Multiple sclerosis: glatiramer acetate inhibits monocyte reactivity in vitro and in vivo

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Summary
It is widely assumed that glatiramer acetate (GA), an approved agent for the immunomodulatory treatment of multiple sclerosis, acts primarily as an antigen for T lymphocytes. Recent studies, however, indicated that in vitro, GA directly inhibits dendritic cells, a rare but potent type of professional antigen-presenting cell (APC). To investigate whether these in vitro observations are relevant to the actions of GA in vivo, we studied the effects of GA on monocytes, the major type of circulating APC. In a first series of experiments, we investigated the effects of GA on monocyte reactivity in vitro. Monocytes were stimulated with ligands for Toll-like receptor (TLR)-2 (peptidoglycan and lipoteichoic acid), TLR-4 [lipopolysaccharide (LPS)] and TLR-5 (flagellin), as well as two proinflammatory cytokines (interferon-γ and granulocyte–monocyte colony-stimulating factor). Monocyte activation was measured by induction of the surface markers signalling lymphocytic activation molecule (SLAM), CD25 and CD69 (detected by cyt fluorometry), and by production of monocyte-derived tumour necrosis factor (TNF)-α (detected by enzyme-linked immunospot assay). GA had a broad inhibitory effect on all measures of monocyte reactivity, regardless of which stimulator was used. It is unlikely that this reflects a simple toxic effect, because monocyte viability and CD14 expression were unaffected. In a second series of experiments, we investigated the properties of monocytes cultured ex vivo from eight GA-treated multiple sclerosis patients, eight untreated multiple sclerosis patients and eight healthy subjects. We found that LPS-induced SLAM expression and TNF-α production were significantly reduced in monocytes from GA-treated patients compared with controls. These results demonstrate for the first time that GA inhibits monocyte reactivity in vitro and in vivo, significantly extending the current concept of the mechanism of action of GA.

Keywords: multiple sclerosis; glatiramer acetate; immunotherapy; monocyte; ex vivo assay

Abbreviations: APC = antigen-presenting cell; DC = dendritic cell; EDSS = Expanded Disability Status Scale; Elispot = enzyme-linked immunospot; FACS = fluorescence activated cell sorting; GM-CSF = granulocyte–monocyte colony-stimulating factor; GA = glatiramer acetate; IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; LTA = lipoteichoic acid; PBMC = peripheral blood mononuclear cell; PGN = peptidoglycan; SLAM = signalling lymphocytic activation molecule; TLR = Toll-like receptor; TNF = tumour necrosis factor


Introduction
Glatiramer acetate (GA, Copaxone), a random copolymer composed of alanine, glutamic acid, lysine and tyrosine, is an approved agent for the immunomodulatory treatment of multiple sclerosis. Clinical and magnetic imaging studies indicated that GA treatment reduces the activity of multiple sclerosis lesions (Johnson et al., 1995, 2000; Mancardi et al., 1998; Ge et al., 2000; Comi et al., 2001; Filippi et al., 2001; Wolinsky et al., 2001; Ziemssen et al., 2001). Over recent years, a number of studies have addressed the immunological basis of the clinical effects of GA in animal models and...
kinase receptor gp145 trkB in multiple sclerosis lesions

derived neurotrophic factor (BDNF) to neurons, which

1998). In addition, the GA-reactive T cells can deliver brain-

in vitro

properties of APCs

cells. Indeed, there is recent evidence that GA might affect

properties in such a way that they preferentially induce TH2

possibilities. First, GA might have a primary effect on T

process is called ‘bystander suppression’ (Aharoni et al.,

1998). In addition, the GA-reactive T cells can deliver brain-
derived neurotrophic factor (BDNF) to neurons, which
upregulate the corresponding full-length signalling tyrosine

kinase receptor gp145 trkB in multiple sclerosis lesions

(Ziemssen et al., 2002). In this way, the recruited TH2 T cells

can suppress neighbouring autoaggressive TH1 cells. This

process is called ‘bystander suppression’ (Aharoni et al.,

1998). The mechanism(s) of the therapeutically induced TH1 to

TH2 cytokine shift of GA-reactive T cells is unknown.

