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Glatiramer Acetate (Copolymer-1, Copaxone) Promotes Th2 Cell Development and Increased IL-10 Production Through Modulation of Dendritic Cells

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Glatiramer acetate (GA; copolymer-1, Copaxone) suppresses the induction of experimental autoimmune encephalomyelitis and reduces the relapse frequency in relapsing-remitting multiple sclerosis. Although it has become clear that GA induces protective degenerate Th2/IL-10 responses, its precise mode of action remains elusive. Because the cytokine profile of Th cells is often regulated by dendritic cells (DC), we studied the modulatory effects of GA on the T cell regulatory function of human DC. This study shows the novel selective inhibitory effect of GA on the production of DC-derived inflammatory mediators without affecting DC maturation or DC immunostimulatory potential. DC exposed to GA have an impaired capacity to secrete the major Th1 polarizing factor IL-12p70 in response to LPS and CD40 ligand triggering. DC exposed to GA induce effector IL-4-secreting Th2 cells and enhanced levels of the anti-inflammatory cytokine IL-10. The anti-inflammatory effect of GA is mediated via DC as GA does not affect the polarization patterns of naive Th cells activated in an APC-free system. Together, these results reveal that APC are essential for the GA-mediated shift in the Th cell profiles and indicate that DC are a prime target for the immunomodulatory effects of GA. The Journal of Immunology, 2003, 170: 4483–4488.

Dendritic cells (DC)† make up a family of highly specialized cells that upon activation by pathogens or their products mature into professional APC. Immature sentinel DC sample peripheral tissues (e.g., skin, lung, and gut epithelia) for incoming pathogen-derived Ags. Thus activated, they mature and migrate through the lymphatics toward secondary lymphoid organs. During maturation, DC lose their capacity to internalize and process Ags, but up-regulate their costimulatory molecules (e.g., CD80, CD86, and CD40) to become potent immunogenic APC for naive CD4+ Th cells (1). In the T cell areas of lymphoid organs, mature effector DC not only present pathogen-derived peptides to T cells, but also adapt the class of immune response to the type of invading pathogen by driving the development of protective effector Th cell subsets. For instance, DC that have been activated by intracellular pathogens or their compounds, commit CD4+ Th cells to become protective IFN-γ-producing effector Th1 cells. Alternatively, helminths or certain extracellular pathogens induce DC that drive the development of protective IL-4-, IL-5-, and IL-13-producing effector Th2 cells. Whereas chronic activation of Th1 cells can cause immunopathology and organ-specific autoimmune disease, Th2 cells can mediate allergic and atopic disease (2–5).

An important DC-derived factor driving Th1 responses is bioactive IL-12p70 (2, 3). IL-12p70 can be secreted by sentinel DC in response to several pathogens (bacteria, viruses) or to pathogen-derived products (LPS, DNA, dsRNA) (6–10). However, during the priming of naive Th cells in the lymphoid organs, the secretion of IL-12p70 is induced upon interaction between CD40 on the DC and the rapidly induced CD40 ligand (CD40L, CD154) on the activated Th cell (6, 9, 11, 12). The levels of IL-12p70 production upon this CD40L-dependent activation are subject to regulation, reflecting the type of pathogen that activated the DC at its sentinel stage in the peripheral tissue concerned (4, 5). We and others have shown that in addition to the type of pathogen, immune mediators and drugs may also determine the levels of IL-12 produced by CD40L-activated mature DC. For instance, the immune mediators PGE2, histamine, and IL-10; and the anti-inflammatory drugs, glucocorticoids, and β2-agonists all prime DC for reduced IL-12p70 production upon CD40L-dependent activation, thus resulting in DC with enhanced Th2-promoting capacity (13–19). This has important implications for the design of therapeutic strategies aimed at counteracting Th1-associated immune pathologies.

Glatiramer acetate (GA), also known as copolymer-1, Cop-1, and Copaxone, is a synthetic random polymer of the amino acids l-alanine, l-glutamate, l-lysine, and l-tyrosine (20). GA was shown to suppress the induction of experimental autoimmune encephalomyelitis (EAE) in response to the encephalitogenic Ags myelin basic protein (MBP), proteolipid protein (21), and myelin oligodendrocyte glycoprotein (20–22). More recently, GA was also shown to inhibit type II collagen-reactive T cells in vitro (23) and to prevent graft vs host disease (24, 25) and transplant rejection (26). The potential of GA as a therapeutic agent in multiple sclerosis (MS) has been further substantiated by the reduction of relapse frequency in relapsing-remitting MS patients and by the reduced appearance of new lesions in gadolinium-enhanced magnetic resonance imaging (27–30).

