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The Evolving Mechanisms of Action of Glatiramer Acetate

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Glatiramer acetate (GA) is a synthetic amino acid copolymer that is approved for treatment of relapsing remitting multiple sclerosis (RRMS) and clinically isolated syndrome (CIS). GA reduces multiple sclerosis (MS) disease activity and has shown comparable efficacy with high-dose interferon-β. The mechanism of action (MOA) of GA has long been an enigma. Originally, it was recognized that GA treatment promoted expansion of GA-reactive T-helper 2 and regulatory T cells, and induced the release of neurotrophic factors. However, GA treatment influences both innate and adaptive immune compartments, and it is now recognized that antigen-presenting cells (APCs) are the initial cellular targets for GA. The anti-inflammatory (M2) APCs induced following treatment with GA are responsible for the induction of anti-inflammatory T cells that contribute to its therapeutic benefit. Here, we review studies that have shaped our current understanding of the MOA of GA.

Multiple sclerosis (MS) is a chronic, progressive, and disabling disorder characterized by immune-mediated demyelination, inflammation, and neurodegenerative tissue damage in the central nervous system (CNS). Relapsing remitting MS (RRMS), characterized by flares followed by partial or complete remission, is the most common MS subtype with ∼85% MS patients presenting to their physicians with this disease pattern. The first MS flare, which is not always recognized, is referred to as clinically isolated syndrome (CIS). Approximately 60%–70% of patients with RRMS evolve to secondary progressive MS over time. About 10%–15% of patients have an insidious progressive course from onset, without recognized flares, and are classified as having a primary progressive course.

Whereas MS is not curable, to date, 12 disease-modifying therapies (DMTs) are available for RRMS treatment, partially addressing different aspects of the immune pathophysiology. Glatiramer acetate (GA), together with β interferons (IFN-βs), is considered first-line treatment for RRMS (Marta and Giovannoni 2012), and is administered subcutaneously or intramuscularly. Although considered the safest medications, they have modest efficacy (Bruck et al. 2013). Second-line drugs have been developed, some that show greater efficacy, including natalizumab (Tysabri), fingolimod (Gilenya), alemtuzumab (Lemtrada), ocrelizumab (Ocrevus) and mitoxantrone (Novantrone), but may have greater potential toxicities. Three of the newer medications, gilenya, teriflunomide (Au-

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bagio), and dimethylfumarate (Tecfidera) are administered orally.

GA (Copaxone, Teva Pharmaceuticals), formerly known as copolymer-1, is a synthetic amino acid polymer that was approved in 1996 in the United States and in 2001 in Europe for treatment of RRMS and, later, in 2014 for CIS. GA was discovered in an attempt to generate antigens mimicking myelin basic protein (MBP), a major protein component of the myelin sheath that is considered one of the candidate myelin autoantigens in MS. Specifically, it was thought that GA would induce experimental autoimmune encephalomyelitis (EAE), the most commonly used animal model for MS. Although GA was found to be immunogenic, it was not encephalitogenic, and prevented myelin protein-induced EAE in various species (Teitelbaum et al. 1971, 1973, 1974, 1996). Those results led first to open-label MS trials with GA (Abramsky et al. 1977; Bornstein et al. 1982) and later placebo-controlled trials (Bornstein et al. 1987; Johnson et al. 1995). Since its approval for MS treatment, GA has remained popular for treatment of MS, especially considering some of the potentially life-threatening side effects of other competitors (Miller et al. 2008).

Whereas the mechanism of action (MOA) of GA has been investigated extensively in both mice and humans, it is still not fully understood. GA is able to modulate multiple processes involving both the innate and adaptive immune system, including the expansion of anti-inflammatory M2 monocytes, T helper (Th)2 cells, and regulatory T (Treg) cells.

CHEMISTRY AND PHARMACOKINETIC PROFILE

GA is composed of the acetate salts of four amino acids, L-glutamate, L-lysine, L-alanine, and L-tyrosine (GLAT), with an average molar fraction of 0.141, 0.427, 0.095, and 0.338, respectively, resulting in a mixture of many synthetic peptides of an average length of 45–200 amino acids with an average molecular weight ranging from 4000 to 9000 Da. Lysine—the basic amino acid common to these copolymers—is essential for therapeutic benefit, as "gat"-iramer acetate, lacking lysine, was ineffective in preclinical EAE studies. The enormous number of potentially active epitopes (10^{30}) in GA prevents the isolation of specific active peptide, and has long created a challenge for its characterization by available methodologies (Varkony et al. 2009). Using deep analytical methodologies capable of characterizing complex proteins and previously developed to sequence heparin copolymers (Venkataraman et al. 1999), which permitted the development of a generic version of Lovenox (Ozug et al. 2012), Momenta Pharmaceuticals (Cambridge, MA) generated a generic version of Copaxone, leading to the approval of Glatopa (Glatiramer Acetate injection, Sandoz, Princeton, NJ) by the Food and Drug Administration (FDA) in 2015.