Theoretically, there are two (not necessarily exclusive)

possibilities. First, GA might have a primary effect on T

cells, for example by virtue of its properties as an ‘altered

pæptide ligand’, or by the special conditions of GA present-

ing in the skin. Secondly, GA might exert a primary effect

on antigen-presenting cells (APCs), e.g. by altering their

properties in such a way that they preferentially induce TH2

cells. Indeed, there is recent evidence that GA might affect

the properties of APCs in vitro (Hussien et al., 2001; Vieira

et al., 2003). In this regard, the findings reported by Vieira

et al. (2003) are especially intriguing. Using in vitro cultures,

these authors found that GA affects the T-cell-stimulating

properties of dendritic cells (DCs). After in vitro treatment

with GA, DCs have an impaired capacity to secrete TH1-
polarizing factors, and therefore preferentially induce TH2

cells (Vieira et al., 2003). Whether these observations extend
to other types of APC, and whether they are relevant in vivo

presently is unknown.

The main aim of our present study was to search for
evidence that GA affects the function of APCs in vivo. We
focused our analysis on monocytes, because DCs constitute a
very rare subset of APCs which can only be studied after
prolonged expansion in culture, and which are therefore
inaccessible for direct ex vivo analysis. In contrast, mono-
cytes represent a major subset of professional APCs which

can be easily obtained from peripheral blood and are readily

accessible for ex vivo investigations.

In a first step of analysis, we performed a detailed series of
experiments to identify the possible effects of GA on different
patterns of monocyte activation in vitro. For monocyte
activation, we used (i) four ligands for three distinct Toll-like

receptors (TLRs); and (ii) the proinflammatory cytokines

IFN-γ and granulocyte–monocyte colony-stimulating factor

(GM-CSF). As markers of monocyte activation, we looked at

(i) the expression of activation-related surface molecules

including signalling lymphocytic activation molecule

(SLAM) (CD150), CD25 and CD69; and (ii) production of

tumour necrosis factor (TNF-α). These experiments revealed

that GA broadly affects monocyte activation by different

ligands and pathways.

In the second step, we addressed the possible effects of GA

on monocytes in vivo. To this end, we compared the

properties of ex vivo monocytes from untreated and GA-
treated subjects. ‘Ex vivo’ means that the monocytes were

exposed to GA only in vivo, but not in vitro. Based on the

experiments from the first step, we chose lipopolysaccharide

(LPS)-induced SLAM expression and TNF-α secretion as

‘read-out’ to test the monocyte stimulation thresholds in vitro.

Our main observation is that monocytes cultured ex vivo from

GA-treated patients were indeed significantly less susceptible
to activation than monocytes from untreated patients and

normal controls. These results demonstrate, we believe for
the first time, that GA treatment in vivo leads to a systemic
alteration of the properties of circulating monocytes, raising
important new questions regarding the mechanism of action

of GA.

Materials and methods

Subjects and cell samples

Blood was drawn from healthy individuals, GA-treated multiple
sclerosis patients and untreated multiple sclerosis patients after
their informed consent. This study has been approved by the local ethics
committee of the Ludwig Maximilians University of Munich. All
patients had definite multiple sclerosis (McDonald et al., 2001). All
GA-treated patients (n = 8) had a relapsing–remitting disease course.
At the time of sampling, they had injected 20 mg of GA
subcutaneously (s.c.) daily for at least 1 year, with a mean treatment
duration of 34.4 ± 16.9 months [mean Expanded Disability Status
Scale (EDSS) at time of sampling 1.75 ± 1.2; mean age 31.8 ± 8.4
years] (Table 1). The untreated group included four patients with
relapsing–remitting multiple sclerosis, two with primary progressive
multiple sclerosis and two with secondary progressive multiple
sclerosis (five women and three men; mean EDSS 3.5 ± 1.9; mean age
47.1 ± 10.5 years). None of these patients was treated with
immunosuppressive or immunomodulatory therapy during at least 3
months preceding the study. The group of healthy donors included
four men and four women with a mean age of 35.1 ± 11.9 years.