It is generally accepted that the therapeutic effects of GA are due to its immunomodulatory effects on T cells (for a review, see Ref.
31). GA has been shown to bind promiscuously to class II MHC molecules in vitro (32–34), thus competing with MBP-derived peptides for the MBP-specific TCR (35–37). Interestingly, GA induces a Th1 to Th2 shift accompanied by the production of the anti-inflammatory cytokine IL-10 both in vitro and in vivo (34, 38–44). Hitherto, the actual mechanism underlying the induction of these protective Th cell responses remains elusive. Because the cytokine profile of Th cells is often regulated by DC, we have studied the modulatory effects of GA on the T cell regulatory function of human DC.

In this study we show that GA exerts its anti-inflammatory action on DC by potently inhibiting the production of the major Th1-polarizing factor IL-12p70 and of the proinflammatory cytokines TNF-α and IL-8 in response to inflammatory signals. Moreover, although GA does not phenotypically affect the maturation of DC, exposure of DC to GA during their maturation results in a stably reduced IL-12p70-producing capacity in response to subsequent activation by the T cell-derived signal CD40L. This results in the polarization of precursor naive Th cells into effector IL-4-producing Th2 cells and is accompanied by enhanced secretion of the anti-inflammatory cytokine IL-10.

Materials and Methods

Reagents

Human rGM-CSF (sp. act. 1.11 × 10⁴ U/mg) was a gift of Schering-Plough (Uden, The Netherlands). Human rIFN-γ (sp. act. 8 × 10⁴ U/mg) was a gift of Dr. P. H. van der Meide (UCyTech, Utrecht, The Netherlands). Human rIL-2 was obtained from Cetus (Emeryville, CA). Human rIL-4 (sp. act. 1 × 10⁵ U/mg) was obtained from Pharmacia Biotechnology Hannover (Hannover, Germany). Escherichia coli-derived LPS was obtained from Difco (Detroit, MI) and was used at an optimal concentration of 100 ng/ml. Superantigen Staphylococcus aureus enterotoxin B (SEB) was obtained from Sigma-Aldrich (St. Louis, MO) and was used at a final concentration of either 10 or 100 pg/ml as indicated in Figs. 3 and 4. Water-soluble GA (COP-1; Sigma-Aldrich) was used at the final concentrations of 1, 3, and 10 μg/ml based on pilot experiments. Mouse anti-human CD3 mAb (CLB-T3/3) and mouse anti-human CD28 mAb (CLB-CD80/1) were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands) and were used at the final concentrations of 1 and 2 μg/ml, respectively, unless indicated otherwise (Fig. 5).

Generation of immature DC from peripheral blood monocytes, induction of DC cytokine production, and induction of DC maturation

Immature CD11c⁺CD14⁻ DC were generated from peripheral blood monocytes cultured in IMDM (Life Technologies, Paisley, U.K.) containing gentamicin (86 μg/ml; Duchaef, Haarlem, The Netherlands), 1% FCS (HyClone Laboratories, Logan, UT), GM-CSF (500 U/ml), and IL-4 (250 U/ml) (6). At day 6, immature DC (2 × 10⁵ cells/well) were stimulated with LPS in either the absence or the presence of IFN-γ (10 ng/ml) in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in IMDM containing 10% FCS in a final volume of 200 μl. GA (1, 3, or 10 μg/ml) was added either alone or simultaneously with LPS ± IFN-γ. Supernatants were harvested after 24 h. Alternatively, at day 6, immature DC were induced to mature on a 2-day exposure to LPS (100 ng/ml) in the absence or in the additional presence of GA (1, 3, or 10 μg/ml). All subsequent tests were performed after harvesting the cells at day 8 and after extensive washing (4 times in 10 ml of culture medium) to remove residual factors. Mature DC (2 × 10⁶ cells/well) were stimulated with the CD40L-transfected J558 cell line (2 × 10⁵ cells; a gift of Dr. P. Lane, University of Birmingham, U.K.) in 96-well flat-bottom culture plates (Costar) in IMDM containing 10% FCS in a final volume of 200 μl. Supernatants were harvested after 24 h. The levels of IL-12p70, IL-6, IL-8, IL-10, and TNF-α were measured by ELISA (see below).

