Control of functional T helper cell polarization by dendritic cells

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Glucocorticoids (GC) are known to affect the immune response at several stages. However, little is known about how GC influence the initiation of the specific immune response at the level of dendritic cells (DC), the highly professional APC for T cells. Therefore, we studied whether GC modulate the cytokine production and T cell stimulatory function of DC. In LPS-stimulated DC, GC strongly reduced the secretion of the Th1-skewing factor IL-12p70 and, to a lesser extent, the production of the proinflammatory cytokines IL-6 and TNF-α. Regarding the T cell stimulatory function of DC, GC did not influence the cell surface expression of HLA-DR or the costimulatory molecules CD40 and CD80 and did not influence the ability of DC to take up antigen. Consequently, GC pretreatment of DC indeed did not affect their ability to stimulate CD4⁺ Th cell proliferation in response to superantigen. However, as a result of their defective production of bioactive IL-12, GC-pretreated DC have a reduced ability to promote the production of IFN-γ in CD4⁺ Th lymphocytes, as shown by the observation that IFN-γ production could be restored by exogenous IL-12. In contrast, GC treatment of DC enhanced the secretion of the antiinflammatory cytokine IL-10 and the type 2 cytokine IL-5 by the T cells. It is concluded that, in addition to their role as potent inhibitors of inflammation via the direct suppression of cytokine production in T cells, GC may further inhibit T cell mediated inflammation indirectly via the suppression of IL-12 production by DC.

INTRODUCTION

Glucocorticoids (GC) are potent antiinflammatory and immunosuppressive agents that are widely used in the treatment of inflammatory disorders, such as autoimmune and allergic diseases (1). Generally, the immunosuppressive effects of GC have been ascribed to their inhibitory effect on Th cell proliferation, due to the inhibition of IL-2 production (2) and the downregulation of signal transduction through the IL-2R (3). In addition, several in vitro studies reported on the downregulatory effects of GC on the secretion of both the Th1 cell-associated cytokine IFN-γ and the type 2 cytokines IL-4 and IL-5 (4-6). In contrast, IL-4 production was reported to be upregulated by GC (2) when T cells were stimulated in the presence of APC. Therefore, GC may not only affect T cells directly, but also indirectly via APC.

The studies on the effects of GC on APC have focused mainly on monocytes and macrophages, and showed the ability of GC to downregulate the secretion of several soluble mediators such as IL-1β (7), IL-6 (7,8), IL-8 (9), IL-12p40 (10, 11), TNF-α (12), granulocyte-macrophage (GM)-CSF (7, 13), and prostaglandins (14). Moreover, GC can interfere with the activation of monocytes and macrophages not only by reducing their phagocytic capacity and the IFN-γ–induced increase of the expression of class II MHC molecules (10, 15) but also by upregulating the expression of the cell surface mannose receptor (MR) on macrophages (16).

Although monocytes and macrophages can present antigen (Ag) to Th cells, dendritic cells (DC) are regarded as professional APC during the onset of the immune response, since they have the particular ability to activate naive Th lymphocytes (17, 18). In addition, DC take up Ag very efficiently and express constitutively high levels of both costimulatory molecules of the B7 family and adhesion molecules required for efficient Ag-presentation (19). The secretion of DC-derived immunoregulatory and immunomodulatory cytokines plays a crucial role in the cascade of events during the priming of naive Th cells. In this respect, IL-12 is an important Th1-skewing factor, because of its ability to induce potently the production of IFN-γ in Th cells (20, 21). IL-12 is a heterodimeric cytokine composed of a 35-kD subunit (p35) and a 40-kD subunit (p40) that have to be produced within the same cell to assemble the biologically active IL-12p70 molecule (22). The levels of bioactive IL-12 are limited and determined mainly by regulation of the expression of the p35 subunit (23).
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Until now, the effects of GC on DC have been poorly studied. Murine studies showed that GC downregulate the ability of in vitro cultured DC to stimulate T cells due to either insufficient expression of B7 family costimulatory molecules or inefficient uptake and/or processing of Ag (24, 25). GC are used as topical drugs to inhibit inflammation. For that purpose, they are applied on peripheral tissues containing immature DC that are able to activate infiltrating T cells. Since modification of DC function may affect the outcome of the specific immune response, the effect of GC on human DC was studied using in vitro-generated monocyte-derived DC as an experimental model to study DC function, which has been generally accepted since it was first reported (26). Based on their frequent clinical application and potency, we tested the synthetic GC clobetasol-17-propionate (CP) and the naturally occurring GC hydrocortisone (HC) as representatives of strong and weak GC, respectively. The effects of these GC on the ability of DC to secrete immunoregulatory and proinflammatory cytokines, to take up Ag, and to stimulate both CD4⁺ Th cell proliferation and the production of type 1 and type 2 cytokines in response to superantigen was investigated.