Peripheral blood mononuclear cells (PBMCs) were isolated on a
discontinuous density gradient (Lymphoprep, Nycomed, Oslo,
Norway). Viable cells were counted by trypan blue (Sigma-
Aldrich) exclusion and resuspended in culture medium [RPMI
1640 supplemented with 5% fetal calf serum (FCS), 1% glutamine and 1% penicillin/streptomycin; Gibco]. One batch of FCS was used throughout the study.

Reagents and antibodies

The following reagents were used: human IFN-γ (Roche, Mannheim, Germany), GM-CSF (R&D, Wiesbaden-Nordenstadt, Germany), flagellin from *Helicobacter pylori* (IBT, Reutlingen, Germany), peptidoglycan (PGN) from *Staphylococcus aureus* (Fluka, Sigma-Aldrich, Schnelldorf, Germany), lipoteichoic acid (LTA) from *Staphylococcus aureus*, and LPS from *Escherichia coli* 0111:B4 (Sigma-Aldrich). LPS-free reagents, water (BioWhittaker, Verviers, Belgium), phosphate-buffered saline (PBS; Gibco, Karlsruhe, Germany) and bovine serum albumin (BSA; Sigma) were used to prepare the aliquots. GA (Batch-No. 242992899) was from Teva Pharmaceutical Industries Ltd, Petah Tiqva, Israel.

The following antibodies were used for fluorescence activated cell sorting (FACS) analyses: phycoerythrin (PE)-labelled anti-SLAM, peridinin chlorophyll protein (PerCP)-labelled anti-human CD14, and fluorescein isothiocyanate (FITC)-labelled anti-human CD25 and CD69 (all from Becton Dickinson, Heidelberg, Germany). All the corresponding isotype controls were from Becton Dickinson.

**FACS staining and analysis**

The cells were labelled with the predetermined appropriate antibody dilution or with the corresponding isotype controls. FACS stainings were analysed on a FACSscan using Cell-Quest software (Becton Dickinson). Monocytes were gated in forward/side scatter. The quadrants were set on the relative isotype controls. The in vitro experiments were repeated at least three times.

**Statistical analysis**

The GA dose dependence of the percentage of SLAM-positive monocytes and the frequency of TNF-α-producing cells were analysed with linear regression. The t test for independent samples was used to compare GA-treated multiple sclerosis patients, untreated multiple sclerosis patients and healthy controls. All P values given are two-sided and subject to a significance level of 5%.

**Results**

In vitro culture with GA inhibits monocyte activation via different TLR ligands and inflammatory cytokines

In previous experiments, we have characterized monocyte responses to (i) different bacterial TLR ligands (TLR-2 ligands PGN and LTA; TLR-4 ligand LPS; and TLR-5 ligand...
flagellin), and (ii) inflammatory cytokines (IFN-γ and GM-CSF). Monocyte activation was measured by (i) induction of surface activation markers (CD25, CD69 and SLAM); and (ii) cytokine [TNF-α production (Farina et al., 2004)]. We found that SLAM was induced preferentially by stimulation with TLR ligands but not inflammatory cytokines, whereas CD25 and CD69 were induced by both TLR ligands and inflammatory cytokines. Further, TNF-α production was induced more strongly by TLR ligands than by IFN-γ and GM-CSF (Farina et al., 2004). These results laid the basis for our present study of the effects of GA on monocyte activation.

In a typical dose–response curve, a plateau of activation was reached with LPS concentrations >1000 pg/ml. At plateau, GA had no detectable inhibitory effect on monocyte reactivity. For optimal detection of the inhibitory effect of GA, we found that low to intermediate concentrations (150–1000 pg/ml) of LPS had to be used. PBMCs from healthy donors were pre-incubated for 1.5 h with four different concentrations of GA, and stimulated overnight with different concentrations of LPS. Figure 1 shows one of three representative experiments for TNF-α production (Fig. 1A) and SLAM induction (Fig. 1B). At each LPS concentration, the percentage of SLAM-positive monocytes and the frequency of TNF-α-producing cells was strongly reduced (P < 0.05 for TNF-α production by 300, 600 and 1000 pg/ml LPS; P < 0.05 for SLAM induction by 600 and 1000 pg/ml LPS). As shown in Fig. 1, the inhibitory effect of GA was dose dependent. We conclude that GA inhibits monocyte responses induced with the TLR-4 ligand LPS.

