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Commentary

Glatiramer acetate for treatment of MS: Regulatory B cells join the cast of players

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ABSTRACT

Glatiramer acetate (GA, copolymer-1, Copaxone®) is a Food and Drug Administration-approved drug for the treatment of relapsing–remitting multiple sclerosis (MS). However, its mechanism of action remains ill-defined. The available evidence indicates that GA induces antigen-presenting cells with anti-inflammatory properties and promotes the generation of immunoregulatory T cells that suppress pathogenic T cells. A new study by Kala et al. (2010) now shows that B lymphocytes, which are best known for their antibody-secreting properties, contribute to the beneficial effects of GA against experimental autoimmune encephalomyelitis (EAE), the animal model of MS. This commentary discusses these new findings in the context of the pathogenesis of MS and EAE, the emerging immunoregulatory role of B cells in autoimmunity, and the relevance of B cells as targets for immunotherapy in MS.

Introduction

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are primarily mediated by T lymphocytes that produce pro-inflammatory cytokines such as IFN-γ, TNF-α, and IL-17 in response to autoantigens expressed in the central nervous system (CNS) (Bhat and Steinman, 2009; El-behi et al., 2010; Goverman, 2009). In healthy individuals, autoantigen-specific T cells are kept in check by a variety of regulatory mechanisms, including immunosuppressive antigen-presenting cells (APCs) and regulatory T cells (Tregs) (Goverman, 2009; McFarland and Martin, 2007). Multiple subsets of Tregs have been implicated in suppressing pathogenic T cells in MS and EAE (Cvetanovich and Hafler, 2010; Zozulya and Wiendl, 2008): IL-4- and IL-10-producing T helper 2 (Th2) cells, TGF-β-producing Th3 cells, $CD4^+CD25^+$ T cells expressing the forkhead transcription factor Foxp3, $CD8^+$ suppressor T cells, and natural killer T cells. Emerging evidence indicates that B lymphocytes, which are best known for their capacity to produce antibodies, can impact the pathogenesis of MS and EAE as well (Franciotta et al., 2008; McLaughlin and Wucherpfennig, 2008).

A better understanding of the immunoregulatory circuits that normally protect against the development of CNS autoimmunity should guide the development of improved immunotherapies for MS and other autoimmune disorders. The disease-modifying drug glatiramer acetate (GA, copolymer-1, Copaxone®) interrupts the pathogenic process in MS by reinforcing these immunoregulatory networks (Arnon and Aharoni, 2009; Blanchette and Neuhaus, 2008; Liblau, 2009; Schrempf and Ziemssen, 2007; Weber et al., 2007a). Prior studies have shown that GA promotes the immunoregulatory functions of both innate and adaptive components of the immune system, including dendritic cells, monocytes, and Tregs. The new study by Kala et al. (2010) shows that GA also promotes regulatory properties in B lymphocytes. This commentary will first review the current knowledge of the mechanism of action of GA for treatment of MS and the role of B cells in the development of autoimmunity in the CNS. It will then discuss the new findings of Kala et al. (2010) that provide evidence for a contribution of regulatory B cells (Bregs) in the protective effects of GA in EAE and possibly MS.

GA and its effects on MS

GA was first synthesized nearly 40 years ago as a research tool to facilitate the reproducible induction of EAE in rodents (Arnon, 1996; Teitelbaum et al., 1971). At the time, EAE was usually induced by immunization of animals with crude myelin preparations derived from the spinal cord of guinea pigs or other animals. Thus, with the goal of standardizing methods to induce EAE, efforts were made to synthesize molecular mimetics of myelin basic protein (MBP), a major product of oligodendrocytes that has been posited to function as an autoantigen in MS. GA is a standardized mixture of polypeptides with an average length of 40 to 100 residues, synthesized from four amino

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acids, namely glutamic acid, lysine, alanine and tyrosine (G-L-A-T), in a random order and at a defined molar ratio of approximately 1.5:3.6:4.6:1.0, as found in MBP. However, instead of inducing EAE, GA protected against EAE induced in response to crude myelin preparations (Teitelbaum et al., 1971). These serendipitous findings prompted a pilot trial, which provided evidence for a beneficial effect of GA in MS (Bornstein et al., 1987). The results from this trial were confirmed in a large randomized clinical trial (Johnson et al., 1995), which led to the regulatory approval of GA for treatment of relapsing–remitting MS in 1996.

