺ T cells induced by TCR peptide vaccination share some similarities to our MHC class Ia restricted CD8 T cells. These Qa-1 restricted CD8 $\alpha\alpha$ +TCR $\alpha\beta$ Tregs also target encephalitogenic CD4⁺ T cells and their functionality is dependent on IFN- γ and perforin[46, 47]. Additional regulatory mechanisms have been identified for Qa-1-restricted CD8⁺ T cells. Qa-1-restricted CD44+CD122+CD8⁺ T cells suppress follicular T-helper cells (T_{FH}) and autoantibody production in systemic lupus erythematosus (SLE)-like disease [48]. Qa-1 compatibility has been shown to be important for suppression of DTH responses. It is suggested that regulatory CD8⁺ T cells suppress DTH responses by producing immunosuppressive TGF- β , as antibodies to TGF- β 1, 2 and 3 inhibit the suppression of DTH by regulatory CD8⁺ T cells [49].

The indispensable role of regulatory CD8 T cells in maintaining immune quiescence in MS

The strongest evidence for the in vivo relevance of the regulatory functions of CD8⁺ T cells comes from our own studies on MS patients. Although MS is thought to be mediated and regulated by CD4⁺ T-cells, several reports from others and us present novel evidence for a role of CD8⁺ T-cells in down-modulation of autoimmune demyelination. Classically, neuroantigen-specific, MHC Class I restricted CD8⁺ T cells are believed to have a pathogenic role in disease, with reports demonstrating in vitro cytotoxic killing of oligodendrocytes. The most common clinical form of MS is relapsing-remitting multiple sclerosis (RRMS). Relapses are clearly defined attacks of deteriorating neurologic function and are followed by periods of partial or complete recovery. Relapses in MS patients are associated with heightened inflammatory immune response in the CNS and periphery including elevated levels of pro-inflammatory cytokines TNF- α , IFN- γ and IL-6 [50] and decreased levels of the anti-inflammatory cytokine IL-10 [51], increased expression of CCR5 and CXCR3 [52] on circulating T cells, deficient CD4+CD25⁺ Treg function [53], upregulation of adhesion molecules on T-cells, B-cells and NK cells [54], higher levels of MMP-2 and MMP-14 in monocytes, and a significantly increased fraction of CD20⁺ B cells. Although the role of CD4+CD25⁺FoxP3⁺ Tregs in maintaining immune quiescence in MS has been known for a while now, scientists have, only recently, started appreciating the regulatory role of CD8⁺ T cells in MS. We identified an unexpected and novel association of the immune regulatory function of neuroantigen-specific CD8⁺ T cells with MS clinical state. Similar to healthy controls, CD8⁺ T cells from quiescent MS patients have neuroantigen-specific suppressor ability. Interestingly, CD8⁺ T cells obtained during an

acute clinical relapse have significantly lower neuroantigen-specific suppressor ability [9]. The clinical implication of this finding is underscored by the fact that recovery of CD8-mediated suppression in MS patients is correlated with the distance in time from an acute clinical episode. This would also imply that correction of the neuroantigen-specific CD8+ suppressor deficit would correlate with recovery from an acute relapse. In keeping with this model, paired samples obtained from MS patients during relapse and quiescence show restoration of neuroantigen-specific CD8+ T cell regulatory function [9, 19]. Percentages of CD8+ T cells and their proliferative capacity in response to neuroantigens during exacerbation and quiescence is not different, suggesting that differences in CD8+ T cell mediated neuroantigen-specific suppression is attributed to functionally different CD8+ T cells. Our studies also show that MS exacerbation is associated with the loss of terminally differentiated CD8+ T cells [19], the subpopulation that harbors the neuroantigen-specific suppressor CD8+ T cells. Other studies have also identified a regulatory role of CD8+ T cells during MS exacerbation. MS exacerbation was associated with a deficiency in CD8+ T cells co-expressing CD25 and FoxP3 in blood and CSF [27]. Taken together, these studies points towards a much more complex immunological picture during acute MS exacerbations and it appears that a combined deficit in CD4+ and CD8+ T cell regulatory function underlies MS exacerbations. We speculate that under relatively non-inflammatory conditions, such as in healthy controls and during MS quiescence, neuroantigen-specific CD8+ T cells, by virtue of their IFN- γ and perforin, keep the pathogenic CD4+ T cells in check and confer anti-inflammatory properties on APCs. Due to as yet unknown mechanisms, once this regulation is lost, inflammatory processes take over and this results in clinical relapses in MS patients.

From Bench to Bedside: Lessons Learned from Copaxone® Therapy

Glatiramer acetate (GA) or Copaxone® (registered trademark of Teva Pharmaceutical Industries Ltd.), is a random copolymer of glutamic acid, lysine, alanine, and tyrosine that emerged more than 30 years ago through experiments in EAE. The peptide copolymer was initially synthesized to mimic the encephalitogenic components of myelin basic protein (MBP). However, it was found that GA inhibited EAE in rodents and primates [55–58]. More importantly, in clinical trials [59–62], GA also reduced the rate of exacerbation and decreased the number of new lesions in patients with RRMS. Due to its efficacy and minimal side effects in MS patients, the mechanism by which the drug acts provides novel insights into the pathways important in MS pathogenesis. Several immunologic effects of GA in MS patients have been reported, including immune deviation of CD4+ T cell responses from Th1 to Th2 [63–68], induction of CD4+CD25+FoxP3 regulatory T cells [69], modulation of APC function by inducing type II monocytes [70] and induction of IL-10 producing B cells [71, 72]. For over three decades it was presumed that responses to GA were restricted to the CD4+ T cells, and therefore, mechanistic studies of GA immunotherapy focused predominantly on the CD4+ T cell subset. Using flow cytometric proliferation assays, we provided the first direct immunophenotypic evidence that proliferative responses to GA are present in both CD4+ and CD8+ T cell compartments in healthy donors and MS patients [10]. Interestingly, whereas the CD4+ GA-specific T cell responses were comparable in all groups, the CD8+ responses were significantly lower in