We also investigated the effects of GA on monocyte activation stimulated with the TLR-2 ligands PGN and LTA, and the TLR-5 ligand flagellin. As with LPS, GA had no detectable inhibitory effect at very high concentrations of these ligands. We measured dose–response curves for each ligand and determined the half-maximal stimulating concentrations. Again, the monocyte response was measured in terms of SLAM induction and TNF-α production by FACS and Elispot assay, respectively. After pre-incubation with 50 µg/ml GA, SLAM induction was inhibited by 66.8 ± 37.4 (PGN stimulation), 80.4 ± 30.3 (flagellin) and 87 ± 18.3% (LTA) (average values ± SD; data not shown). Similarly, GA inhibited TNF-α production by monocytes stimulated with half-maximal doses of the different TLR ligands (Fig. 2; P = 0.002 for PGN, P < 0.0001 for LTA and flagellin).

GA inhibited not only TLR-stimulated, but also IFN-γ and GM-CSF-stimulated TNF-α release (Fig. 2, P = 0.001 for IFN-γ and P < 0.0001 for GM-CSF), although in this case high concentrations of inflammatory cytokines were necessary for monocyte stimulation. In three different experiments, the inhibitory effect of GA on TNF-α production was consistently greater when TLR ligands were used as activators (87–97% inhibition), compared with stimulation with IFN-γ or GM-CSF (47–58% inhibition) (Fig. 2).

We also investigated the effects of GA on two additional markers of monocyte activation, CD25 and CD69. These molecules are newly induced on monocytes by stimulation with TLR ligands or inflammatory cytokines. Table 2 shows the results of one (out of three) representative FACS experiment. Pre-incubation with 50 µg/ml GA reduced the
fraction of activated monocytes expressing CD25 or CD69, regardless of which activator was used for stimulation (Table 2). Although the magnitudes of monocyte activation and of the inhibitory effect of GA varied between the different stimuli, GA consistently reduced the induction of CD25 and CD69 by all the different stimuli (CD69: 70% average inhibition for all activators; CD25: 70% average inhibition for LPS, LTA and PGN, 50% average inhibition for flagellin and IFN-γ, 30% inhibition for GM-CSF). In contrast, CD14, which is constitutively expressed on monocytes, was unaffected by GA (Fig. 3).

**Treatment with GA in vivo leads to a reduction of monocyte reactivity ex vivo**

The experiments described in the previous section indicated that GA inhibits monocyte activation by TLR ligands and inflammatory cytokines in vitro. To investigate whether in vivo treatment with GA also affects monocyte properties,

**Table 2 GA blocks induction of activation markers on monocytes**

<table>
<thead>
<tr>
<th></th>
<th>CD25*</th>
<th>CD69*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>-GA</td>
<td>+GA**</td>
</tr>
<tr>
<td>No stimulus</td>
<td>0.4%</td>
<td>1.2%</td>
</tr>
<tr>
<td>LPS 0.15 ng/ml</td>
<td>24.1%</td>
<td>0.8%</td>
</tr>
<tr>
<td>LTA 0.25 μg/ml</td>
<td>17.4%</td>
<td>7.8%</td>
</tr>
<tr>
<td>PGN 0.25 μg/ml</td>
<td>24.6%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Flagellin 0.6 μg/ml</td>
<td>5.3%</td>
<td>3.3%</td>
</tr>
<tr>
<td>IFN-γ 1000 U/ml</td>
<td>5.9%</td>
<td>3.5%</td>
</tr>
<tr>
<td>GM-CSF 100 ng/ml</td>
<td>19.8%</td>
<td>15.3%</td>
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*The expression of the indicated activation markers on monocytes was determined by FACS and expressed as percentage of positive cells; **pre-incubation with 50 μg/ml GA.