Mechanism of action of GA in MS

Multiple mechanisms have been proposed for the protective effects of GA in MS and EAE (Arnon and Aharoni, 2009; Blanchette and Neuhaus, 2008; Liblau, 2009; Schrempf and Ziemssen, 2007; Weber et al., 2007a). Initial studies focused on the capacity of GA to bind promiscuously with major histocompatibility complex (MHC) class II molecules on APCs, without the need for intracellular processing (Fridkis-Hareli and Strominger, 1998). Thus, binding of GA with MHC class II was shown to compete with binding of MBP-derived peptides and antagonize T cell responses *in vitro* (Aharoni et al., 1999). Furthermore, in addition to its function as an antagonist of MBP-specific T cell responses, it has been suggested that GA can function as an altered peptide ligand to induce regulatory cytokine production in T cells (Gran et al., 2000).

There is ample evidence that GA induces the generation of GA-specific Th2 cells that produce IL-4 and IL-10, and possibly Th3 cells that produce TGF-β (Duda et al., 2000; Neuhaus et al., 2000). These Th2 and Th3 cells can suppress the pathogenic effects of autoantigen-specific Th1 and Th17 cells. However, the precise mechanism of this suppression remains unclear. While studies with EAE have shown that GA-specific Th2 cells can enter the CNS (Aharoni et al., 2000), it is unlikely that sufficient amounts of GA are available in the CNS to activate these cells *in situ*. Although some GA-specific T cell lines can cross-react with MBP, this does not appear to be the norm (Aharoni et al., 2000; Chen et al., 2001). Nevertheless, it has been hypothesized that GA-specific Th2 cells exhibit broad cross-reactivity with myelin-derived antigens and possibly other autoantigens (Liblau, 2009). Secretion of anti-inflammatory cytokines by these GA-reactive Th2 cells subsequently leads to suppression of pathogenic, autoantigen-specific Th1 and Th17 cells in the CNS through "bystander suppression." Furthermore, more recent studies have shown that these GA-specific T cells can produce neurotrophic factors such as BDNF (brain-derived neurotrophic factor) or induce production of these factors in other cell types of the CNS (Arnon and Aharoni, 2009; Blanchette and Neuhaus, 2008). These neurotrophic factors can promote neuronal protection and repair, without impinging on the inflammatory process (Kerschensteiner et al., 1999; Linker et al., 2010), which likely contributes to the capacity of GA to halt or reverse some of the neuronal damage inflicted during EAE and MS.

The idea that Th2 cells are required for the suppressive effects of GA in MS has been challenged by studies investigating the role of Th2 cell-derived cytokines in the capacity of GA to suppress EAE induced in C57BL/6 mice following immunization with a myelin oligodendrocyte glycoprotein (MOG) peptide (Jee et al., 2006). GA moderately suppressed EAE but failed to induce Th2-polarized responses in these animals. Furthermore, GA was also protective in IL-4-deficient, IL-10-deficient and IL-4/IL-10 double-deficient mice. Therefore, it is likely that mechanisms other than Th2 cells contribute to the therapeutic effects of GA. Indeed, several research groups showed that GA expands or promotes the activity of Foxp3-expressing Tregs *in vitro* and *in vivo* (Aharoni et al., 2010; Hong et al., 2005; Jee et al., 2007; Putheti et al., 2003). Furthermore, Tregs from GA-treated mice were more effective than Tregs from untreated mice in preventing EAE upon adoptive transfer (Jee et al., 2007).