Isolation of naive CD4⁺CD45RA⁺CD45RO⁻ Th cells

Naïve CD4⁺CD45RA⁺CD45RO⁻ Th cells were isolated from peripheral blood leucocytes, with the negative selection human CD4⁺CD45RO⁻ columns kit (R&D Systems, Minneapolis, MN). This method yielded highly purified naïve Th cells as assessed by flow cytometry (>98% CD4⁺CD45RA⁺CD45RO⁻, <1% CD14⁺, <1% CD20⁺, data not shown).

APC-dependent and -independent priming of cytokine production in maturing Th cells

Naïve Th cells (2 × 10⁵ cells/well) were cocultured in 96-well flat-bottom culture plates (Costar) in IMDM containing 10% FCS with allogeneic mature DC (5 × 10⁵ cells) in the presence of SEB (10 pg/ml or 100 pg/ml) in a final volume of 200 μl. Before the coculture, DC were induced to mature by exposure to LPS in either the absence or the presence of GA (1, 3, or 10 μg/ml). Alternatively, naïve Th cells (1 × 10⁵ cells/200 μl) were stimulated in the absence of DC with soluble anti-CD3 and anti-CD28 mAbs in the absence or the presence of GA (1, 3, or 10 μg/ml) in 96-well flat-bottom culture plates in IMDM containing 10% FCS. Both the APC-dependent and in the APC-independent activation protocols, IL-2 (10 U/ml) was added on day 5, and the cultures were further expanded for another 9 days. On day 14, resting memory Th cells were harvested, washed, and restimulated with PMA (Sigma-Aldrich) and ionomycin (Sigma-Aldrich) in the presence of brefeldin A (Sigma-Aldrich) to detect the intracellular production of IL-4 and IFN-γ. Alternatively, at day 14 resting Th cells were restimulated with soluble anti-CD3 and anti-CD28 mAbs (13), and the concentrations of IL-10 in 72-h supernatants were determined by ELISA (see below).

Induction and measurement of proliferative response in naïve Th cells

Naïve Th cells (2.5 × 10⁵ cells/200 μl) were cocultured in 96-well flat-bottom culture plates (Costar) with increasing numbers of allogeneic DC (10⁻¹–10⁰) or with autologous DC in the additional presence of 100 pg/ml SEB. Cell proliferation was determined by incorporation of [³H]ThdR (Radiochemical Center, Amersham Pharmacia, Little Chalfont, U.K.) by liquid scintillation spectroscopy after a pulse with 13 KBq/well during the last 16 h of a 3-day (SEB) or 7-day (MLR) culture.

Evaluation of cytokine production at the single-cell level

To evaluate intracellular cytokine expression, day 14 resting Th cells were harvested, washed, and restimulated with PMA (10 ng/ml) and ionomycin (1 μg/ml) for 6 h in the additional presence of brefeldin A (10 μg/ml). Thereafter, the cells were washed in PBS, fixed for 15 min at room temperature in PBS containing 2% paraformaldehyde, and stained in permeabilization buffer (PBS containing 0.5% saponin, 1% BSA, and 0.05% NaN₃) with FITC-labeled mouse anti-human IFN-γ (IgG2b) and PE-labeled mouse anti-human IL-4 (IgG1) or the respective isotype-matched controls from BD Pharmingen, San Diego, CA. Subsequently, the cells were washed, suspended in PBS containing 1% BSA and 0.05% NaN₃, and analyzed by flow cytometry. Data were analyzed using WinMDI software (http://facs.scripps.edu/).

Evaluation of cytokine production by ELISA

Determination of IL-12p70 concentrations in culture supernatants was performed by specific solid-phase sandwich ELISA as described previously (6). Pairs of specific mAbs and recombinant cytokine standards were obtained from BioSource International (Camarillo, CA) for the determination of IL-6, IL-8, and TNF-α, and from BD Pharmingen for the determination of IL-10. The detection limits of these ELISAs are as follows: IL-6, 20 pg/ml; IL-8, 30 pg/ml; IL-10, 25 pg/ml; IL-12p70, 3 pg/ml; and TNF-α, 20 pg/ml.

Statistical analysis

Data were analyzed for statistical significance with the GraphPad InStat software (version 3.00; GraphPad, San Diego, CA) using ANOVA followed by Dunnett’s multiple comparisons test. A p value of <0.05 was considered to be the level of significance.