we assessed LPS-stimulated SLAM induction and TNF-α production by monocytes from GA-treated multiple sclerosis patients and controls. We compared PBMCs from eight GA-treated multiple sclerosis patients, eight untreated multiple

![Figure 3](image3.png)

**Fig. 3** GA-mediated effects on CD25 (A) and CD14 (B) expression by LPS-stimulated monocytes. LPS (0.15 ng/ml) and GA (50 μg/ml) were used. Upper panels (A) represent dot-blots of CD25 expression (ordinate) versus forward scatter (abscissa). GA reduced the proportion of CD25+ monocytes from 34.7% (without GA, upper left) to 3.1% (with GA, upper right). Lower panels (B) represent dot-blots of CD14 expression (ordinate) versus forward scatter (abscissa). There was no detectable change of CD14 expression in the presence of GA (lower right). The two markers were analysed in parallel in one experiment using PBMCs from one healthy donor.

![Figure 4](image4.png)

**Fig. 4** LPS-stimulated SLAM induction on monocytes cultured ex vivo from eight healthy donors, eight untreated multiple sclerosis patients and eight GA-treated multiple sclerosis patients. SLAM induction was measured by FACS. The monocytes had been exposed to GA only during in vivo treatment, not in vitro. Monocytes from GA-treated patients showed significantly reduced responses. *P = 0.002 healthy controls versus GA-treated patients; P = 0.017 untreated patients versus GA-treated patients; P = 0.185 healthy controls versus untreated patients. The right panel shows means from each of the left panels. The P values at lower LPS concentrations were not significant.
The major new observation reported herein is that in treated multiple sclerosis patients, GA induces systemic functional changes of circulating monocytes. Monocytes from GA-treated multiple sclerosis patients are significantly less susceptible to LPS stimulation than monocytes from untreated multiple sclerosis patients and normal control subjects. This reduced monocyte reactivity was observed in terms of both LPS-induced expression of the surface activation molecule SLAM, and also LPS-induced secretion of the cytokine TNF-α.

These changes were observed with ‘ex vivo’ monocytes, which had been exposed to GA only in vivo, but not in vitro. The results are fully consistent with our parallel observations on monocytes cultured in the presence of GA in vitro. After pre-incubation with GA in vitro, monocytes were less susceptible to a broad range of diverse stimuli, including ligands of different TLRs, and also different stimulatory cytokines (IFN-γ and GM-CSF).

Discussion

sclerosis patients and eight healthy donors. Note that in these experiments, the monocytes were not exposed to GA in vitro.

Figure 4 compares LPS-induced SLAM expression on monocytes from each group of patients and control subjects. Despite some inter-individual variability, cells from GA-treated patients were clearly less susceptible to SLAM induction than cells from the control groups. For example, at 1000 pg/ml LPS, the mean proportion of SLAM-expressing monocytes was 22.1 ± 4.4% (mean ± SD) for GA-treated patients compared with 37.2 ± 9.5% for healthy subjects (P = 0.002), and with 31.1 ± 8.0% for the untreated multiple sclerosis group (P = 0.017). The two control groups were not significantly different (Fig. 4).

We also compared LPS-induced TNF-α production by monocytes from the different groups. Figure 5 shows the dose–response curves for each subject as detected by Elispot assay. Spontaneous release was similar in the three groups (P = 0.41 for healthy controls versus GA-treated patients; P = 0.81 for healthy controls versus untreated patients; P = 0.23 for untreated patients versus GA-treated patients). However, PBMCs from GA-treated individuals were less susceptible to in vitro activation even at a low concentration of LPS (P = 0.015 and P = 0.013 at 150 pg/ml LPS when compared with healthy subjects and untreated multiple sclerosis patients, respectively). At increasing LPS concentrations, the differences became more evident (P = 0.002 and P = 0.0001 when compared with healthy donors, P = 0.033 and P = 0.001 when compared with untreated multiple sclerosis patients at 600 and 1000 pg/ml LPS, respectively). There were no significant differences between the two control groups at any of the different LPS concentrations (P values ranging from 0.29 to 0.69).