GA is hydrolyzed at the site of injection, where it can interact both with antigen-presenting cells (APCs) and lymphocytes (Ziemssen et al. 2001). Some material is then presumed to either reach draining lymph nodes or general circulation. Data obtained from animal models using radiolabeled doses of GA showed that the highest levels were achieved in the stomach and thyroid, and the lowest were in the CNS. The hydrophilic nature of GA and its metabolites might therefore prevent it from crossing the blood–brain barrier, suggesting that the therapeutic effect would preferentially occur in periphery (Carter and Keating 2010). In animals, the main route of elimination was shown to be urinary excretion.

MECHANISMS OF ACTION

Modulation of the Adaptive Responses

MOAs of GA that promote immunomodulation and neuroprotection have been described (Fig. 1). Those mechanisms are not mutually exclusive, and several may contribute to the efficacy of the drug.

Given the molecular resemblance to fragments of MBP, GA was shown to bind to major histocompatibility complex (MHC) class II molecules that bind MBP (Fridkis-Hareli et al. 1994, 1997; Fridkis-Hareli and Strominger 1998). Therapeutic effects of GA have been attributed,

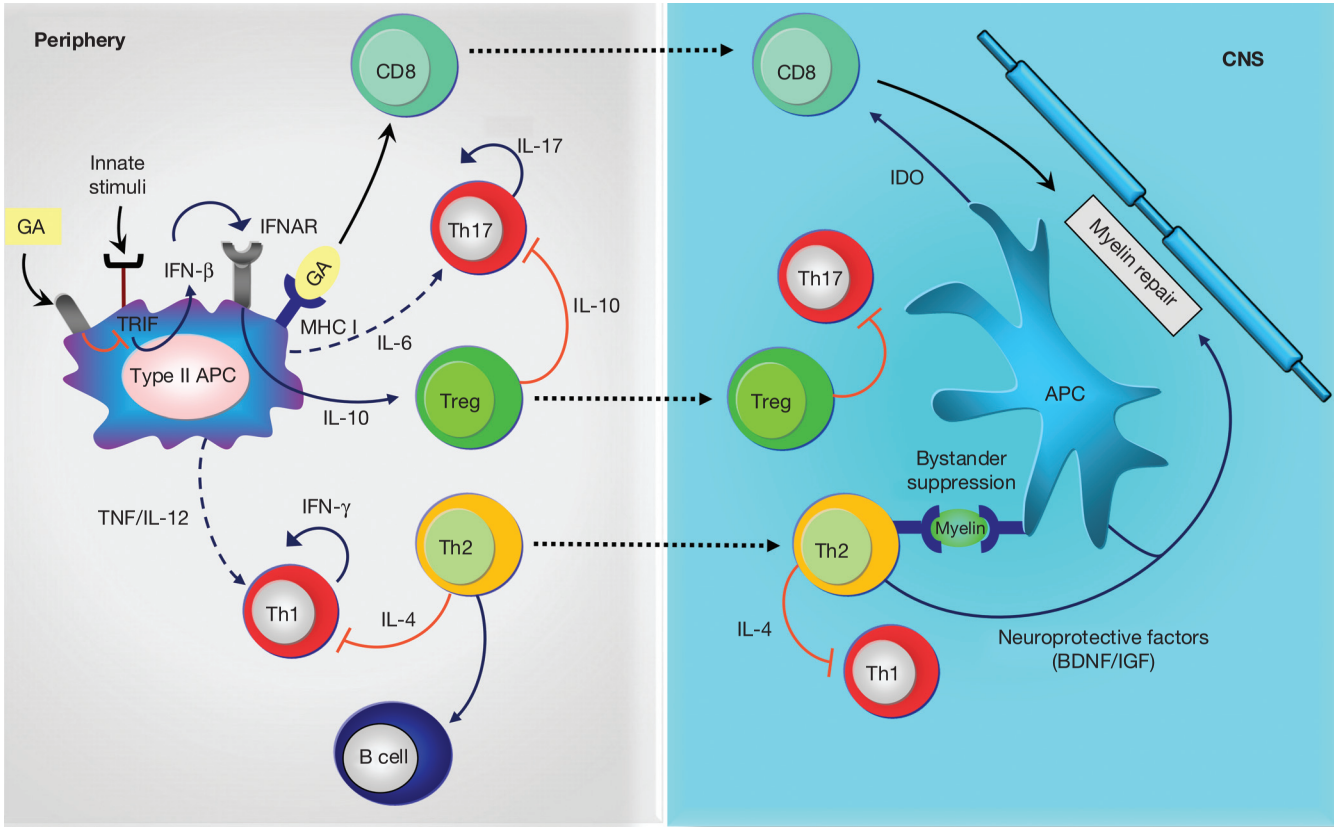

Figure 1. Anti-inflammatory mechanisms induced by glatiramer acetate (GA). GA treatment on antigen-presenting cells (APCs) leads to anti-inflammatory differentiation. Treatment modulates innate stimuli and is associated with down-regulation of type I interferon (IFN), increased T helper (Th)2, and regulatory T (Treg) cell differentiation. Reactivation of GA-reactive Th2 cells in periphery through presentation of myelin antigens is associated with bystander suppression. Th2 cells also modulate B-cell activation. Treg cells down-regulate secretion of proinflammatory cytokines by effector T (Teff) cells both in periphery and in the central nervous system (CNS). $CD8^+$ T cells are generated by antigen presentation of GA in periphery and migrate to the CNS where they contribute to inhibiting myelin degradation. IL, Interleukin; TNF, tumor necrosis factor; IFNAR, interferon-receptor; MHC, major histocompatibility complex; BDNF, brain-derived neurotrophic factor; IGF, insulin-like growth factor; IDO, indoleamine-2,3-dioxygenase; solid lines, cytokines produced by the representative cells; dashed lines, reduced production of cytokines; red lines: inhibitory cytokines.

in part, to the ability of antigenic sequences in hydrolyzed GA peptides to act as altered peptide ligands (APLs), antagonizing the activation of MBP-specific T-cell clones (Teitelbaum et al. 1992; Aharoni et al. 1999). However, those results were later challenged by use of a stereoisomer of GA that retained the ability to bind to MHC II, but yet failed to suppress EAE (Aharoni et al. 1997), and by a report showing that the anti-inflammatory properties of GA were preserved in cells devoid of MHC II expression (Weber et al. 2007).