untreated MS patients. Of paramount importance, we showed a distinct upregulation of CD8⁺ T cell responses to GA in MS patients after GA treatment [10]. We further showed that GA-induced CD8⁺ T cells possess suppressor functions in both human MS [11] and EAE [12]. Interestingly, this suppressor ability is deficient in untreated MS patients and is significantly upregulated in GA-treated patients [11]. This was an exciting and novel discovery that not only suggested that immunomodulatory effect of GA may be mediated by GA-induced CD8⁺ T cells but also highlighted the regulatory role of CD8⁺ T cells in management of MS. The inability of GA to suppress EAE in CD8-deficient mice [12] is further proof that CD8⁺ T cells are absolutely required for the therapeutic effects of GA. The GA-epitope recognized by these suppressor CD8⁺ T cells is a matter of future research. Our finding that significant improvement in GA-specific CD8⁺ suppressor function can be seen as early as 12h after GA therapy initiation in MS patients [17], suggests rapid proliferation/expansion of preexisting cells. However, sustained increase in the suppressor function, as seen 7 months post therapy initiation, might be induced in CD8⁺ T cells by tolerogenic APCs. This evidence comes from EAE studies, where GA-specific suppressor CD8⁺ T cells could not be generated in IDO deficient mice [12], suggesting that the expression of this tolerogenic protein in APCs is clearly required for induction of regulatory behavior in GA-specific CD8⁺ T cells. Concomitantly, GA treatment results in significant increase in plasmacytoid dendritic cells (PDCs) in the blood at 3 months post therapy initiation (unpublished data). It remains to be seen whether IDO is upregulated in PDCs, which subsequently induce suppressor function in CD8⁺ T cells. Like MOG specific CD8⁺ T cells, GA-specific CD8⁺ T cell-suppressive function is dependent on IFN- γ and perforin expression [12]. In MS patients, GA-reactive CD8⁺ T cells express and produce IFN- γ and their suppressive activity is mediated by IFN- γ in a mitogen driven in vitro proliferation assay. Our RNA sequencing studies show that STAT1, a signaling mediator of IFN- γ , is activated in CD8⁺ T cells obtained from the blood of MS patients 12h after GA therapy initiation (unpublished finding). Concomitantly, this time point is also associated with significant increase in CD8⁺ T cell suppressive potential in GA receiving MS patients.

Unlike MOG-specific CD8⁺ T cells, GA-specific CD8⁺ T cell-suppressive function is dependent on non-classical MHC class I presentation (likely Qa-1 in mouse and HLA-E in humans). In this regard, GA-induced CD8⁺ suppressor T cells appear very similar to Qa-1-restricted suppressor/cytotoxic CD8⁺ T cells described in murine systems. GA therapy of MS presents indisputable evidence that restoration/upregulation of CD8⁺ T cell mediated suppression, which is deficient in MS patients as shown by us and others, is a safe and effective way of preventing exacerbations in MS, and that, CD8 T cell can be used as an effective cell based immunosuppressive therapy.

Conclusions and Perspectives: Model of CD8-Mediated Disease Regulation

Antigen-specific immune regulation has high therapeutic relevance in autoimmune diseases, which are clearly associated with defects in the regulatory arm of the immune system. Cellular therapies with targeted antigenic specificities present an attractive treatment strategy for the management of such diseases. There is no doubt that effector and regulatory functions are two parallel arms of CD8⁺ T cell biology, and while their effector role is well characterized, much work remains to be done to better understand their regulatory functions.

Due to the presence of considerable heterogeneity in regulatory CD8⁺ T cell lineages, there is a pressing need for the identification of specific immunological and/or molecular markers associated with them. We are just beginning to understand the mechanism of CD8⁺ T cell mediated immune regulation in the context of autoimmune disease. Studies from our group have uncovered two novel populations of regulatory CD8⁺ T cells, with distinct antigenic specificities. CNS-specific “autoregulatory” CD8⁺ T cells and GA therapy-induced regulatory CD8⁺ T cells have some similar and some distinct biologic features (Table 4). Overall, a model of CD8-mediated antigen-specific regulation of autoimmune demyelinating disease is presented in Fig 1. In MS, regulatory CD8⁺ T cells (naturally existing or therapeutically induced) likely regulate inflammatory responses in the periphery as well as in the CNS. Neuroantigen-specific CD8⁺ T cells get primed by APCs cross-presenting neuroantigens in the context of MHC class Ia. IFN- γ and perforin from these activated CD8⁺ Tregs can cause suppression and/or cytotoxic killing of pathogenic CD4⁺ T cells. Additionally, CD8⁺ Tregs can also target activated pro-inflammatory APCs. IFN- γ from CD8 Tregs can potentially induce IDO expression in APCs, making them tolerogenic. These activated CD8 Tregs can cross the blood brain barrier and enter into the CNS to exert their anti-inflammatory effects on encephalitogenic CD4⁺ T cells and pro-inflammatory APCs. Future studies are needed to elucidate as to how CD8-Tregs can target CD4⁺ T cells in an MHC class I-restricted manner. This would require the presentation of CNS antigen on the CD4⁺ T cells, likely through passive loading of digested myelin components at the site of pathology or via trogocytosis (i.e. membrane exchange) between APC and CD4⁺ T cells. CD8-mediated immunomodulation in the CNS can potentially halt the inflammatory cascade in the CNS. It remains to be definitively established why such CNS-specific CD8⁺ T cells might not target oligodendrocytes, which are theoretically capable of presenting myelin antigens in context with MHC class I. Further elucidation of the immune regulatory mechanisms in the context of this model will form an important basis in the design of novel immune therapeutic strategies either targeting or utilizing CD8⁺ T cells.