Results

GA is a selective inhibitor of cytokine production by tissue-type sentinel DC

Because GA is therapeutically applied s.c., we first addressed the question of whether GA influences the sentinel function of tissue-type DC. To this end, immature DC were activated by exposure to LPS either in the absence or in the presence of increasing doses of GA. Fig. 1 depicts the regulatory effect of GA on the production of several DC-derived inflammatory mediators. The secretion of the

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Th1-polarizing cytokine IL-12p70 was clearly inhibited by the presence of GA in a dose-dependent manner. Similarly, although to a lesser extent, GA reduced the secretion of the chemoattractant IL-8 and of the proinflammatory cytokine TNF-α. In contrast, IL-6 secretion was hardly affected in the range of GA doses tested, suggesting a selective role for GA in the regulation of DC cytokine production. The production of IL-10 by the DC was not detectable in any condition. In the absence of LPS, GA by itself did not induce DC cytokine production (data not shown).

**Effect of GA on cytokine production by immature DC**

We and others have shown previously that certain immune mediators and anti-inflammatory drugs that inhibit IL-12p70 production in sentinel DC also prime maturing DC for a stable effector phenotype with a reduced capacity to secrete IL-12p70 in response to subsequent CD40L-dependent activation (13–19). To study whether GA exerts a similar effect, DC were matured by exposure to LPS during 48 h in the absence or the presence of increasing doses of GA. Subsequently, DC were thoroughly washed to remove residual factors, and stimulated with CD40L-transfected J558 cells. Fig. 2 shows that maturation of DC in the presence of GA resulted in a dose-dependent reduction of their capacity to secrete IL-12p70 in response to subsequent activation by CD40L. A comparable effect was observed for TNF-α, whereas IL-8 secretion was inhibited to a much lesser extent. In contrast, IL-6 production was hardly affected, again suggesting that GA selectively regulates DC function. The production of IL-10 by the DC was not detectable in any condition. Similar results were obtained when DC maturation was induced by the combination of IL-1β and TNF-α instead of LPS (data not shown), suggesting that the effect of GA does not critically depend on microenvironmental conditions.

Maturation-associated phenotypical changes are characterized by the up-regulation of class II MHC, the costimulatory molecules CD40, CD80, CD86, OX40L, ICAM-1, and the acquisition of the mature DC marker CD83. DC matured with LPS (or with IL-1β/ TNF-α) acquired these mature phenotype markers irrespective of the presence of GA (data not shown). Mature DC are the exclusive effector APC for lymph node-based naïve Th cells and consequently for the initiation of specific immune responses (1). Therefore, we addressed the question of whether exposure of maturing DC to GA affects their immunostimulatory capacity toward naïve Th cells, by testing the capacity of GA-treated DC to induce proliferation of either allogeneic naïve Th cells or autologous naïve Th cells in response to SEB. Fig. 3 shows that exposure of DC to GA during maturation did not impair their immunostimulatory potential as judged by the similar proliferation rates of naïve Th cells in response to DC exposed to increasing doses of GA both in the MLR and in the SEB models. These results are in line with the unaffected acquisition of the mature phenotype. Together, these data suggest that in the presence of GA, DC become genuine effector APC with a stably reduced capacity to produce IL-12p70.

![FIGURE 1](#) GA is a selective inhibitor of cytokine production by immature DC. Day 6 immature DC were stimulated with LPS in the absence or the presence of the indicated concentrations of GA. The concentrations of IL-12p70 (induced in the presence of IFN-γ), IL-6, IL-8, and TNF-α in 20-h supernatants were evaluated by ELISA. Results, expressed as the mean ± SD of triplicate cultures, are from one experiment representative of six. Data were analyzed for statistical significance using ANOVA followed by Dunnett’s multiple comparisons test. *, p < 0.05; #, p < 0.01.

![FIGURE 2](#) DC exposed to GA during their maturation have a reduced IL-12p70-producing capacity upon subsequent CD40L-dependent activation. Day 6 immature DC were induced to mature by a 2-day exposure to LPS in the absence or the presence of the indicated concentrations of GA. After 48 h, at day 8, matured DC were washed thoroughly to remove residual factors. DC were then stimulated with the CD40L-transfected J558 cell line, and 20-h supernatants were collected for cytokine measurement by ELISA. Results, expressed as the mean cytokine concentration ± SD of triplicate cultures, are from 1 experiment representative of 10. Data were analyzed for statistical significance using ANOVA followed by Dunnett’s multiple comparisons test. *, p < 0.05; #, p < 0.01.