Repeated GA immunization induces a deviation from a proinflammatory Th1 immune response to an "anti-inflammatory" Th2 phenotype, characterized by the secretion of interleukin (IL)-4, -5, -10, -13, -27 and even transforming growth factor (TGF)-β (Miller et al. 1998; Aharoni et al. 1999, 2003; Duda et al. 2000; Farina et al. 2001; Mindur et al. 2016). Because GA does not directly penetrate the CNS blood–brain barrier, it was thought that the immunomodulatory functions are mediated by peripheral GA-induced Th2 cells that enter the CNS, which then become reactivated following recognition of myelin antigens. GA-induced Th2 cells not only down-regulate the response MBP-specific T cells, but also other encephalitogenic antigens, including myelin oligodendrocyte protein (MOG) and proteolipid protein (PLP)-reactive T cells in a process called "bystander suppression" (Aharoni et al. 1998; Neuhaus et al. 2000; Sela and Teitelbaum 2001; Dhib-Jalbut 2002; Hestvik et al. 2008).

Recent studies, however, have challenged the requirement for both antigen specificity and Th2 differentiation for GA-mediated immunomodulation. First, it was observed that GA-specific Th2 cells that did not cross-react with MBP, PLP, or MOG-suppressed EAE. Therefore, the capability of regulating EAE by GA-reactive T cells occurred independent of antigen specificity (Weber et al. 2007). Similarly, another study that examined T-cell lines from MS patients treated with GA showed that Th2 differentiation occurred independently of antigen specificity (Allie et al. 2005). Additional evidence against the need for myelin antigen cross-reactivity of GA-reactive T cells was provided by studies performed in inflammatory diseases unrelated to CNS pathogenicity. Indeed, GA treatment showed some clinical efficacy in various T-cell-dependent models of inflammation, including experimental autoimmune uveoretinitis (Zhang et al. 2000), inflammatory bowel disease (IBD) (Aharoni et al. 2005b, 2007; Gur et al. 2006; Neesse et al. 2009) and graft rejection (Arnon and Aharoni 2004). Further, experiments with IL-4- and IL-10-deficient mice show that GA still had beneficial effects in suppressing EAE in the absence of these Th2 cytokines (Jee et al. 2006), suggesting that other mechanisms come into play to fully explain the MOA of GA.

Treg cells play an important role in the pathogenesis of autoimmune diseases, and have been shown to be functionally impaired in MS patients (Venken et al. 2008). In addition to inducing Th2 T cells, GA also increases the frequency (Hong et al. 2005; Weber et al. 2007) and function of $CD4^+CD25^+FoxP3^+$ Tregs (Jee et al. 2007; Haas et al. 2009). GA was also shown to down-regulate Th17 T-cell differentiation (Aharoni et al. 2010), considered as one of the main pathogenic drivers for CNS autoimmune diseases.