Acknowledgments

This work was supported, in part, by grant awards (to NJK) from the NIH and National MS Society. We thank Drs. Elliot Frohman, Benjamin Greenberg and E. Torage Shivapour as well as the staff and patients of the UT Southwestern Medical Center and University of Iowa MS Clinics for their support of our studies. We also acknowledge Drs. Vladimir Badovinac and Michael Crawford for their review of this manuscript.

References

1. Anderson DW, Ellenberg JH, Leventhal CM, Reingold SC, Rodriguez M, Silberberg DH. Revised estimate of the prevalence of multiple sclerosis in the United States. *Annals of neurology*. 1992; 31(3):333–6.10.1002/ana.410310317 [PubMed: 1637140]
2. Arnason BG. Relevance of experimental allergic encephalomyelitis to multiple sclerosis. *Neurologic clinics*. 1983; 1(3):765–82. [PubMed: 6209540]
3. Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. *Annual review of immunology*. 1992; 10:153–87.10.1146/annurev.iy.10.040192.001101
4. McFarlin DE, McFarland HF. Multiple sclerosis (first of two parts). *The New England journal of medicine*. 1982; 307(19):1183–8.10.1056/NEJM198211043071905 [PubMed: 6750404]
5. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by

- micromanipulation and single cell polymerase chain reaction. *The Journal of experimental medicine*. 2000; 192(3):393–404. [PubMed: 10934227]
6. Crawford MP, Yan SX, Ortega SB, Mehta RS, Hewitt RE, Price DA, et al. High prevalence of autoreactive, neuroantigen-specific CD8+ T cells in multiple sclerosis revealed by novel flow cytometric assay. *Blood*. 2004; 103(11):4222–31.10.1182/blood-2003-11-4025 [PubMed: 14976054]
 7. Antel J, Bania M, Noronha A, Neely S. Defective suppressor cell function mediated by T8+ cell lines from patients with progressive multiple sclerosis. *J Immunol*. 1986; 137(11):3436–9. [PubMed: 2946761]
 8. Balashov KE, Khoury SJ, Hafler DA, Weiner HL. Inhibition of T cell responses by activated human CD8+ T cells is mediated by interferon-gamma and is defective in chronic progressive multiple sclerosis. *The Journal of clinical investigation*. 1995; 95(6):2711–9.10.1172/JCI117973 [PubMed: 7769110]
 9. Baughman EJ, Mendoza JP, Ortega SB, Ayers CL, Greenberg BM, Frohman EM, et al. Neuroantigen-specific CD8+ regulatory T-cell function is deficient during acute exacerbation of multiple sclerosis. *Journal of autoimmunity*. 2011; 36(2):115–24.10.1016/j.jaut.2010.12.003 [PubMed: 21257291]
 10. Karandikar NJ, Crawford MP, Yan X, Ratts RB, Brenchley JM, Ambrozak DR, et al. Glatiramer acetate (Copaxone) therapy induces CD8(+) T cell responses in patients with multiple sclerosis. *The Journal of clinical investigation*. 2002; 109(5):641–9.10.1172/JCI14380 [PubMed: 11877472]
 11. Tennakoon DK, Mehta RS, Ortega SB, Bhoj V, Racke MK, Karandikar NJ. Therapeutic induction of regulatory, cytotoxic CD8+ T cells in multiple sclerosis. *J Immunol*. 2006; 176(11):7119–29. [PubMed: 16709875]
 12. Tyler AF, Mendoza JP, Firan M, Karandikar NJ. CD8 T Cells Are Required For Glatiramer Acetate Therapy in Autoimmune Demyelinating Disease. *PloS one*. 2013; 8(6):e66772.10.1371/journal.pone.0066772 [PubMed: 23805274]
 13. York NR, Mendoza JP, Ortega SB, Benagh A, Tyler AF, Firan M, et al. Immune regulatory CNS-reactive CD8+T cells in experimental autoimmune encephalomyelitis. *Journal of autoimmunity*. 2010; 35(1):33–44.10.1016/j.jaut.2010.01.003 [PubMed: 20172692]
 14. Tsai S, Shameli A, Yamanouchi J, Clemente-Casares X, Wang J, Serra P, et al. Reversal of autoimmunity by boosting memory-like autoregulatory T cells. *Immunity*. 2010; 32(4):568–80.10.1016/j.immuni.2010.03.015 [PubMed: 20381385]
 15. Brimnes J, Allez M, Dotan I, Shao L, Nakazawa A, Mayer L. Defects in CD8+ regulatory T cells in the lamina propria of patients with inflammatory bowel disease. *J Immunol*. 2005; 174(9):5814–22. [PubMed: 15843585]
 16. Cho BA, Sim JH, Park JA, Kim HW, Yoo WH, Lee SH, et al. Characterization of effector memory CD8+ T cells in the synovial fluid of rheumatoid arthritis. *Journal of clinical immunology*. 2012; 32(4):709–20.10.1007/s10875-012-9674-3 [PubMed: 22367266]
 17. Ayers CL, Mendoza JP, Sinha S, Cunnusamy K, Greenberg BM, Frohman EM, et al. Modulation of immune function occurs within hours of therapy initiation for multiple sclerosis. *Clin Immunol*. 2013; 147(2):105–19.10.1016/j.clim.2013.02.015 [PubMed: 23578552]
 18. Ortega SB, Kashi VP, Tyler AF, Cunnusamy K, Mendoza JP, Karandikar NJ. The disease-ameliorating function of autoregulatory CD8 T cells is mediated by targeting of encephalitogenic CD4 T cells in experimental autoimmune encephalomyelitis. *J Immunol*. 2013; 191(1):117–26.10.4049/jimmunol.1300452 [PubMed: 23733879]
 19. Cunnusamy KBE, Franco J, Ortega SB, Sinha S, Chaudhary P, Greenberg BM, Frohman EM, Karandikar NJ. Disease Exacerbation of Multiple Sclerosis is Characterized by Loss of Terminally Differentiated Autoregulatory CD8+ T cells. *Clinical Immunology*. 2014 In Press.
 20. Biegler BW, Yan SX, Ortega SB, Tennakoon DK, Racke MK, Karandikar NJ. Glatiramer acetate (GA) therapy induces a focused, oligoclonal CD8+ T-cell repertoire in multiple sclerosis. *Journal of neuroimmunology*. 2006; 180(1–2):159–71.10.1016/j.jneuroim.2006.07.015 [PubMed: 16935352]

