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Glucocorticoids Affect Human Dendritic Cell Differentiation and Maturation

Lorenzo Piemonti,1* Paolo Monti,1* Paola Allavena,2† Marina Sironi,3 Laura Soldini,3 Biagio Eugenio Leone,8 Carlo Socci,* and Valerio Di Carlo*

Because dendritic cells (DC) play a major role in the initiation of T cell-mediated immunity, we studied the effects of glucocorticoids, well-known inhibitors of the immune and inflammatory response, on the differentiation and maturation of human DC. DC were differentiated from human monocytes by culture with GM-CSF and IL-4 for 7 days with and without dexamethasone (Dex). Cells treated with Dex (10−8 M) (Dex-DC) developed a characteristic dendritic morphology; however, membrane phenotype analysis demonstrated that they were not fully differentiated. Dex-DC expressed low levels of CD1a and, unlike untreated cells, high levels of CD14 and CD16. Molecules involved in Ag presentation (CD40, CD86, CD54) were also impaired. In contrast, molecules involved in Ag uptake (mannose receptor, CD32) and cell adhesion (CD11/CD18, CD54) were up-regulated. After exposure to TNF-α or CD40 ligand, Dex-DC expressed lower levels of CD83 and CD86 than untreated cells. Dex-DC showed a higher endocytic activity, a lower APC function, and a lower capacity to secrete cytokines than untreated cells. Overall, these results indicate that DC differentiated in the presence of Dex are at a more immature stage. Moreover, Dex also partially blocked terminal maturation of already differentiated DC. In conclusion, our data suggest that glucocorticoids may act at the very first step of the immune response by modulating DC differentiation, maturation, and function. The Journal of Immunology, 1999, 162: 6473–6481.

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3 Abbreviation used in this paper: DC, dendritic cells; Dex, dexamethasone; GC, glucocorticoid; LY, Lucifer yellow; MR, mannose receptor; TT, tetanus toxin; CD40L, CD40 ligand; Ctr, untreated; FI, fluorescence intensity; CFII, comparative FI index; DX, dextran.
protocols. Here we report that exposure of monocytes to dexamethasone (Dex) together with GM-CSF and IL-4 inhibit their differentiation to DC. Moreover, Dex partially blocked terminal maturation of already differentiated DC.

Materials and Methods

Cytokines and reagents
Human recombinant GM-CSF (sp. act. 1.1 x 10^4 U/µg) was obtained from Novartis (Basel, Switzerland). Human recombinant IL-4 (sp. act. >2 x 10^4 U/mg) and human recombinant TNF-α (sp. act. >2 x 10^4 U/mg) were obtained from PeproTech (London, U.K.). Water-soluble Dex was obtained from Sigma (St. Louis, MO). Human recombinant IL-2 was obtained from Chiron (Milan, Italy). Human recombinant IL-10 was obtained from Schering-Plough (Kenilworth, NJ). LPS was obtained from Sigma (Escherichia coli 0127:B8).

DC culture
Highly enriched monocytes (>80% CD14+) were obtained from buffy coats of 20 blood donors (through the courtesy of Centro Trasfusionale, Ospedale San Raffaele, Milan, Italy) by Ficoll and Percoll gradients and purified by adherence. Monocytes were cultured for 7 days at 1 x 10^6/ml in 24-well Multitray tissue culture plates (Falcon, Becton Dickinson, Rutherford, NJ) in RPMI (Biochrom, Berlin, Germany) in 10% FCS (HyClone, Logan, UT) supplemented with 50 ng/ml GM-CSF, 10 ng/ml IL-4, and with (Dex-DC) or without (Ctrl-DC) different concentrations of Dex. In the control group (GM-CSF plus IL-4), the cell yield was about 80% of input cells. All the cultures were tested for the presence of endotoxin (<0.05 U/ml; endotoxin test).

DC maturation
TNF-α (10 ng/ml) or LPS (1 µg/ml) was added to induce maturation of DC. Alternatively, J558L cells transfected with CD40L (J558LcmCD40L) were used to induce CD40 triggering on DC. Untransfected J558L cells were used for control cultures. J558L after irradiation (10,000 rad) were seeded together with DC at a 1:1 ratio in 24-well culture plates in culture medium (1 x 10^6 cells/well). Cells were recovered after 48-72 h of culture.