Although the function and clinical relevance of $CD8^+$ T cells in GA treatment is not yet fully understood, GA-specific $CD8^+$ T cells show similar suppressive functions as $CD4^+$ Tregs. In EAE, adoptive transfer of GA-induced $CD8^+$ T cells results in amelioration of the disease. Indoleamine-2,3-dioxygenase (IDO), a tryptophan-metabolizing enzyme that is strongly up-regulated in lymphoid tissues by proinflammatory molecules, is required for the generation of these cells (Tyler et al. 2013). This suppressive activity is impaired in naïve MS patients (Karandikar et al. 2002), but restored following GA treatment, which was induced by both proliferative responses and enhanced cytotoxic ability (Karandikar et al. 2002; Biegler et al. 2006; Tennakoon et al. 2006).

Anti-Inflammatory Effects of GA on APCs

Studies have suggested that the immunomodulatory effects of GA are not limited to the adaptive immune compartment, but rather result in

a broad, antigen-independent modulation of bone marrow–derived (myeloid) APCs, which are cells within the innate immune system.

GA was found to be able to switch APC activation from a proinflammatory M1 to an anti-inflammatory M2 differentiation (Hussien et al. 2001; Jung et al. 2004; Weber et al. 2004, 2007; Sanna et al. 2006; Molnarfi et al. 2015). GA-treated monocytes and macrophages can produce increased amounts of IL-10, TGF-β, secreted IL-1 receptor antagonist (sIL-1Ra), and, conversely, lower levels of IL-12, IL-1β, and tumor necrosis factor (TNF)-α (Vieira et al. 2003; Jung et al. 2004; Kim et al. 2004; Weber et al. 2004, 2007; Burger et al. 2009; Carpintero et al. 2010). Type II differentiation was also observed with CNS cell types, in which GA promoted the phagocytic activity of microglia and increased the secretion of IL-10 while decreasing TNF-α (Chabot et al. 2002; Pul et al. 2011), suggesting a general effect on myeloid monocytic cells. The relevance of the in vitro observations was validated in GA-treated MS patients. Reduction of cell activation and cytokine secretion was observed in circulating monocytes (Kim et al. 2004; Weber et al. 2004; Carpintero et al. 2010; Pul et al. 2012) and also in plasmacytoid dendritic cells (DCs) of MS patients (Stasiolek et al. 2006).

A growing body of evidence suggests GA-induced type II (M2) differentiation of myeloid cells may be primarily responsible for the effects observed on T cells, including the promotion of Th2 and Treg differentiation. Expansion of Th2 and Treg cells was also observed in vivo, following adoptive transfer of M2 monocytes, and was associated with a reduction of EAE severity (Weber et al. 2007). However, modulations of the T-cell compartment appear to occur independently of antigen specificity (Kantengwa et al. 2007; Weber et al. 2007). Thus, whereas GA mediates a primary effect on APC independent of T cells, M2 APC-induced Treg T-cell populations appear to be the effector cells of GA-mediated immune modulation.

Although the molecular mechanisms required for GA-mediated M2 differentiation and therapeutic efficacy have remained elusive, GA has been hypothesized to interact with cell-surface receptors, leading to the activation of second messengers and regulatory signaling pathways. It was originally speculated that the interaction of GA with either CD11b or MHC II, both expressed at the surface of myeloid cells, might contribute to GA-mediated modulation (Stapulionis et al. 2008; Toker et al. 2011). However, two studies (Weber et al. 2007; Molnarfi et al. 2015) using CD11b- and MHC II–deficient mice showed that neither CD11b expression nor MHC II expression were required for GA-mediated M2 differentiation. Although the receptor responsible for M2 differentiation has yet to be identified, PI3K (Carpintero et al. 2010; Molnarfi et al. 2015), but not cyclic adenosine monophosphate (cAMP), has been shown to participate in M2 polarization by GA, supporting a central role for PI3K/Akt in regulating inflammatory responses. It was also observed that GA treatment was associated with inhibition of signal transducers and activators of transcription (STAT)1 in type II monocytes, indicating that GA affected at least one proinflammatory signaling pathway within these cells (Fig. 2) (Weber et al. 2007). Interestingly, GA has been shown to inhibit monocyte reactivity in response to engagement of Toll-like receptors (TLRs) (Weber et al. 2004, 2007), suggesting that modulation of innate signaling could represent a principal MOA of GA. TLR engagement mainly triggers activation of either myeloid differentiation primary response gene 88 (MyD88) or Toll-IL-1 receptor domain–containing adaptor-inducing IFN-β (TRIF), which are important in CNS autoimmunity (Guo et al. 2008; Prinz et al. 2008; Prod'homme and Zamvil 2008). Signaling via TRIF leads to activation of IFN regulatory factor 3 (IRF3) transcription factor and subsequent production of IFN-β. Signaling through TRIF, IRF3, or the type I IFN-receptor (IFNAR) also influences development of Th17 cells and EAE (Guo et al. 2008; Prinz et al. 2008; Fitzgerald et al. 2014). Using various genetically modified mouse strains, as well as human monocytes, Molnarfi and colleagues (Molnarfi et al. 2015) showed that GA inhibited the TRIF-dependent pathway, resulting in a reduction of IFN-β production (Fig. 2). This observation is consistent with the earlier demonstration that STAT1 phosphorylation is reduced on acti-