21. Biegler BW, Yan SX, Ortega SB, Tennakoon DK, Racke MK, Karandikar NJ. Clonal composition of neuroantigen-specific CD8+ and CD4+ T-cells in multiple sclerosis. *Journal of neuroimmunology*. 2011; 234(1–2):131–40.10.1016/j.jneuroim.2011.02.001 [PubMed: 21397340]
22. Racke MK, Lovett-Racke AE, Karandikar NJ. The mechanism of action of glatiramer acetate treatment in multiple sclerosis. *Neurology*. 2010; 74 (Suppl 1):S25–30.10.1212/WNL.0b013e3181c97e39 [PubMed: 20038760]
23. Ratts RB, Karandikar NJ, Hussain RZ, Choy J, Northrop SC, Lovett-Racke AE, et al. Phenotypic characterization of autoreactive T cells in multiple sclerosis. *Journal of neuroimmunology*. 2006; 178(1–2):100–10.10.1016/j.jneuroim.2006.06.010 [PubMed: 16901549]
24. Ratts RB, Lovett-Racke AE, Choy J, Northrop SC, Hussain RZ, Karandikar NJ, et al. CD28-CD57+ T cells predominate in CD8 responses to glatiramer acetate. *Journal of neuroimmunology*. 2006; 178(1–2):117–29.10.1016/j.jneuroim.2006.06.001 [PubMed: 16837065]
25. Jiang H, Canfield SM, Gallagher MP, Jiang HH, Jiang Y, Zheng Z, et al. HLA-E-restricted regulatory CD8(+) T cells are involved in development and control of human autoimmune type 1 diabetes. *The Journal of clinical investigation*. 2010; 120(10):3641–50.10.1172/JCI43522 [PubMed: 20877010]
26. Carvalheiro H, da Silva JA, Souto-Carneiro MM. Potential roles for CD8(+) T cells in rheumatoid arthritis. *Autoimmunity reviews*. 2013; 12(3):401–9.10.1016/j.autrev.2012.07.011 [PubMed: 22841983]
27. Correale J, Villa A. Role of CD8+ CD25+ Foxp3+ regulatory T cells in multiple sclerosis. *Annals of neurology*. 2010; 67(5):625–38.10.1002/ana.21944 [PubMed: 20437560]
28. Davila E, Kang YM, Park YW, Sawai H, He X, Pryshchep S, et al. Cell-based immunotherapy with suppressor CD8+ T cells in rheumatoid arthritis. *J Immunol*. 2005; 174(11):7292–301. [PubMed: 15905576]
29. Tada Y, Ho A, Koh DR, Mak TW. Collagen-induced arthritis in CD4- or CD8-deficient mice: CD8+ T cells play a role in initiation and regulate recovery phase of collagen-induced arthritis. *J Immunol*. 1996; 156(11):4520–6. [PubMed: 8666829]
30. Penninger JM, Neu N, Timms E, Wallace VA, Koh DR, Kishihara K, et al. The induction of experimental autoimmune myocarditis in mice lacking CD4 or CD8 molecules [corrected]. *The Journal of experimental medicine*. 1993; 178(5):1837–42. [PubMed: 8228830]
31. Stuart PM, Summers B, Morris JE, Morrison LA, Leib DA. CD8(+) T cells control corneal disease following ocular infection with herpes simplex virus type 1. *The Journal of general virology*. 2004; 85(Pt 7):2055–63.10.1099/vir.0.80049-0 [PubMed: 15218191]
32. Jiang H, Zhang SI, Pernis B. Role of CD8+ T cells in murine experimental allergic encephalomyelitis. *Science*. 1992; 256(5060):1213–5. [PubMed: 1375398]
33. Lee YH, Ishida Y, Rifa'i M, Shi Z, Isobe K, Suzuki H. Essential role of CD8+CD122+ regulatory T cells in the recovery from experimental autoimmune encephalomyelitis. *J Immunol*. 2008; 180(2):825–32. [PubMed: 18178821]
34. Rifa'i M, Kawamoto Y, Nakashima I, Suzuki H. Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. *The Journal of experimental medicine*. 2004; 200(9):1123–34.10.1084/jem.20040395 [PubMed: 15520244]
35. Crucian B, Dunne P, Friedman H, Ragsdale R, Pross S, Widen R. Alterations in levels of CD28-/CD8+ suppressor cell precursor and CD45RO+/CD4+ memory T lymphocytes in the peripheral blood of multiple sclerosis patients. *Clinical and diagnostic laboratory immunology*. 1995; 2(2):249–52. [PubMed: 7697540]
36. Najafian N, Chitnis T, Salama AD, Zhu B, Benou C, Yuan X, et al. Regulatory functions of CD8+CD28- T cells in an autoimmune disease model. *The Journal of clinical investigation*. 2003; 112(7):1037–48.10.1172/JCI17935 [PubMed: 14523041]
37. Hu D, Ikizawa K, Lu L, Sanchirico ME, Shinohara ML, Cantor H. Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nature immunology*. 2004; 5(5):516–23.10.1038/ni1063 [PubMed: 15098030]
38. Jiang H, Braunstein NS, Yu B, Winchester R, Chess L. CD8+ T cells control the TH phenotype of MBP-reactive CD4+ T cells in EAE mice. *Proceedings of the National Academy of Sciences of*