The present data show a strong inhibitory effect of GC on the ability of human DC to secrete the Th1-polarizing factor IL-12p70 and, to a lesser extent, the proinflammatory cytokines IL-6 and TNF-α. However, GC did not affect the uptake of Ag, the expression of class II MHC and costimulatory molecules, or the stimulatory capacities toward CD4⁺ Th cells. Due to their reduced IL-12p70 levels, GC-pretreated DC do not promote the type 1 cytokine IFN-γ; rather these DC stimulate the secretion of the antiinflammatory cytokine IL-10 and the type 2 cytokine IL-5 by CD4⁺ Th lymphocytes in the presence of superantigen.

MATERIALS AND METHODS

Cytokines, Antigens, and reagents
Human rGM-CSF (sp. act. 1.11x10⁷ U/mg) was a gift of Schering-Plough (Uden, The Netherlands). Human rIFN-γ (sp. act. 8x10⁷ U/mg) was a gift of Dr. P. H. van der Meide (Biomedical Primate Research Center, Rijswijk, The Netherlands). Human rIL-4 (sp. act. 1x10⁸ U/mg) was obtained from Pharma Biotechnologie Hannover (Hannover, Germany). Human rIL-12 (sp. act. 1.7x10⁸ U/mg) was a gift of Dr. M. K. Gately (Hoffmann-La Roche, Nutley, NJ).
Fixed *Staphylococcus aureus* Cowan strain I (SAC) (Calbiochem, San Diego, CA) was used at a final concentration of 75 µg/ml. LPS (Difco, Detroit, MI) was used at a final concentration of 100 ng/ml. Superantigen *Staphylococcus aureus* enterotoxin B (SEB) (Sigma, St. Louis, MA) was used at a final concentration of 1 ng/ml.

The GC CP (Sigma) and HC (Sigma) were kept as stock solutions of $10^{-2}$ M in 95% ethanol, stored at -80°C, and diluted into complete medium just before use. A 1:10^4 final dilution of 95% ethanol was included as a vehicle control in all experiments.

**In vitro generation of dendritic cells from peripheral blood monocytes**

Venous blood from healthy donors was collected by venipuncture in sodium-heparin containing tubes (VT100H; Venoject, Terumo Europe, Leuven, Belgium). PBMC were isolated by density centrifugation with Lymphoprep (Nycomed, Torshov, Norway). Subsequently, PBMC were centrifuged on a Percoll (Pharmacia, Uppsala, Sweden) gradient consisting of three density layers (1.076, 1.059 and 1.045 g/ml). The light density fraction floating on the middle layer, which contained predominantly monocytes, was seeded in 24-well culture plates (Costar, Cambridge, MA) at a density of 5x10^5 cells/well. After 30 min of incubation at 37°C, nonadherent cells were removed; adherent cells were cultured in Iscove's modified Dulbecco's medium (Life Technologies, Paisley, U.K.) containing gentamycin (86 µg/ml; Duchefa, Haarlem, The Netherlands) and 1% FCS (HyClone, Logan, UT) and supplemented with GM-CSF (500 U/ml) and IL-4 (250 U/ml) to obtain DC as described previously (26). At day 3, the media, including the supplements, were refreshed. At day 6, CD1a^+CD14^- DC were obtained.