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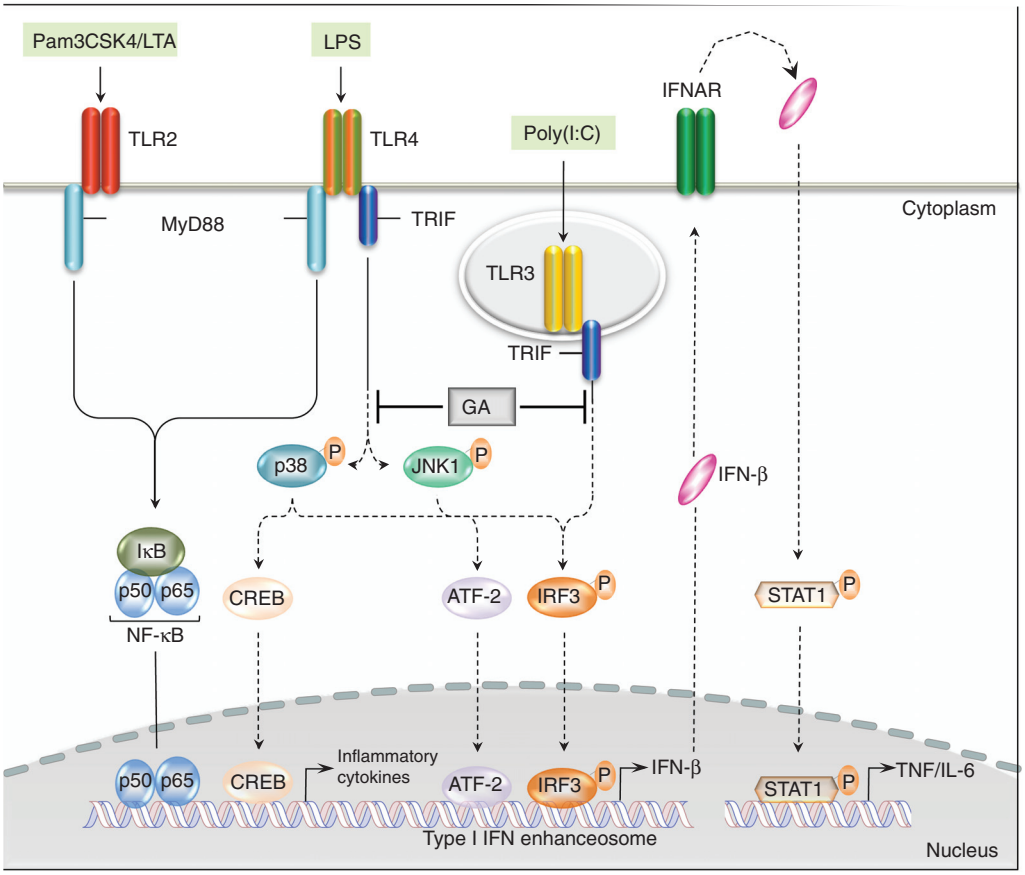

Figure 2. Glatiramer acetate (GA) treatment modulates type I interferon (IFN) production. GA treatment downregulates Toll-IL-1 receptor domain–containing adaptor-inducing IFN-β (TRIF) signaling on antigen-presenting cells (APCs), leading to decreased activation of IFN regulatory factor 3 (IRF3) and ATF-2, and subsequent DNA binding of the type I IFN enhanceosome (Molnarfi et al. 2015). Reduction of IFN-β production results in decreased signal transducers and activators of transcription (STAT)1 phosphorylation and activation of proinflammatory cytokines. LPS, Lipid peroxidation; IFNAR, interferon-receptor; TLR, Toll-like receptor; MyD88, myeloid differentiation primary response gene 88; TNF, tumor necrosis factor.

vation in type II monocytes (Weber et al. 2007). These findings provide a key anti-inflammatory mechanism connecting innate and adaptive immune modulation in GA therapy.

In addition to inducing M2 myeloid cells, GA can also modulate B cells and promote differentiation of regulatory B cells (Kala et al. 2010). B cells from GA-treated mice show an increased production of IL-10, reduced expression of costimulatory molecules, and diminished proliferation of MOG-specific T cells. GA treatment also inhibited B-cell release of proinflammatory cytokines, including IL-6, IL-12, and TNF-α (Begum-Haque et al. 2010). Although these results suggest that B cells may be important to the protective effects of GA in CNS autoimmunity, it is yet to be shown whether this effect is primarily achieved by an altered B-cell APC function, or through the modulation of the cytokine environment or T-cell activation.