![FIGURE 3](#) DC exposed to GA are not impaired in their capacity to activate naïve Th cell proliferation. DC were matured with LPS in the absence (○) or the presence of increasing doses of GA (1 μg/ml, ●; 3 μg/ml, ■; or 10 μg/ml, ▲). After extensive washing, increasing numbers of DC were used to stimulate either 2.5 × 10^4 allogeneic naïve Th cells (MLR) or 2.5 × 10^4 autologous naïve Th cells in the presence of 100 pg/ml SEB. The proliferative response was determined either at day 3 (MLR) or day 7 (MLR) of coculture by [3 H]TdR incorporation. Results, expressed as the mean cpm ± SD of triplicate cultures, are from one experiment representative of four.
DC matured in the presence of GA induce Th2 cells accompanied by high levels of IL-10

The levels of DC-derived IL-12p70 play a major role in Th1 polarization (2, 3). Hence, we studied to what extent the exposure of DC to GA hampers the early commitment of naive Th cells to become Th1 cells. To this aim, effecter DC were generated by maturation with LPS in the absence or the presence of increasing doses of GA. After 48 h the cells were thoroughly washed and used to stimulate allogeneic naive Th cells or autologous naive Th cells in the presence of SEB. The polarization of effector Th cells was evaluated by determining the production ratios of signature Th1 (IFN-γ) to Th2 (IL-4) cytokines at the single-cell level. Fig. 4A shows that DC that were matured in the presence of increasing doses of GA promoted the development of IL-4-producing effector Th cells and inhibited the generation of IFN-γ-producing Th cells. The dose-dependent shift in the ratio of IFN-γ-producing cells to IL-4-producing cells positively correlated with the dose-dependent inhibitory effect of GA on the IL-12p70-producing capacity of DC and indicates that GA modulates DC-derived IL-12p70 to control the polarization profiles of inflammatory Th cells. We subsequently evaluated whether this Th2 shift is accompanied by the induction of IL-10-producing Th cells. Fig. 4B shows that exposure of DC to GA during maturation resulted in the generation of Th cells with a strongly enhanced capacity to secrete the anti-inflammatory cytokine IL-10. Together, these results indicate that GA modulates the APC function of DC to induce anti-inflammatory Th cells producing IL-4 and/or IL-10.

To further investigate to what extent this anti-inflammatory effect of GA depends on the presence of APC, naive Th cells were activated in an APC-free system with soluble mAbs directed against CD3 and CD28 in the absence or the presence of increasing doses of GA. The obtained resting effector Th cells were restimulated and evaluated for their production of IFN-γ and IL-4 at the single cell level by flow cytometry. Fig. 5 shows that in the absence of APC, GA was unable to affect the balance between Th1 and Th2 cells, indicating that DC are critical for the GA-mediated shift in the Th cell profile.

**Discussion**

The present study shows for the first time that GA induces anti-inflammatory Th cell responses by modulating the APC function of DC and not by direct effects on the Th cells. GA selectively inhibits the production of DC-derived inflammatory mediators without affecting DC maturation and the DC immunostimulatory potential. DC exposed to GA during maturation have an impaired and stable capacity to secrete the major Th1-polarizing cytokine IL-10.

It is generally accepted that GA exerts its effects by modulating T cell function (reviewed in Ref. 31). With respect to the binding of GA to immune cells, in vitro experiments have shown that GA binds promiscuously to class II MHC (32–34), thereby competing with MBP-derived peptides for the MBP-specific TCR (35–37). This is an implausible setting in vivo. GA is rapidly degraded into small peptides (45), and it is unlikely that 20 mg of GA administered s.c. would be capable of exerting TCR antagonism by displacing relevant auto-Ags in both the CNS and the immune system (46). GA is well tolerated, and s.c. application seldom results in
skin reactions (28). However, there are indications of immune activation in vivo that suggest the active participation of APC in mediating the effects of GA. A recent study reported lymphadenopathy in 30% of MS patients following GA administration (47). Moreover, a recent report supports our observations that the anti-inflammatory effects of GA are mediated via the APC. Wiesemann et al. (48) showed that GA induces IL-5 and IL-13 production in naive Th cells only if the cells are activated in the presence of CD14+ cells. This effect is dependent on Ag presentation as demonstrated by TCR blocking Abs. It is noteworthy that GA induces a Th1 to Th2 shift accompanied by the production of the anti-inflammatory cytokine IL-10 both in vitro and in vivo (34, 38–44).