FACS analysis
Cell staining was performed using mouse mAbs followed by FITC-conjugated affinity-purified, isotype-specific goat anti-mouse Abs (Ancell, Bayport, MN). The following mAbs were used: L243 (IgG2a, anti-MHC class II), TS1/18 (IgG1, anti-CD18), TS1/22 (IgG1, anti-CD11a), 17a (anti-CD11b), 3.2 (anti-CD32), and IV.3 (anti-CD64) (obtained from American Type Culture Collection, Manassas, VA); HK14 (IgG2a, anti-MHC class II), UC7M-1 (IgG2a, anti-CD14), and W6/32 (IgG2a, anti-MHC I) (obtained from Sigma); SK9 (IgG2b, anti-CD1a) and L306 (IgG2a, anti-CD58) (obtained from Becton Dickinson, San Jose, CA); B73.1 (IgG2a, anti-CD16) and PAM-1 (IgG1, anti-mannose receptor (MR) produced by immunizing mice with human alveolar macrophages (44)) (a kind gift of Dr. P. Allavena, Milan, Italy); BB1 (IgM, anti-CD80), BU66 (IgG1, anti-CD86), and EA-5 (IgG1, anti-CD40) (obtained from Ancell); HB15a (IgG2b, anti-CD83) (obtained from Immunotech, Marseille, France); CBR-IC3/1 (IgG1, anti-CD50) (obtained from Alexis Corporation, Nottingham, U.K.); and DX2 (IgG1, anti-CD95) (obtained from PharMingen, San Diego, CA). Results are expressed as the percent of positive cells or as fluorescence intensity (FI), calculated according to the formula: FI = mean fluorescence (sample) / mean fluorescence (control). The comparison of fluorescence intensity between two different groups was calculated as comparative FI index (CFI) according to the formula: CFI = (FI sample B/FI sample A).

Endocytosis
MR-mediated endocytosis was measured as the cellular uptake of FITC-DX (FITC-D) and quantified by flow cytometry. Approximately 2 x 10^6 cells for sample were incubated in media containing FITC-DX (1 mg/ml) (m.w. 40,000; Sigma) for 0, 60, and 120 min. After incubation, cells were washed twice with PBS to remove excess dextran and fixed in cold 1% formalin. The quantitative uptake of FITC-DX by the cells was determined using FACS. At least 8000 cells per sample were analyzed. Fluid-phase endocytosis via membrane ruffling was measured as the cellular uptake of 1 mg/ml of Lucifer yellow (LY) dipotassium salt (Sigma) and quantified by flow cytometry.

Electron microscopy
DC were processed for electron microscopy. DC were fixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Then, DC were postfixed with 1% OsO4 in cacodylate buffer at 4°C for 1 h, dehydrated in graded ethanol up to propylene-oxide, and finally embedded in an Epon-Araldite mixture. Well-preserved areas were identified by light microscopy of semithin sections (0.5 mm). Subsequently, serial ultrathin sections (80 nm) were mounted on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and finally examined with a Zeiss CEM 902 electron microscope (Oberkochen, Germany).

Calculations and statistical analysis
Data were expressed as mean ± SD. Comparisons were performed by Student’s t test. Values of p < 0.05 were considered statistically significant.
Results

Dex interferes with the differentiation of DC from human monocytes

To investigate the effect of Dex on DC differentiation from human monocytes, we cultured monocytes in the presence of GM-CSF, IL-4 (Ctr-DC), and different concentrations (10^{-10} M to 10^{-6} M) of Dex (Dex-DC). At concentration higher than 10^{-8} M, Dex significantly affected cell recovery (cells yield as percentage of Ctr cells: 10^{-7} M, 75 ± 6; 10^{-6} M, 50 ± 8; n = 20) (Fig. 1). The standard concentration of Dex chosen for the study was 10^{-8} M (cells yield as percentage of Ctr cells: 92 ± 13; n = 20). Upon culture with GM-CSF and IL-4, the cells became nonadherent, clustered, with abundant cytoplasm and protruding veils typical of DC (6, 11). In the presence of Dex 10^{-8} M, DC showed a more irregular external surface with more cytoplasmic projections and more endocytic vacuoles (Fig. 2). The presence of Dex during the differentiation of DC from monocytes induced a modification of membrane phenotype (Fig. 3). Ctr-DC expressed high levels of CD1a and were negative or low positive for CD14 and CD16, while Dex-DC were negative or low positive for CD1a but expressed high levels of CD14 and CD16 (CFII Dex vs Ctr: CD1a = 0.26; CD14 = 14.9; CD16 = 6.78; n = 20) (Table I). This effect