**Isolation of CD4^+ Th cells**

Highly purified CD4^+ Th cells (>98% as assessed by flow cytometry, data not shown) were obtained by incubating the PBL, which had been harvested from the lower interface of the Percoll gradient, with anti-CD4 coated Dynabeads (Dynal AS, Oslo, Norway), followed by treatment with Detachabeads (Dynal AS) according to the manufacturer's instructions.
Stimulation of DC in the presence or absence of GC
CD1a+ DC (4x10^4 cells/well) were stimulated in 96-well flat-bottom culture plates (Costar) in Iscove's modified Dulbecco's medium containing 10% FCS in a final volume of 200 µl. The following stimuli were used: LPS (final concentration 100 ng/ml) and SAC (final concentration 75 µg/ml). Both modes of stimulation were performed in the absence or presence of IFN-γ (10^3 U/ml). The GC used in this study were CP and HC. Pilot experiments demonstrated that 10^{-8} M CP strongly reduced IL-12p70 production by stimulated DC, whereas a 100- to 1000-fold higher concentration of HC was needed to observe a similar reduction. Therefore, the concentrations of CP used for future experiments were between 10^{-11} and 10^{-8} M whereas HC was used in a concentration range of 10^{-8}-10^{-6} M. Supernatants were harvested after 24 h and stored at -20°C until the levels of IL-12p70, IL-10, IL-6, and TNF-α were measured by ELISA.

GC pretreatment of DC followed by stimulation in the absence of GC
CD1a+ DC were cultured for 2 days in GM-CSF (500 U/ml) in the presence of CP (10^{-10} M or 10^{-8} M), HC (10^{-8} M or 10^{-6} M), or vehicle. To study the stability of the observed cytokine production profiles, DC were cultured for another 2 days in GM-CSF (500 U/ml) alone after removal of the GC. To stimulate the DC, the cells were harvested, washed thoroughly, counted and the viability was determined. A difference in viability between GC-treated and untreated DC was not observed in any of the experiments. DC (4x10^4/200µl) were stimulated with LPS (final concentration 100 ng/ml) in the absence of GC. Supernatants were harvested after 24 h and stored at -20°C until the levels of IL-12p70, IL-10, IL-6, and TNF-α were measured by ELISA.

Ag uptake assay
CD1a+ DC were incubated at 37°C for 30 min in the presence of culture medium containing either 0.2 µg/ml of albumin-FITC conjugate (BSA-FITC, 1:12 molar ratio; Sigma) or 0.2 µg/ml of α-D-mannosylated-albumin-FITC conjugate (manBSA-FITC, 1:2.5 molar ratio; Sigma). Negative controls were incubated with the respective Ag at 4°C for 30 min. Ag uptake was stopped by extensive cold washing, and cell surface
fluorescence was quenched with trypan blue (Sigma). Ag uptake was evaluated by flow cytometry.

**Induction of proliferative response in CD4\(^+\) Th cells**
CD4\(^+\) Th cells (5x10\(^4\) cells/200µl) were cocultured in 96-well flat-bottom culture plates (Costar) with different concentrations of vehicle- or GC-treated DC (as indicated in Fig. 5) coated with SEB (final concentration 1 ng/ml). Cell proliferation was assessed by the incorporation of \((^{3}\text{H})\)-TdR (Radiochemical Centre, Amersham, Little Chalfont, U.K.) after a pulse with 13 KBq/well during the last 16 h of 5-day culture after stimulation, as measured by liquid scintillation spectroscopy.