- the United States of America. 2001; 98(11):6301–6.10.1073/pnas.101123098 [PubMed: 11353822]
39. Correale J, Villa A. Isolation and characterization of CD8+ regulatory T cells in multiple sclerosis. *Journal of neuroimmunology*. 2008; 195(1–2):121–34.10.1016/j.jneuroim.2007.12.004 [PubMed: 18234356]
 40. Pannemans K, Broux B, Goris A, Dubois B, Broekmans T, Van Wijmeersch B, et al. HLA-E restricted CD8+ T cell subsets are phenotypically altered in multiple sclerosis patients. *Multiple sclerosis*. 2013;10.1177/1352458513509703
 41. Shi Z, Okuno Y, Rifa'i M, Endharti AT, Akane K, Isobe K, et al. Human CD8+CXCR3+ T cells have the same function as murine CD8+CD122+ Treg. *European journal of immunology*. 2009; 39(8):2106–19.10.1002/eji.200939314 [PubMed: 19609979]
 42. Lu L, Cantor H. Generation and regulation of CD8(+) regulatory T cells. *Cellular & molecular immunology*. 2008; 5(6):401–6.10.1038/cmi.2008.50 [PubMed: 19118505]
 43. Jurgens B, Hainz U, Fuchs D, Felzmann T, Heitger A. Interferon-gamma-triggered indoleamine 2,3-dioxygenase competence in human monocyte-derived dendritic cells induces regulatory activity in allogeneic T cells. *Blood*. 2009; 114(15):3235–43.10.1182/blood-2008-12-195073 [PubMed: 19625705]
 44. Martini M, Testi MG, Pasetto M, Picchio MC, Innamorati G, Mazzocco M, et al. IFN-gamma-mediated upmodulation of MHC class I expression activates tumor-specific immune response in a mouse model of prostate cancer. *Vaccine*. 2010; 28(20):3548–57.10.1016/j.vaccine.2010.03.007 [PubMed: 20304037]
 45. Wang Z, Hong J, Sun W, Xu G, Li N, Chen X, et al. Role of IFN-gamma in induction of Foxp3 and conversion of CD4+ CD25- T cells to CD4+ Tregs. *The Journal of clinical investigation*. 2006; 116(9):2434–41.10.1172/JCI25826 [PubMed: 16906223]
 46. Beeston T, Smith TR, Maricic I, Tang X, Kumar V. Involvement of IFN-gamma and perforin, but not Fas/FasL interactions in regulatory T cell-mediated suppression of experimental autoimmune encephalomyelitis. *Journal of neuroimmunology*. 2010; 229(1–2):91–7.10.1016/j.jneuroim.2010.07.007 [PubMed: 20708278]
 47. Tang X, Maricic I, Purohit N, Bakamjian B, Reed-Loisel LM, Beeston T, et al. Regulation of immunity by a novel population of Qa-1-restricted CD8alphaalpha+TCRalphabeta+ T cells. *J Immunol*. 2006; 177(11):7645–55. [PubMed: 17114434]
 48. Kim HJ, Verbinen B, Tang X, Lu L, Cantor H. Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. *Nature*. 2010; 467(7313):328–32.10.1038/nature09370 [PubMed: 20844537]
 49. Cone RE, Chattopadhyay S, Sharafieh R, Lemire Y, O'Rourke J, Flavell RA, et al. T cell sensitivity to TGF-beta is required for the effector function but not the generation of splenic CD8+ regulatory T cells induced via the injection of antigen into the anterior chamber. *International immunology*. 2009; 21(5):567–74.10.1093/intimm/dxp023 [PubMed: 19325036]
 50. Ozenci V, Kouwenhoven M, Huang YM, Kivisakk P, Link H. Multiple sclerosis is associated with an imbalance between tumour necrosis factor-alpha (TNF-alpha)- and IL-10-secreting blood cells that is corrected by interferon-beta (IFN-beta) treatment. *Clinical and experimental immunology*. 2000; 120(1):147–53. [PubMed: 10759776]
 51. Huang WX, Huang P, Link H, Hillert J. Cytokine analysis in multiple sclerosis by competitive RT-PCR: A decreased expression of IL-10 and an increased expression of TNF-alpha in chronic progression. *Multiple sclerosis*. 1999; 5(5):342–8. [PubMed: 10516778]
 52. Calabresi PA, Martin R, Jacobson S. Chemokines in chronic progressive neurological diseases: HTLV-1 associated myelopathy and multiple sclerosis. *Journal of neurovirology*. 1999; 5(1):102–8. [PubMed: 10190696]
 53. Frisullo G, Nociti V, Iorio R, Patanella AK, Caggiula M, Marti A, et al. Regulatory T cells fail to suppress CD4T+-bet+ T cells in relapsing multiple sclerosis patients. *Immunology*. 2009; 127(3):418–28.10.1111/j.1365-2567.2008.02963.x [PubMed: 19016907]
 54. Sellner J, Koczi W, Harrer A, Oppermann K, Obregon-Castrillo E, Pilz G, et al. Glatiramer acetate attenuates the pro-migratory profile of adhesion molecules on various immune cell subsets in