The majority of patients treated with GA have been shown to develop GA-reactive antibodies, which, unlike antibodies against IFN-β, do not seem to interfere with the MOA of GA (Teitelbaum et al. 2003; Karussis et al. 2010; Sellebjerg et al. 2012). Moreover, relapse-free pa-

tients develop higher immunoglobulin (Ig)G titer (Brenner et al. 2001), and GA-specific antibodies in an animal model of CNS demyelinating disease were shown to promote myelin repair (Ure and Rodriguez 2002), suggesting a possible beneficial effect rather than a neutralizing one. Antibodies belong to the IgG class with a bias toward higher IgG1 titers than IgG2 (Brenner et al. 2001; Basile et al. 2006), followed by increased IgG4 titers over time, probably reflecting a switch toward a Th2 response (Farina et al. 2002).

Neuroprotective Effects

A growing body of evidence also suggests that GA may exert neuroprotective activities. In EAE, GA treatment has been associated with a reduction of axonal damage and degeneration, as well as with an increase of myelin repair (Gilgun-Sherki et al. 2003; Aharoni et al. 2008). Neuroprotective effects of GA were also supported by the increased axonal integrity observed in GA-treated patients, either by magnetic resonance imaging (MRI) with a reduction of severe lesions ("black holes") (Filippi et al. 2001) or brain proton magnetic resonance spectroscopy (1H-MRS) (Khan et al. 2008).

GA has been shown to be able to increase secretion of various neurotrophic factors, including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT)-3, NT-4, insulin-like growth factor (IGF)-1, and IGF-2. Neuroprotective effects of GA have been observed in various conditions, from neuroinflammation (EAE and MS) to neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) (Kipnis et al. 2000; Angelov et al. 2003; Benner et al. 2004; Aharoni et al. 2005c, 2007; Frenkel et al. 2005; Butovsky et al. 2006; Laurie et al. 2007; Ben-Zeev et al. 2011).

Increased expression of IGF and NT was observed in astrocytes and neurons in situ, suggesting a neuroprotective effect of GA in lesions (Aharoni et al. 2005a). Remyelination induced by GA has also been associated with increased proliferation, differentiation, and survival of oligodendrocyte precursor cells (OPCs), which was specifically related to elevated levels of both IGF-1 and BDNF (Skihar et al. 2009).

BDNF is expressed on most immune cells, as well as activated astrocytes, and is involved in the survival and differentiation of neurons and glial cells. BDNF was found to be up-regulated in both Th1 and Th2 GA-specific T-cell lines in vitro (Kipnis et al. 2000; Ziemssen et al. 2002; Chen et al. 2003) and has also been shown to modulate EAE severity (Linker et al. 2010). BDNF was up-regulated in the CNS of GA-treated mice, whether adoptively transferred with GA-specific T cells (Aharoni et al. 2003) or directly injected with GA (Aharoni et al. 2005a). In addition, induction of BDNF by GA is also observed in MS patients (Azoulay et al. 2005; Blanco et al. 2006; Sarchielli et al. 2007).

A phase III trial, enrolling 943 patients randomized with either GA or placebo for 36 months, addressed the influence of GA in primary progressive MS (PPMS) and did not show a clear clinical benefit in this population (Wolinsky et al. 2007). Furthermore, because it is a matter of debate whether PPMS is prominently caused by neurodegeneration (Steinman and Zamvil 2016), one can question whether the observed reduction in myelin injury occurs primarily from the release of BDNF or from the blockade of inflammatory pathways and restoration of the integrity of the blood–brain barrier, especially because both effects might occur simultaneously (Lalive et al. 2011).

CLINICAL ASPECTS

FDA approval of GA for RRMS was based upon results of the placebo-controlled trials, one of which was a multicenter phase III study that enrolled 251 patients. The study's primary end point was the mean relapse rate (RR), which was reduced by 29% in the GA-treated group compared with the placebo group (Johnson et al. 1995). The study was extended by up to 11 months in a double-blind fashion and showed a 32% reduction in RR in the treatment group and, conversely, an increase in the proportion of patients that were relapse free (Johnson et al. 1998). At the end of the extension study, 83% of the originally randomized patients were reen-

rolled in an open-label study to determine the effect of long-term treatment. At the 8-year time point, GA-treated patients showed clinical improvements compared to the placebo group. The annualized relapse rate (ARR) was 0.43 in the GA group versus 0.52 in the placebo group, and the proportion of patients with stable or improved expanded disability status scale (EDSS) was 65.3% in GA-treated patients versus 50.4% in the placebo group (Johnson et al. 2003, 2005). After 10 years, patients receiving GA experienced an increase of mean EDSS score of 0.5 (±1.65), an 80% decline in RR compared with placebo, and 91% of patients remained ambulatory without assistance (Ford et al. 2006). No toxicity was reported after up to 22 years of treatment, confirming the safety profile of GA.