It is well established that the cytokine profile of Th cells is often regulated by DC. Hitherto, there is no information regarding the effects of GA on the effector function of DC. Very recently, Husser et al. (49) reported that the presence of GA during the in vitro generation of immature DC from human peripheral blood monocytes inhibited the capacity of these immature DC to produce biologically inactive IL-12p40. This study left the question whether GA could directly modulate the production of bioactive IL-12p70 of immature DC or could modulate the maturation of these DC and consequently their capacity to induce Th1 responses unanswered. The data reported in this study show for the first time that DC are critically involved in the mechanisms underlying the immunomodulatory effects of GA on Th cells. In addition to the direct inhibitory effect of GA on the secretion of inflammatory cytokines, in particular IL-12p70 production by immature DC in response to LPS, GA primes maturing DC for deficient IL-12p70 production in response to subsequent CD40 triggering. The levels of IL-12p70 secreted by the DC are of key importance in determining the class of the primary immune response (2, 3). By inhibiting the IL-12p70-producing capacity of DC, GA suppresses the development of Th1-associated profiles. The immunostimulatory potential of DC primed by GA is not compromised, probably because GA does not affect their maturation status. In this study we demonstrate that the Th cells that develop in response to the GA-primed DC secrete enhanced levels of IL-10. It is well established that at low IL-12p70 levels, autocrine IL-4 production boosts the development of Th2 cells (2, 3). In contrast, the nature of the signal(s) driving the induction of high-level IL-10-secreting cells in low IL-12p70 conditions is unknown. In experiments using supernatants of activated DC we observed that GA-modulated DC induce Th cell polarization via as yet unknown soluble factor(s) (data not shown). Moreover, in experiments using neutralizing anti-IL-10 mAb during the activation of naive Th cells by these DC, the acquisition of the high-level IL-10-producing capacity was not prevented (data not shown). The putative role for other candidate molecules responsible for the induction of high-level IL-10-producing T cells, such as the glucocorticoid-induced TNFR family-related protein and its ligand (50), needs further investigation.

The actual molecular mechanisms by which GA affects the production of immune mediators by DC and consequently directs the generation of anti-inflammatory Th cells remains elusive. Recently, it was described that GA inhibits the IL-1-dependent activation of NF-kB in astroglial cells (51). Indeed, the NF-kB family of transcription factors is involved in the regulation of activation of DC and of cytokine production in myelomonocytic cells (1, 52, 53). In this respect, NF-kB family member RelA and IxB (53) may be targets for the GA regulatory effect. We have preliminary evidence suggesting that activation of DC in the presence of GA (1–10 µg/ml) results in reduced translocation of RelA to the nucleus with retention of RelA in the cytoplasmic compartment (our unpublished results). Whether this effect of GA on RelA is mediated through IxB is not yet clear. These issues are currently under investigation.

We have shown in this study that DC are pivotal in the transmission of protective Th2/IL-10 responses, a mechanism that has not been described before to explain the anti-inflammatory effects of GA. GA-primed DC will drive the development of anti-inflammatory effector Th cells from naive T cells responding to any Ag presented by these DC. The notion that this effect of GA on DC is Ag-nonspecific may explain that GA is not only effective in EAE (34, 38, 39) but also in graft vs host disease (25) and transplant rejection (26) models as well as in MS patients (40–44). At the same time, however, this finding does not exclude the fact that this polypeptide has additional Ag-specific effects. In the specific case of EAE and MS, GA-primed DC may activate GA-specific Th cells that will further compete with, e.g., MBP-specific Th cells for binding to MBP peptides presented in the context of class II MHC. In contrast, APC loaded with GA may also compete with APC loaded with MBP peptides for MBP-specific TCR, and may help to shift the cytokine profiles of these inflammatory MBP-specific T cells.

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References

13. Kalinski, P., J. H. Schuitemaker, C. M. Hilken, and M. L. Kapsenberg. 1998. Prostaglandin E 2 induces the maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. J. Immunol. 161:2004.
adherent cell culture treated with IL-10 prime naive CD4+ T cells to secrete IL-4.