- multiple sclerosis. *Clinical and experimental immunology*. 2013; 173(3):381–9.10.1111/cei.12125 [PubMed: 23611040]
55. Keith AB, Arnon R, Teitelbaum D, Caspary EA, Wisniewski HM. The effect of Cop 1, a synthetic polypeptide, on chronic relapsing experimental allergic encephalomyelitis in guinea pigs. *Journal of the neurological sciences*. 1979; 42(2):267–74. [PubMed: 90129]
 56. Teitelbaum D, Meshorer A, Hirshfeld T, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. *European journal of immunology*. 1971; 1(4):242–8.10.1002/eji.1830010406 [PubMed: 5157960]
 57. Teitelbaum D, Webb C, Bree M, Meshorer A, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis in Rhesus monkeys by a synthetic basic copolymer. *Clinical immunology and immunopathology*. 1974; 3(2):256–62. [PubMed: 4141659]
 58. Teitelbaum D, Webb C, Meshorer A, Arnon R, Sela M. Suppression by several synthetic polypeptides of experimental allergic encephalomyelitis induced in guinea pigs and rabbits with bovine and human basic encephalitogen. *European journal of immunology*. 1973; 3(5):273–9.10.1002/eji.1830030505 [PubMed: 4128127]
 59. Abramsky O, Teitelbaum D, Arnon R. Effect of a synthetic polypeptide (COP 1) on patients with multiple sclerosis and with acute disseminated encephalomyelitis. Preliminary report. *Journal of the neurological sciences*. 1977; 31(3):433–8. [PubMed: 845620]
 60. Bornstein MB, Miller AI, Teitelbaum D, Arnon R, Sela M. Multiple sclerosis: trial of a synthetic polypeptide. *Annals of neurology*. 1982; 11(3):317–9.10.1002/ana.410110314 [PubMed: 7092185]
 61. Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology*. 1995; 45(7):1268–76. [PubMed: 7617181]
 62. Johnson KP, Brooks BR, Ford CC, Goodman A, Guarnaccia J, Lisak RP, et al. Sustained clinical benefits of glatiramer acetate in relapsing multiple sclerosis patients observed for 6 years. Copolymer 1 Multiple Sclerosis Study Group. *Multiple sclerosis*. 2000; 6(4):255–66. [PubMed: 10962546]
 63. Brenner T, Arnon R, Sela M, Abramsky O, Meiner Z, Riven-Kreitman R, et al. Humoral and cellular immune responses to Copolymer 1 in multiple sclerosis patients treated with Copaxone. *Journal of neuroimmunology*. 2001; 115(1–2):152–60. [PubMed: 11282165]
 64. Duda PW, Krieger JI, Schmied MC, Balentine C, Hafler DA. Human and murine CD4 T cell reactivity to a complex antigen: recognition of the synthetic random polypeptide glatiramer acetate. *J Immunol*. 2000; 165(12):7300–7. [PubMed: 11120865]
 65. Duda PW, Schmied MC, Cook SL, Krieger JI, Hafler DA. Glatiramer acetate (Copaxone) induces degenerate, Th2-polarized immune responses in patients with multiple sclerosis. *The Journal of clinical investigation*. 2000; 105(7):967–76.10.1172/JCI8970 [PubMed: 10749576]
 66. Farina C, Then Bergh F, Albrecht H, Meinel E, Yassouridis A, Neuhaus O, et al. Treatment of multiple sclerosis with Copaxone (COP): Elispot assay detects COP-induced interleukin-4 and interferon-gamma response in blood cells. *Brain: a journal of neurology*. 2001; 124(Pt 4):705–19. [PubMed: 11287371]
 67. Neuhaus O, Farina C, Yassouridis A, Wiendl H, Then Bergh F, Dose T, et al. Multiple sclerosis: comparison of copolymer-1- reactive T cell lines from treated and untreated subjects reveals cytokine shift from T helper 1 to T helper 2 cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(13):7452–7. [PubMed: 10861011]
 68. Qin Y, Zhang DQ, Prat A, Pouly S, Antel J. Characterization of T cell lines derived from glatiramer-acetate-treated multiple sclerosis patients. *Journal of neuroimmunology*. 2000; 108(1–2):201–6. [PubMed: 10900354]
 69. Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(18):6449–54.10.1073/pnas.0502187102 [PubMed: 15851684]