A limitation of the pivotal trial was that it did not include sufficient monitoring by MRI. The effect of GA on MRI parameters was subsequently addressed in a European/Canadian trial, which consisted of two studies, each lasting 9 months. The first treatment phase, involving 239 RRMS subjects, was randomized, double-blind, and placebo controlled. Patients were injected daily with either 20 mg GA or placebo, and were followed with monthly brain MRIs (Comi et al. 2001). GA-treated patients showed a significant reduction in total gadolinium (Gd)-enhancing lesions compared with placebo, number and volume of new T2 lesions, brain atrophy progression, as well as clinical efficacy measured by reduction of mean RR. The short duration of the study prevented assessment of treatment effects on disability progression. A substudy of the European/Canadian Imaging Study, showed that, at 8 months, 15.6% in the GA group and 31.4% in the placebo group had evolved into permanent black holes, representing permanently damaged tissue (Filippi et al. 2001). Further analysis of the imaging data from this trial confirmed the reduction in accumulation of brain atrophy (Sormani et al. 2004).

In the second, open-label, phase of the European/Canadian study, patients were only administered with GA. The effect of treatment was sustained, with a 54% reduction in the mean number of Gd-enhancing lesions for those switching from placebo to GA and a further 24.6% reduction for those remaining on GA (Wolinsky et al. 2002). A long-term follow-up over a period of 5.8 years did not show significant differences for any MRI parameters between originally GA- or placebo-treated subjects, but showed an increased proportion of patients not requiring walking aids in the treated group, suggesting a favorable impact on long-term disease evolution (Rovaris et al. 2007).

GA treatment also reduces risk of conversion from CIS to clinically definite MS (CDMS). Here, GA was studied in the 36-month, placebo-controlled, randomized, double-blind, phase III (PreCISe) trial. Four hundred eighty-one patients with a monofocal CIS and two or more T_2 brain lesions (≥6 mm) were randomly assigned to either daily subcutaneous (SC) GA 20 mg or placebo. The primary end point was the time to conversion to CDMS, whereas secondary end points included measures of MS activity by MRI parameters. GA reduced both the risk of conversion to CDMS and the number of new T_2 lesions (Comi et al. 2009).

In summary, these three trials consistently showed the efficacy of GA in the treatment of patients with RRMS or CIS, showing an approximately 30% reduction in RR and benefits on MRI measures of disease activity.

Comparator Trials

GA, along with various IFN-βs, is considered first-line therapy in the treatment of MS. Three large, multicenter randomized clinical trials have been performed to compare the efficacy and safety of GA and IFN-β in RRMS patients (Mikol et al. 2008; Cadavid et al. 2009; O'Connor et al. 2009).

The REGARD study was a randomized, open-label trial comparing SC IFN-β1a (Rebif; EMD Millipore) three times a week to daily SC GA 20 mg in 764 RRMS patients. No differences between the two treatment groups were observed after 96 weeks, either in the time to relapse or the number or volume of active T_2 lesions. Whereas the IFN-β1a group presented lower Gd-enhancing lesions compared with

the GA group, GA-treated patients showed significantly less brain atrophy than the IFN-treated group (Mikol et al. 2008).

The BEYOND study involved 2244 RRMS patients, randomized to either 250 μg SC or 500 IFN-β1b (Betaseron) every other day or daily SC GA 20 mg over a minimum of 2 years. Results did not show statistically significant differences between groups in ARR, disability progression, and most MRI parameters (Gd-enhancing lesions, T_1 lesions, normalized brain volume) (O'Connor et al. 2009).

Finally, the BECOME study compared radiological efficacy of SC IFN-β1b 250 μg every other day with daily SC GA 20 mg, over a period of 2 years in 75 RRMS and CIS patients (Cadavid et al. 2009). Similar to the other studies, results did not show significant differences between groups in the number of combined active lesions per patient per scan at year 1.

Although the three large head-to-head trials were designed with the intent to show superiority of high-dose IFN, they ended up demonstrating that GA has comparable efficacy to high-dose IFN-βs in the treatment of RRMS, both from a clinical and radiological perspective. The only notable difference is that GA displayed a better protection against brain-volume loss, whereas IFN-βs showed fewer Gd-enhancing lesions.

Among all the other MS therapeutics, the efficacy of GA was only compared with Tecfidera (BG-12) in the phase III CONFIRM trial (Fox et al. 2012). This multicenter, placebo-controlled trial involved 1417 RRMS patients randomized either to BG-12 240 mg twice a day, BG-12 240 mg three times a day, GA 20 mg SC daily, or placebo, and treated for 2 years. Although the study was not powered to address the comparison between BG-12 and GA, both BG-12 doses showed similar or superior results compared with GA across all end points, although neither drug significantly reduced disability progression.