**Induction of cytokine production in CD4\(^+\) Th cells**
CD4\(^+\) Th cells (5x10\(^4\) cells/200µl) were cocultured with 3x10\(^4\) vehicle- or GC-treated DC coated with SEB (final concentration 1 ng/ml) in 96-well flat-bottom culture plates (Costar), in either the presence or absence of an excess of exogenous IL-12 (100 U/ml). Supernatants were harvested after 4 days and the levels of IFN-\(\gamma\), IL-4, IL-5, and IL-10 were measured by ELISA.

**Cytokine measurements**
Measurements of IFN-\(\gamma\), IL-4, IL-5, IL-10, and IL-12p70 levels in culture supernatants were performed by a specific solid-phase sandwich ELISA as described previously (27). Measurements of IL-6 and TNF-\(\alpha\) were performed by ELISA using pairs of specific mAbs and recombinant cytokine standards obtained from BioSource International (Camarillo, CA). The limits of detection of these ELISA are as follows: IFN-\(\gamma\), 100 pg/ml; IL-4, 60 pg/ml; IL-5, 50 pg/ml; IL-6, 20 pg/ml; IL-10, 25 pg/ml; IL-12p70, 3 pg/ml; and TNF-\(\alpha\), 20 pg/ml.

**Analysis of expression of cell surface molecules by flow cytometry**
The mouse anti-human mAbs against the following molecules were used: CD1a (OKT6; Ortho Diagnostic System, Beerse, Belgium), CD40 (EA-5; a gift of Dr. T. LeBien, University of Minnesota, Minneapolis, MN), CD80 (B7-24 (28); kindly provided by Innogenetics N.V., Ghent, Belgium), HLA-DR (L243; Becton Dickinson, San José, CA), and MR (15-2 (16); a gift of Dr. F. Noorman, Gaubius Laboratory,
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Netherlands Central Organization for Applied Scientific Research for Prevention and Health, Leiden, The Netherlands), followed by FITC-conjugated goat anti-mouse mAb (Jackson Immunoresearch Laboratories, West Grove, PA).

Statistical analysis
Data were analyzed for statistical significance (InStat, Version 2.02, GraphPad, San Diego, CA) using ANOVA followed by Dunnett’s multiple comparisons test. The stability of the effect of GC on cytokine production by GC-pretreated DC at 2 days after GC withdrawal (see Fig. 2) was evaluated using the Student’s t-test. A P value of <0.05 was considered as the level of significance.

RESULTS
Direct effect of GC on cytokine secretion by DC
Since DC are the professional effective APC and their secretion of immunoregulatory and proinflammatory cytokines plays a crucial role during T cell priming (17, 18), we investigated whether cytokine production by in vitro generated DC is affected by GC. To address this issue, the effect of increasing concentrations of CP and HC on IL-12, IL-6, and TNF-α production was compared. As is shown in Fig. 1, the stimulation of DC with LPS (100 ng/ml) in the presence of increasing concentrations of GC resulted in a dose-dependent inhibition of IL-12p70, IL-6, and TNF-α production.

![Figure 1](image-url)  
Figure 1. Inhibitory effect of GC on LPS-induced cytokine production by DC. DC (4x10⁶ cells/200μl) were stimulated with 100 ng/ml LPS combined with the indicated concentrations of CP (A) or HC (B). Results are expressed as the mean percentage cytokine (IL-12p70, ■; IL-6, ○; or TNF-α, ▲) concentration of triplicate cultures in the 24 h supernatants from one representative experiment of three. Cultures stimulated with LPS in the presence of GC are compared with respective cultures stimulated with LPS in the presence of vehicle (100%) ± SD. At 100%, IL-12p70 production (in the presence of IFN-γ) was 1.8 ± 0.4 ng/ml, IL-6 was 1.3 ± 0.03 ng/ml, and TNF-α was 3.5 ± 0.03 ng/ml. Data were analyzed for statistical significance using ANOVA followed by Dunnett’s multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001.
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Even at the low concentration of $10^{-11}$ M, CP (Fig. 1A) inhibited the secretion of the IL-12p70 heterodimer by ~ 50% ($P<0.01$); in contrast, no significant reduction in either IL-6 or TNF-α production was observed at this dose. High concentrations of CP ($10^{-8}$ M) almost completely abrogated the production of IL-12p70 ($P<0.001$) but affected IL-6 and TNF-α to a lesser extent. Also HC was found to affect DC-derived cytokines (Fig. 1B), but 100- to 1000-fold higher concentrations were needed for an effect that was comparable with that of CP.