70. Weber MS, Prod'homme T, Youssef S, Dunn SE, Rundle CD, Lee L, et al. Type II monocytes modulate T cell-mediated central nervous system autoimmune disease. *Nature medicine*. 2007; 13(8):935–43.10.1038/nm1620
71. Begum-Haque S, Sharma A, Christy M, Lentini T, Ochoa-Reparaz J, Fayed IF, et al. Increased expression of B cell-associated regulatory cytokines by glatiramer acetate in mice with experimental autoimmune encephalomyelitis. *Journal of neuroimmunology*. 2010; 219(1–2):47–53.10.1016/j.jneuroim.2009.11.016 [PubMed: 20034680]
72. Kala M, Rhodes SN, Piao WH, Shi FD, Campagnolo DI, Vollmer TL. B cells from glatiramer acetate-treated mice suppress experimental autoimmune encephalomyelitis. *Experimental neurology*. 2010; 221(1):136–45.10.1016/j.expneurol.2009.10.015 [PubMed: 19879259]
73. Jurewicz A, Biddison WE, Antel JP. MHC class I-restricted lysis of human oligodendrocytes by myelin basic protein peptide-specific CD8 T lymphocytes. *J Immunol*. 1998; 160(6):3056–9. [PubMed: 9510211]
74. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. *The American journal of pathology*. 2008; 172(1):146–55.10.2353/ajpath.2008.070690 [PubMed: 18156204]
75. Annibaldi V, Ristori G, Angelini DF, Serafini B, Mechelli R, Cannoni S, et al. CD161(high)CD8+T cells bear pathogenetic potential in multiple sclerosis. *Brain: a journal of neurology*. 2011; 134(Pt 2):542–54.10.1093/brain/awq354 [PubMed: 21216829]
76. Jilek S, Schluep M, Rossetti AO, Guignard L, Le Goff G, Pantaleo G, et al. CSF enrichment of highly differentiated CD8+ T cells in early multiple sclerosis. *Clin Immunol*. 2007; 123(1):105–13.10.1016/j.clim.2006.11.004 [PubMed: 17188575]
77. Bania MB, Antel JP, Reder AT, Nicholas MK, Arnason BG. Suppressor and cytolytic cell function in multiple sclerosis. Effects of cyclosporine A and interleukin 2. *The Journal of clinical investigation*. 1986; 78(2):582–6.10.1172/JCI112612 [PubMed: 2942563]
78. Huseby ES, Liggitt D, Brabb T, Schnabel B, Ohlen C, Goverman J. A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *The Journal of experimental medicine*. 2001; 194(5):669–76. [PubMed: 11535634]
79. Ford ML, Evavold BD. Specificity, magnitude, and kinetics of MOG-specific CD8+ T cell responses during experimental autoimmune encephalomyelitis. *European journal of immunology*. 2005; 35(1):76–85.10.1002/eji.200425660 [PubMed: 15593305]
80. Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, et al. Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol*. 2001; 166(12):7579–87. [PubMed: 11390514]
81. Mars LT, Bauer J, Gross DA, Bucciarelli F, Firat H, Hudrisier D, et al. CD8 T cell responses to myelin oligodendrocyte glycoprotein-derived peptides in humanized HLA-A*0201-transgenic mice. *J Immunol*. 2007; 179(8):5090–8. [PubMed: 17911594]
82. Saxena A, Bauer J, Scheikl T, Zappulla J, Audebert M, Desbois S, et al. Cutting edge: Multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. *J Immunol*. 2008; 181(3):1617–21. [PubMed: 18641296]
83. Saitoh O, Abiru N, Nakahara M, Nagayama Y. CD8+CD122+ T cells, a newly identified regulatory T subset, negatively regulate Graves' hyperthyroidism in a murine model. *Endocrinology*. 2007; 148(12):6040–6.10.1210/en.2007-0300 [PubMed: 17823258]
84. Endharti AT, Okuno Y, Shi Z, Misawa N, Toyokuni S, Ito M, et al. CD8+CD122+ regulatory T cells (Tregs) and CD4+ Tregs cooperatively prevent and cure CD4+ cell-induced colitis. *J Immunol*. 2011; 186(1):41–52.10.4049/jimmunol.1000800 [PubMed: 21098236]
85. Wang R, Han G, Song L, Wang J, Chen G, Xu R, et al. CD8+ regulatory T cells are responsible for GAD-IgG gene-transferred tolerance induction in NOD mice. *Immunology*. 2009; 126(1):123–31.10.1111/j.1365-2567.2008.02884.x [PubMed: 18624731]

Figure 1. Model for neuroantigen-specific or GA-induced CD8+ T cell-mediated regulation of multiple sclerosis

MHC class Ia or HLA-E-restricted CD8+ T cells use a combination of IFN- γ mediated-immune modulation and perforin-mediated cytotoxic mechanisms to curb inflammatory responses associated with immune cells. These CD8-Tregs can be induced by IDO-expressing APC and further secretion of IFN- γ by CD8-Tregs can potentially act in a positive feedback loop to boost IDO production in APC. The ultimate effect of actions on APC and CD4+ Th1/Th17 cells is the inhibition of pathogenic T cell responses in the periphery and in the CNS, resulting in control of disease pathology.

Table 1

Pathogenic and regulatory role for CD8+ T cells in multiple sclerosis

Subpopulation	Proposed role	Comments	References
Multiple Sclerosis			
CD8 T cell lines from MS patients	Pathogenic: caused lysis of oligodendrocyte in HLA restricted manner	In vitro manipulation of CD8+ T cells to generate cell lines could have changed functional properties of CD8+ T cells	Jurewicz et al [73]
IL17+CD8+ T cells in CNS	Pro-inflammatory	MS patients were either primary or secondary progressive. In these stages of MS, inflammatory cells might be overrepresented in the CNS	Tzartos et al [74]
CD8+CD161+ T cells	Pathogenic: Proinflammatory phenotype suggested by IFN- γ and IL-17 production	It is not clear whether they are MHC class I restricted. IFN- γ from CD8+ T cells has been shown to have regulatory functions	Annibaldi et al [75]
CCR7-CD45RA+ CD8+ T cells	Pathogenic: effectors by definition. Enriched in CSF of MS patients	Pathogenic potential unclear as perforin content less than that present in blood derived CD8+ T cells	Jilek et al [76]
CD8+ T cell lines from progressive MS patients	Suppressor	Persistently reduced suppressive potential of CD8+ T cell lines from MS patients	Antel et al [7]
CD8+ T cells from progressive MS patients	Suppressor	Mitogen induced CD8 suppressor activity was reduced in MS patients	Bania et al [77]
HLA-E restricted CD8+ T cells	Regulatory. Phenotypically altered in MS patients	Reduced FoxP3 on NKG2C ⁺ CD8 T cells and more proinflammatory cytokines from NKG2A ⁺ CD8+ T cells	Pannemans et al [40]
Neuroantigen-specific CD8+T cells	Regulatory	Suppressive function is deficient during MS relapse	Baughman et al [9] & Cunnusamy et al [19]
GA-specific CD8 T cells	Regulatory	Restored in MS patients initiating Copaxone therapy	Karandikar et al [10] & Tennakoon et al [11]