It has also been reported that GA induces GA-specific $CD8^+$ T cell responses in MS patients, which was associated with an improved clinical response (Farina et al., 2001; Karandikar et al., 2002). These cytotoxic $CD8^+$ T cells might exhibit regulatory properties similar to those of Foxp3-expressing Tregs and/or directly lyse the pathogenic $CD4^+$ T cells that are activated in MS.

GA also provides significant protection against diseases other than MS and EAE, including arthritis, uveoretinitis, inflammatory bowel disease, and graft rejection in mice (Arnon and Aharoni, 2004; Gur et al., 2006). These findings suggested that some of the beneficial effects of GA are due to mechanisms independent of the direct recognition of GA by antigen-specific receptors of the adaptive immune system. Indeed, there is strong evidence that GA directly affects APCs, including dendritic cells and monocytes (Vieira et al., 2003; Weber et al., 2007b). Dendritic cells exposed to GA became impaired for IL-12 production and promoted the induction of IL-4-secreting Th2 cells (Vieira et al., 2003). Similarly, GA promoted the development of anti-inflammatory, type 2 monocytes, which are characterized by reduced secretion of IL-12 and increased secretion of IL-10 and TGF-β (Weber et al., 2007b). This type 2 phenotype in monocytes was induced without the need for GA binding with MHC class II molecules. In turn, these type 2 monocytes promoted the differentiation of Th2 cells and Foxp3-expressing Tregs. Adoptive transfer of GA-induced type 2 monocytes was able to reverse EAE (Weber et al., 2007b). Therefore, these findings provided evidence that cross-reactivity of GA-specific T cells with myelin antigens is not required for the protective effects of GA in EAE.

GA also induced antibody responses in treated MS patients, which were initially predominantly of the IgG1 subclass but then switched towards the IgG4 subclass, suggesting interaction with Th2 cells (Basile et al., 2006; Schrempf and Ziemssen, 2007). These antibodies did not appear to interfere with the clinical efficacy of GA and, in fact, higher titers were detected in relapse-free patients (Brenner et al., 2001). Although these findings suggested that GA-specific antibodies might be beneficial to the mechanism of action of GA, possibly by facilitating neuronal repair, the new study from Kala et al. (2010) indicates that the contribution of B cells in the therapeutic effects of GA in EAE is largely due the acquisition of a regulatory phenotype in these cells. Before discussing the work of Kala et al. (2010) in more depth, it is worthwhile to briefly review the role of B cells in MS and EAE.

B cells and CNS autoimmunity

B cells can play opposing roles in the development of CNS autoimmunity (Kurosaki, 2008). While these cells are best known for their capacity to produce antibodies, they can also function as APCs for T lymphocytes and modulate various immune responses via cytokine and chemokine production. In a seminal study, Janeway and colleagues showed that mice deficient in B cells (due to a targeted mutation in the IgM heavy chain) developed a more severe and chronic course of EAE disease than wild-type animals (Wolf et al., 1996). These findings suggested a suppressive role of B cells in the development of EAE. Subsequent studies provided evidence for a critical role of IL-10 production by B cells in suppressing EAE (Fillatreau et al., 2002). Consistent with these findings, depletion of B cells prior to induction of EAE resulted in disease exacerbation (Matsushita et al., 2008). Importantly, adoptive transfer of an IL-10-producing $CD1d^{hi}CD5^+$ B cell subset was able to prevent this disease exacerbation (Matsushita et al., 2008). B cells expressing CD5 and high levels of CD1d have been shown to exhibit potent immunoregulatory activities by producing high levels of IL-10 that can modulate T cell responses and the antigen-presenting functions of dendritic cells (Bouaziz et al., 2008; Lund and Randall, 2010). The available evidence suggests that the Bregs that suppress EAE are likely specific for myelin antigens (Fillatreau et al., 2002).

In sharp contrast with the results obtained for early B cell depletion, depletion of B cells during progression of EAE potently ameliorated disease (Matsushita et al., 2008). Although pathogenic