An identical downregulation of cytokine production was observed when DC were stimulated with SAC (75 μg/ml) (data not shown). At all conditions, IL-10 production was not detectable.

**Effect of GC pretreatment on cytokine secretion by DC**

We questioned whether the inhibition of IL-12p70 production by GC was a direct effect during DC stimulation or could also be found when DC were pretreated with GC. Therefore, DC were preincubated with GC or vehicle for 2 days, washed and stimulated with LPS (100 ng/ml) in the absence of GC. As is shown in Fig. 2 (filled bars), GC pretreatment also inhibited the production of DC-derived cytokines upon subsequent stimulation. The production of IL-12p70 (Fig. 2A, filled bars) was strongly inhibited ($P<0.001$) by preincubation with either $10^{-10}$ M or $10^{-8}$ M CP. Again, HC appeared to be much less effective and induced a similar IL-12p70 inhibition only at $10^{-8}$ M ($P<0.001$); $10^{-8}$ M HC did not produce any statistically significant effect. The production of IL-6 (Fig. 2B, filled bars) and TNF-α (Fig. 2C, filled bars) was inhibited to a lesser extent compared with IL-12p70. IL-10 production was not detectable in these culture supernatants, which is consistent with the poor ability of DC to produce this cytokine (27).

To assess the stability of the downregulation of cytokine production, GC-pretreated DC were washed extensively and kept in culture in the absence of GC for an additional 2 days before stimulation. As is shown in Fig. 2 (open bars), IL-12p70 and IL-6 were inhibited to the same extent compared with experiments in which they were determined directly after GC pretreatment (filled bars). However, the additional 2 days of culture in the absence of GC partially restored the ability of DC pretreated with HC and low dose CP to produce TNF-α ($P<0.001$ and $P<0.01$, respectively).
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Figure 2. Inhibitory effect of GC treatment on cytokine production by DC is transient for TNF-α. DC were treated for 2 days with the indicated concentrations of GC. After the treatment period, DC were washed and either stimulated directly in the absence of GC with 100 ng/ml LPS (filled bars) or kept in culture for an additional 2 days before similar stimulation (open bars). Results are expressed as the mean percentage IL-12p70 (A), IL-6 (B) and TNF-α (C) concentration of triplicate cultures in the 24 h supernatants from one representative experiment of three. Cytokine production by GC-treated DC is compared with the respective cytokine production by vehicle-treated control DC (100%) ± SD. At 100%, IL-12p70 production (in the presence of IFN-γ) was 2.0 ± 0.2 ng/ml, IL-6 was 3.4 ± 0.3 ng/ml, and TNF-α was 18.7 ± 1.3 ng/ml. Data were analyzed for statistical significance using ANOVA followed by Dunnett’s multiple comparisons test. The stability of the effect of GC on cytokine production by GC-treated DC at 2 days after withdrawal was evaluated using the Student’s t-test. *P<0.05, **P<0.01, ***P<0.001.

Figure 3. Surface phenotype of GC-treated DC. DC were treated for 2 days with GC and analyzed for the expression of cell surface molecules by flow cytometry. A comparison of expression intensity by GC-treated DC with vehicle-treated DC is shown. Data are representative of seven independent experiments.

GC pretreatment does not affect the expression of surface molecules on DC
We studied whether GC pretreatment of DC affects their cell surface expression of the DC-associated marker CD1a, the class II MHC Ag-presenting molecule HLA-DR, the costimulatory molecules CD40 and the B7 molecule CD80, and MR. Although it has been reported previously for monocytes and macrophages that GC interfere with
the cell surface expression of molecules involved in Ag presentation and costimulation (10, 15), GC pretreatment of DC for 2 days did not affect the expression of surface molecules when compared with vehicle-treated control cells (Fig. 3).