Table 2

Pathogenic and regulatory role for CD8+ T cells in EAE

Subpopulation	Proposed role	Comments	References
EAE			
MBP specific CD8+ T cells	Pathogenic		Huseby et al [78]
MOG35-55 specific CD8+ T cells	MOG37-46 specific CD8+ T cells induced adoptive EAE		Ford et al [79], Sun et al [80]
MOG specific HLA- A*0201 specific CD8+ T cells	Aggravated CD4- mediated EAE	Genetically altered mice	Mars et al [81]
Influenza hemagglutinin (HA) specific CD8+ T cells	Pathogenic. Activated HA-specific CD8+ T cells led to inflammatory lesions in optic nerve, spinal cord and brain	Mice are manipulated to express HA in oligodendrocytes	Saxena et al [82]
CD8-deficient mice	Regulatory	Persistent EAE with more relapses	Jiang et al [32]
Qa-1-restricted CD8aa+TCRab+ T cells	Regulatory	Kill activated T cells and prevents EAE	Tang et al [47]
MHC-class Ia-restricted CNS-specific autoregulatory CD8 T cells	Regulatory	Prevent and ameliorate EAE in IFN- γ and perforin dependent manner	Ortega et al [18] & York et al [13]
Qa-1-restricted GA- specific CD8+ T cells	Regulatory	CD8 regulatory function is required for therapeutic benefit of glatiramer acetate in EAE	Tyler et al [12]

Table 3

Regulatory CD8 subtypes in disease models

CD8+ regulatory subtype	Mouse Strain	Disease Model	Mechanism of Suppression
CD8 $\alpha\alpha$ + TCR $\alpha\beta$ +	B10.PL	EAE - MBP TCR-peptide immunization [47]	Apoptotic cell death of disease mediating CD4+ T cells, Perforin
CD8+CD44+CD122+	C57BL/6 (B6) Qa-1 (D227K)	SLE-like disease [48]	Suppress T _{FH} cells via perforin
MOG ₃₅₋₅₅ - CD8+ T cells	C57BL/6	EAE – MOG ₃₅₋₅₅ [18]	Killing of MOG-loaded CD4+ T cells, Perforin Modulation of CD11c+ DC
GA-induced CD8+ T cells	C57BL/6	EAE – MOG ₃₅₋₅₅	Perforin and IFN- γ -mediated modulation Class Ib-restricted?
CD8+CD122+ T cells	BALB/c C57BL/6 RAG2 ^{-/-}	Graves' disease [83] EAE [33], CD4+ T cell-induced Colitis [84]	Disease protection through IL-10 secretion
CD8+CD28-FoxP3+ T cells	B6 CD28 ^{-/-} CD8 ^{-/-}	EAE-MOG [36]	MHC class I-restricted Contact dependent Suppress proliferation of MOG-specific CD4+ T cells. Downregulate costimulatory molecules on APCs
CD8+CD28-CD56+ T cells	NOD-SCID	Rheumatoid Synovitis [28]	Inhibit IFN- γ and TNF- α production Downregulate costimulatory molecule ligands
CD8+Foxp3+	NOD	Insulin-dependent diabetes mellitus (IIDM) [85]	Antigen-specific cellular and humoral suppression

Table 4

CNS-specific vs. GA-induced CD8+ Tregs

Properties	CNS-Specific Autoregulatory CD8+ T cells		GA-Specific Regulatory CD8+ T cells	
	EAE	MS/Humans	EAE	MS/Humans
Induction	Immunization/Disease induction	Present in healthy individuals and MS patients	Immunization with GA	GA CD8 present in healthy subjects; deficient in MS patients Increased post-therapy
MHC restriction	MHC –Class Ia (classical; eg., K ^b D ^b in C57Bl/6 mice)	HLA-A,B,C	Protective properties are non-Class Ia- restricted (likely Qa-1)	Evidence of HLA- E restricted cytotoxicity
Cytokine Profile	IFN- γ , IL-4, TNF- α	IFN- γ ; IL-10	IFN- γ , IL-10, TNF- α	IFN- γ , TNF- α IL-10, TGF- β
Cytotoxic?	Yes	Yes	Yes	Yes
Phenotype		Terminally differentiated (CD27- CD28- CD45RO- CD62L-CD57+)		CD28-/CD57+, Terminally differentiated?
Mechanism(s) of Suppression	1	Requires perforin, granzyme, IFN- γ , but not IL-10 or IL-4	1	Required for mediating effects of GA therapy
	2	Capable of killing pathogenic CD4+ T cells	2	Regulatory function absolutely dependent on IFN- γ & partially on perforin
	3	Modulation of CD11c+ DC	3	Regulatory function is not dependent on IL- 4 or IL-10
			3	Downstream generation of Type II monocytes