As shown in Figs 4 and 5 (right panels), the curves representing the means for GA-treated patients were different from the control curves.
with GA blocked the induction of all investigated surface markers (SLAM, CD25 and CD69), and also of TNF-α release. Several experiments with enriched monocyte populations showed that the inhibitory in vitro effect of GA on SLAM induction occurs in the absence of T cells.

Taken together, these results suggest that GA broadly affects monocyte reactivity. The exact mechanism(s) of the inhibitory effect is presently unknown, and deserves further study. For several reasons, it appears unlikely that GA exerts a (trivial) toxic effect on monocytes. First, monocyte viability was not altered at the GA concentrations used in our in vitro experiments, as seen by propidium iodide uptake. Secondly, although GA reduced the induction of the activation-related surface molecules SLAM, CD25 and CD69, another surface marker of monocytes, CD14 [which is part of the LPS receptor complex (Underhill, 2003)] was completely unaffected by GA treatment in vitro. Furthermore, T cells from GA-treated patients showed the typical, previously described Elispot cytokine responses when stimulated with a wide range of GA concentrations in vitro (Farina et al., 2001, 2002).

The new findings have obvious implications for our understanding of the mechanisms of action of GA. In several previous studies, part of these mechanisms was elucidated in some detail. It is widely assumed that the beneficial effects of GA are mediated mainly by GA-reactive TH2 cells. Previous studies showed that GA treatment induces a gradual shift of GA-reactive T cells from TH1 to TH2 (Miller et al., 1998; Neuhaus et al., 2000; Duda et al., 2000; Farina et al., 2001). Since the GA-reactive T cells are constantly activated by immunization, they can gain access to the CNS (Wekerle et al., 1986; Hickey, 1991). Indeed, GA-reactive T cells have been directly demonstrated in the CNS of adoptively transferred animals (Aharoni et al., 2000). It is assumed further that after local recognition of cross-reactive myelin degradation products, the GA-specific TH2 cells are stimulated to secrete TH2-like cytokines, which suppress neighbouring encephalitogenic T cells via ‘bystander suppression’ (Aharoni et al., 1997, 1998; Neuhaus et al., 2001). Furthermore, the locally activated GA-reactive T cells produce the neurotrophic factor BDNF (Ziemssen et al., 2002). Because the BDNF receptor gp145 trk B is expressed in neurons in the immediate vicinity of multiple sclerosis plaques (Stademmann et al., 2002), these neurons should be responsive to the T-cell-derived BDNF.

The basic tenets of the above scenario, especially the GA-induced TH2 shift of T cells, are widely accepted. It is unclear, however, how the cytokine shift is brought about. The most widely held view is that GA acts mainly on T cells, and that several factors related to the special antigenic properties of GA contribute to the observed TH2 shift. These include the properties of GA as a co-polymer, its route of administration by frequent s.c. injection and perhaps its local presentation to T cells by dermal Langerhans cells. In contrast, our results indicate that GA directly affects the functional properties of ‘professional’ APCs. Consistent with our findings, several recent publications showed that GA had inhibitory effects on TNF-α production by a monocyctic cell line (Li et al., 1998) and on IL-12 secretion by in vitro generated, monocyte-derived DCs (Hussien et al., 2001; Vieira et al., 2003). Furthermore, our results show, as we believe for the first time, that GA reduces monocyte reactivity in treated patients, and that this effect can be observed with ‘ex vivo’ monocyte cultures.