**GC pretreatment does not affect the ability of DC to take up Ag**

Using BSA-FITC and manBSA-FITC in a functional assay, we subsequently assessed the effects of GC on the ability of DC to take up Ag via fluid phase endocytosis or via their specific MR. Regardless of the pretreatment conditions, neither CP nor HC affected the uptake of either BSA-FITC (Fig. 4, left panels) or manBSA-FITC (Fig. 4, right panels). These results clearly show that the endocytic capacity of DC was not affected by preculture with GC. Although MR expression has been reported to be upregulated by GC (16), no differences on MR-mediated uptake were induced; therefore, this observation is in line with the unchanged cell surface expression of MR by GC-treated DC in comparison with control DC (Fig. 3).

**Figure 4. Intracellular accumulation of FITC-conjugated Ag by GC-treated DC.** DC were treated for 2 days with the indicated concentrations of GC and subsequently pulsed for 30 min in medium containing either 0.2 μg/ml BSA-FITC (left panels) or 0.2 μg/ml manBSA-FITC (right panels). Flow cytometry assessment of Ag-uptake at 37°C (open profiles) is compared with negative uptake controls at 4°C (filled profiles). Data are representative of five independent experiments.

**GC pretreatment does not affect the ability of DC to stimulate proliferation of CD4\(^+\) Th cells**

Next, we studied the effects of GC on the proliferation-inducing capacity of DC toward Th cells. To exclude the direct effects of GC on T cells, DC were pretreated
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with GC before coculture with highly purified CD4+ Th cells in the presence of superantigen (SEB; 1 ng/ml). In the absence of either DC or SEB, no activation of T cells could be observed. GC-pretreated DC were as effective as vehicle-pretreated DC in inducing T cell proliferation (Fig. 5). These results indicate that the exposure of DC to GC does not affect their ability to stimulate T cell proliferation in the presence of superantigen. These results are in line with the unaffected expression of HLA-DR and costimulatory molecules described above (Fig. 3).

**Figure 5.** GC pretreatment of DC does not impair their ability to stimulate proliferation of CD4+ Th cells. DC were treated for 2 days with GC (vehicle, •; 10^8 M CP, ○; or 10^6 M HC, ■). After the treatment period, GC were washed away and different concentrations of DC were used to stimulate 5x10^5 CD4+ Th cells in the presence of 1 ng/ml SEB. The proliferative response was determined at day 5 of coculture by [3H]-Tdr incorporation. Data are presented as mean cpm ± SD of triplicate cultures. Results are representative of six independent experiments.

GC pretreated DC do not promote the secretion of the type 1 cytokine IFN-γ by CD4+ Th cells but do stimulate the secretion of IL-10 and IL-5

Since IL-12 is a potent inducer of IFN-γ secretion in activated CD4+ Th cells (20, 21), we investigated whether GC pretreatment of DC influenced the cytokine profile of SEB-triggered CD4+ Th cells. As is shown in Fig. 6A, pretreatment with GC reduced (P<0.001) the ability of DC to stimulate the production of the type 1 cytokine IFN-γ. In contrast, GC-pretreated DC upregulated T cell production of the antiinflammatory mediator IL-10 (Fig. 6B) and the type 2 cytokine IL-5 (Fig 6C). Interestingly, the addition of rIL-12 to these cocultures abolished the reduction of IFN-γ and the upregulation of IL-5 production by the T cells but not the upregulation of IL-10. In both the absence and the presence of exogenous IL-12, IL-4 production was not detectable in the culture supernatants. These results indicate that the use of GC-pretreated DC as APC results in a T cell cytokine shift toward a type 2 profile, which largely due to the reduced production of IL-12.
**DISCUSSION**