Combination Therapies

Considerable effort has been devoted to the identification of therapeutics that may provide additive or synergistic benefit when combined for treatment of MS (Stuve et al. 2006; Metz et al. 2009; Zamvil and Steinman 2011). Although mixed results were obtained when GA was tested in combination with type I IFNs in preclinical studies (Brod et al. 2000; Soos et al. 2002), GA and IFN-β1a were tested in the randomized, double-blind, placebo-controlled, multicenter, phase III CombiRx trial. The study, involving 1008 participants, showed that combining the two most commonly prescribed therapies for MS did not provide any added benefit in reducing disease progression, compared with either agent alone. In fact, GA alone or in combination with IFN-β, showed more efficacy than IFN-β in reducing the risk of relapse (Lublin et al. 2013). Interestingly, although the results of the CombiRx trial did not identify clinical benefit from the combination of GA and intramuscular IFN-β1a, they did not provide clear evidence of antagonism either. It is of interest that one mechanistic study observed GA-induced M2 monocyte polarization through down-regulation of IFN-β signaling and production (Molnarfi et al. 2015), raising the possibility that GA could antagonize IFN-β in some situations.

High-Dose GA and Different Regimens

GA was originally approved as 20 mg once-daily SC, but some data have suggested that higher doses were associated with greater efficacy (Teitelbaum et al. 1999). Efficacy, safety, and tolerability of 40 mg and 20 mg daily doses of SC GA were evaluated in a phase II and III clinical trials in RRMS patients (Cohen et al. 2007; Comi et al. 2011). Although results from the earlier phase II trial suggested that higher dose GA would be more effective (Cohen et al. 2007), the phase III trial (FORTE) found there was no gain in efficacy with higher daily GA, indicating a ceiling had been reached. Similar efficacy and safety profiles were observed for 40 mg and 20 mg daily doses. These findings raised the possibility that it might be possible to achieve similar benefit from less frequent administration of the higher dose.

The efficacy and safety of a three-times weekly 40 mg GA dosing was evaluated in the randomized, placebo-controlled, phase III "GALA"

trial (Khan et al. 2013), which compared that regimen with the approved daily SC 20 mg in treatment of RRMS. Thrice-weekly 40 mg dosing showed comparable results to the pivotal 20 mg trial with a significant (34%) reduction in ARR, which led to the approval of this treatment schedule of GA (Copaxone) by the FDA in 2014. A generic three-times weekly 40 GA version, manufactured by Mylan Pharmaceuticals, was later approved in 2017.

SAFETY PROFILE

GA is considered to have the most favorable adverse effect profile compared with the other MS therapies. In this regard, the safety profile of GA in RRMS has been confirmed by long-term studies for up to 22 years (Ford et al. 2010). The typical flu-like reaction characteristic of IFN-β does not occur with GA and, unlike IFNs, natalizumab, and fingolimod, GA does not cause liver function abnormalities or leukopenia, depression, or fatigue (Simone et al. 2006; Kieseier and Stuve 2011). The most common adverse effect, occurring in 80% of patients, is a local injection site reaction experienced as erythema and pruritus. Up to 10%–15% RRMS patients treated with 20 mg GA daily also experience a self-limited postinjection systemic reaction, characterized by chest tightness, shortness of breath, and palpitations. These reactions are unpredictable and can be mistaken for cardiac ischemia, but are not considered dangerous and only require proper patient education and reassurance from the clinician. The frequency of these immediate postinjection reactions (IPIRs) is lower when using 40 mg three times weekly, in part, because of the lower frequency of administration (Khan et al. 2013).

According to FDA classification of fetal risk as a result of pharmaceuticals, GA is a category B drug in the United States, defined as "no controlled human studies are available but animal studies show either no risk or minimal risk to the fetus" and should be used during pregnancy only if clearly needed. Although there are no well-controlled studies in pregnant women, administration of GA to pregnant rats and rabbits did not result in adverse effects on offspring development. There is no apparent impact of paternal exposure to GA on birth outcome or child health. Overall, GA is considered to be safe, even in early pregnancy, although most clinicians will stop its use during pregnancy.

CONCLUDING REMARKS

Over the last two decades, significant progress has been made both in elucidating the MOA of GA and establishing the long-term clinical benefit to patients. GA has well-characterized immunomodulatory properties, promoting expansion of anti-inflammatory and regulatory Th2 and Treg cells. Animal studies have shown that GA-reactive Th2 cells migrate to the CNS and accumulate at the site of active lesions. Thus, GA-reactive T cells provide the effector arm in treatment. However, more recent evidence indicates that APCs are the initial target of GA, and it is the modulation of the APC compartment to anti-inflammatory (M2) phenotype that is responsible for both expansion of regulatory Th2 and Treg cells. Although challenged by the development of various emerging therapies, due to both the excellent long-term safety profile of GA and its comparable clinical benefit to other first-line therapies, it is anticipated that GA will continue to be used in MS therapy in the future.

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