What are the implications of these observations? First, GA may alter the properties of APCs in such a way that they preferentially induce TH2 responses. This is supported by the study of Vieira et al. (2003) who demonstrated that GA-treated DCs induce IL-4-secreting TH2 cells in vitro. We investigated the effects of GA treatment on the TH2 shift in a subset of patients. Five of six patients showed a pronounced GA-induced IL-4 production by Elispot assay, defined according to Farina et al. (2001) (data not shown). These findings indicate that the GA-induced TH2 shift and the inhibition of monocyte reactivity usually occur concurrently. Secondly, monocyte inhibition per se could be beneficial in multiple sclerosis, independently of the TH2 shift of T cells. Monocytes and macrophages have an important role in the pathogenesis of multiple sclerosis (for a review see Izikson et al., 2002). Although the composition of the inflammatory infiltrate in the CNS varies depending on the type, stage and activity of multiple sclerosis, monocytes/macrophages are thought to be key effectors responsible for tissue damage. They predominate in active multiple sclerosis lesions, and the presence of myelin degradation products inside macrophages is one of the most reliable markers of lesional activity. Toxic products of activated monocytes/macrophages, such as TNF-α, reactive oxygen species and matrix metalloproteinases (Bar-Or et al., 2003), contribute to myelin injury (for a review see Lassmann et al., 2001). Blockade of monocytes is therefore an obvious therapeutic goal.

Thirdly, the direct effect of GA on APCs makes it necessary to reconsider the concept of ‘antigen selectivity’ of GA. Although not formally proven, it is widely believed that GA preferentially affects myelin basic protein (MBP)-specific T-cell responses, at least in experimental allergic encephalomyelitis (EAE) models. However, it has been known for some time that the suppressive effect of GA is not restricted to MBP-induced EAE, but extends to EAE induced with proteolipid protein (PLP) (Teitelbaum et al., 1996) and myelin oligodendrocyte glycoprotein (MOG) (Ben-Nun et al., 1996). Furthermore, GA seems to have a protective effect in an animal model of neurodegeneration (Angelov et al., 2003). These puzzling observations are difficult to reconcile with an antigen-specific, exclusively T-cell-mediated effect of GA. However, they would be consistent with an antigen-independent inhibitory effect on APCs.

Fourthly, although the side effects of GA are generally mild, the newly demonstrated systemic effect on monocytes might raise concern that GA has general immunosuppressive actions, especially on innate (antigen non-specific) immune responses. Up to now, there has been no evidence supporting this notion (Ziemssen et al., 2001). One reason could be that
the GA-induced systemic inhibition of monocyte reactivity can be overcome by strong stimuli as they occur in the clinical setting of infections in vivo. Indeed, in our in vitro experiments, the inhibitory effects of GA could only be demonstrated at low to intermediate concentrations of TLR ligands. High concentrations of TLR ligands (as they might occur in vivo during infection) could overcome the blocking effect of GA. In contrast, GA blocked monocyte responses induced by IFN-γ and GM-CSF although these stimulators were applied at high concentrations. This might imply that the modification of monocyte properties by GA is relevant in the inflammatory environment of multiple sclerosis lesions, where monocytes presumably are stimulated by inflammatory cytokines, not by TLR ligands.

Fifthly and finally, the inhibitory effect of GA detectable in ‘ex vivo’ cultured monocytes might be helpful to monitor the immunological response in GA-treated patients. In previous studies, we established a set of criteria for the identification of immunological responders and non-responders (Farina et al., 2001). In a pilot study, these immunological criteria helped to discriminate between clinical responders and non-responders (Farina et al., 2002). In future studies, it will be interesting to see whether the reduced reactivity of ex vivo monocytes can be added to the previous criteria, and whether these changes correlate with the clinical response.

In conclusion, our findings demonstrate that GA inhibits monocyte responses in vitro, and that similar changes can be demonstrated ex vivo in monocytes obtained from GA-treated patients. When viewed together with recent findings from other investigators, these changes at the APC level might help to explain the well-established TH1 to TH2 cytokine shift observed in GA-reactive T cells of treated patients. In the light of the new findings, it seems necessary to revise the concept that GA selectively targets T cells. The detailed mechanism of the monocyte-inhibiting effect of GA, and its clinical implications need to be explored further in future studies.

After submission of this paper we became aware of two additional studies which suggest that GA directly affects APC function (Jung et al., 2004; Kim et al., 2004).

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References


Jung S, Siglenti I, Graver O, Magnus T, Scarlato G, Toyka K. Induction of