The present study shows that GC are potent inhibitors of production of the bioactive IL-12p70 heterodimer and, to a lesser extent, of the proinflammatory cytokines IL-6 and TNF-α. In contrast, GC did not affect the level of expression of costimulatory molecules by DC or the ability to take up Ag and stimulate the proliferation of CD4+ Th cells in vitro. Largely due to an inhibition of IL-12p70 production, GC downregulate the capacity of DC to induce the secretion of the type 1 cytokine IFN-γ in CD4+ Th cells while upregulating their capacity to promote the secretion of the antiinflammatory cytokine IL-10 and the type 2 cytokine IL-5.

GC inhibit cytokine gene transcription through the activation of their specific cytosolic receptors (29). The actual downregulation of transcription of sensitive genes by GC was found to be due to the functional inhibition of some key transcription factors. GC were shown to inhibit the assembly of c-Fos and c-Jun to form functional activator protein-1 (30). Furthermore, the inhibitory effect of GC on NF-κB was shown to be mediated either via the enhancement of IκBα transcription and synthesis (31, 32), which is known to bind to NF-κB and prevent its migration to the nucleus, or via a direct association with Rel A, the 65-kD NF-κB binding protein (33).
The direct inhibitory effect of GC on T cell-derived cytokines is well documented, but T cells are not the only cell type in which cytokine production is modulated by GC. Several studies reported the downregulatory effect of GC on the secretion of the proinflammatory cytokines IL-1β (7), IL-6 (7, 8) and TNF-α (12) by monocytes and macrophages. Recently, Blotta et al. (10) demonstrated the inhibitory effect of GC on human monocyte-derived IL-12p40 with a concomitant upregulation of IL-10. And very recently, the GC-induced inhibition of IL-12p70 production by human monocytes was also shown (34). However, the effect of GC on human DC-derived bioactive IL-12p70 was not known.

Here, we report on the strong inhibition of IL-12p70 secretion by human DC in response to GC. The positive regulation of the IL-12p40 promoter by NF-κB was reported previously (35), notwithstanding that the regulation of the IL-12p35 promoter remains elusive. Hence, it can be speculated that, in addition to the probable effect of GC on the transcription of the IL-12p40 monomer, which is mediated indirectly via the downregulation of NF-κB, GC may also affect the transcription of IL-12p35 or interfere with the assembly of the two IL-12 subunits to form the bioactive IL-12p70 heterodimer. Since the production of bioactive IL-12 is determined mainly by the expression of the p35 gene (23), knowledge with regard to the regulation of IL-12p35 transcription will help to clarify the direct effects of GC on the secretion of IL-12p70. As GC stimulate IL-10 production in human monocytes (10), which is known to reduce IL-12 synthesis (23), it was suggested that these GC indirectly suppress IL-12 production via the upregulation of IL-10. Although this inhibitory pathway may be relevant for monocytes, in vitro-generated human DC do not produce detectable levels of IL-10, implying that the inhibitory effect of GC on cytokine production by DC cannot be explained by the indirect effect of upregulation of IL-10.

Both IL-6 and TNF-α are important mediators of a wide range of biological activities that play a critical role in the induction of proinflammatory and immune responses. GC inhibited IL-6 as well as TNF-α secretion by DC. The NF-κB binding site within the IL-6 promoter was suggested to be essential for the induction of IL-6 transcription (36). Likewise, NF-κB is involved in the positive regulation of TNF-α gene transcription (37). Ray and Prefontaine (38) showed that the activation of the IL-6 promoter by a combination of NF-IL-6 and Rel A was inhibited by dexamethasone-activated GC receptors. Since NF-κB plays a critical role in
inflammatory reactions, the possible inhibition of cytokine gene transcription as a result of the antagonism between activated GC receptors and NF-κB constitutes a likely mechanism underlying the GC-mediated antiinflammatory and immunosuppressive mode of action.

In contrast to the clear inhibitory effect of GC on DC cytokine production, our study indicated that Ag uptake, either via endocytosis or MR-mediated, was not affected by exposure to GC. Moreover, our data show that the expression of the class II MHC molecule HLA-DR and the costimulatory molecules CD40 and CD80 remained unaffected after the preincubation of DC with GC. Indeed, pretreatment of in vitro-generated DC with GC does not downregulate their stimulatory potential, as suggested by the observation that GC pretreatment did not affect the capacity of DC to stimulate CD4⁺ Th cell proliferation. Therefore, our results are in contrast with recent reports on the reduced ability of GC-treated human monocytes (10) or purified murine splenic DC (24) to stimulate the proliferation of T lymphocytes. In these two studies it was described that GC inhibit the IFN-γ (10) or 24-h-culture- (24) induced upregulation of the cell surface expression of molecules involved in either Ag presentation or costimulation. However, the upregulation of class II MHC and costimulatory molecules as well as an increased T cell stimulatory potential are features of maturation of DC. The possibility cannot be excluded that partial maturation was induced by the culturing conditions in both studies mentioned.

In line with the fact that IL-12 induces Th1 responses via a strong induction of IFN-γ secretion (20, 21), we found that GC-treated DC with a reduced capacity to produce bioactive IL-12p70 induced less IFN-γ in superantigen-stimulated CD4⁺ Th cells. Previously, a similar effect was reported for GC-pretreated human monocytes (10) and murine macrophages (11) when used to stimulate cytokine production by CD4⁺ T lymphocytes. In those studies, it was assumed that the inhibitory effect of GC on the production of IL-12p40 by the APC was representative for the effect on the bioactive heterodimer; however, the regulation of bioactive IL-12 production is determined mainly by the transcription of the p35 subunit (23). Here, we report that the downregulation of bioactive IL-12p70 production by DC in response to GC is indeed responsible for the reduction of IFN-γ secretion by CD4⁺ T cells, and that this reduction can be restored by the addition of exogenous bioactive IL-12 to the cultures during stimulation. Additionally, secretion of the antiinflammatory cytokine IL-10 and
the type 2 helper cytokine IL-5 was promoted when T cells were stimulated with GC-pretreated DC, confirming the studies of Blotta et al. (10) and DeKruyff et al. (11), respectively. IL-12 has been shown to be involved in the downregulation of IL-5 secretion (27, 39) by T cells. The observed upregulation of IL-5 secretion by the T cells in response to stimulation with GC-treated DC was abolished by rIL-12, indicating that the lack of secretion of bioactive IL-12 was responsible for the upregulation of IL-5. Although IL-12 has been also reported to induce T cell derived IL-10 (40), the production of this antiinflammatory mediator by CD4+ Th cells was not sensitive to rIL-12 in our cultures. However, Jeannin et al. (40) made their observation with established T cell lines in an APC-free system. Consequently, this suggests a rather distinct regulatory pathway of IL-10 production by IL-12 compared with the activation of resting T cells in an APC-dependent way as we show here.

By virtue of defective IL-12 production and the subsequent effects on cytokine production by Th cells, GC-pretreated DC should be regarded as antiinflammatory players in the Ag-specific immune system. Although GC are potent direct inhibitors of specific Th cell responses, additional modulation of the immune response may be expected through the effects of GC on DC, rendering them less supportive for inflammatory responses. In this way, the recurrent activation of T cells by GC-treated APC may lead indirectly to a suppression of the secretion of inflammatory type 1 cytokines, favoring the activation of T cells with a type 2 cytokine profile. GC pretreatment may induce increased IgE production (41, 42) and eosinophil numbers (43). Indeed, GC were shown to enhance IgE synthesis in both allergic and nonallergic individuals (44, 45). Despite this effect, therapy with GC appears to be useful in the blocking of allergen-specific reactions. This effect could probably be explained by the dominant antiinflammatory effect mediated not only via the direct inhibition of inflammatory T cell cytokines but also via the inhibition of IL-12 production in professional APC.

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