FIGURE 2. Morphological appearance at electron microscopy of Dex-DC and Ctr-DC. DC were differentiated from monocytes cultured for 7 days in GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) in the absence (left) or presence (right) of Dex 10^{-8} M. In the presence of 10^{-8} M of Dex, DC showed a more irregular external surface with more cytoplasmic projections and more endocytic vacuoles.

FIGURE 3. Flow cytometry analysis of molecules expressed by DC. Monocytes were cultured for 7 days with 50 ng/ml GM-CSF and 10 ng/ml IL-4 in the presence (Dex-DC) or absence (Ctr-DC) of Dex (10^{-8} M). Cells were labeled with the designed mAb and then with FITC-labeled goat anti-mouse-Ig. Shown is a representative experiment.
was dose and time-of-exposure dependent. In fact, the addition of Dex at day 1 and 16 after initiation of the 7-day culture had a lower inhibitory activity (Figs. 4 and 5). A partial conversion to a monocyte/macrophage phenotype was also seen when we added Dex for 7 days to differentiated immature DC. Analysis of MHC class I, MHC class II molecules, and CD80 showed an up-regulation in Dex-DC (CFII Dex vs Ctr: MHC I \(\times 1.61\); MHC II \(\times 1.31\); CD80 = 1.97; \(n = 20\)), while the expression of costimulatory molecules CD40, CD86 (Fig. 5), and CD58 was decreased (CFII Dex vs Ctr: CD86 \(\times 0.67\); CD40 \(\times 0.79\); CD58 \(\times 0.84\); \(n = 20\)). Furthermore, Dex up-regulated adhesion molecules like CD11a, CD11b, CD18, and CD54 and did not influence the expression of CD50. Ctr-DC and Dex-DC were both negative for CD68 and CD83. To evaluate whether the Dex-induced modifications in DC were reversible, DC were cultured for 7 days in the presence of GM-CSF, IL-4, and Dex; then Dex was washed away and cultures were prolonged with GM-CSF and IL-4 for 7 days. We compared these cells with DC obtained after 14 days of culture in GM-CSF, IL-4, and Dex. The modifications induced by Dex, as evaluated by phenotypic analysis, remained constant even after 7 days of culture without Dex (data not shown).

**Dex up-regulates MR-mediated as well as fluid-phase endocytosis**

Immature DC such as the cells derived by culturing monocytes with GM-CSF and IL-4 express a potent ability to uptake external

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**Table I. Phenotype analysis of DC differentiated in the presence of Dex**

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* DC were differentiated from monocytes with GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) with (Ctr-DC) or without Dex \(10^{-8}\) M (Dex-DC) for 7 days. Maturation was induced by culture with TNF-\(\alpha\) (10 ng/ml) or CD40L-transfected cell line (J558LmCD40L) for 48 h. Results are expressed as FI. Data were expressed as mean (\(n = 16\)). NT, Not tested.

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**FIGURE 4.** Dex affects DC differentiation. Membrane phenotype analysis of cells cultured for 7 days in GM-CSF (50 ng/ml) plus IL-4 (10 ng/ml) with or without Dex \(10^{-8}\) M. Cells were labeled with the designed mAb and then with FITC-labeled goat anti-mouse Ig. Results are expressed as FI, calculated according to the formula: FI = mean fluorescence (sample) – mean fluorescence (control). Each panel shows a dose-response relationship (Dex was added at the beginning of the culture at the indicated doses) and the influence of the timing of the addition (Dex \(10^{-8}\) M was added either at the beginning of the culture, day 0, or at day +2 or +6) (\(n = 6\)). *, \(p < 0.05\) vs control.
molecules, essentially via two main mechanisms: a receptor-mediated endocytosis and a fluid-phase endocytosis (macropinocytosis) (7). To study the endocytic activity of DC, two fluorescent markers were used: LY, a nonspecific fluid-phase marker, and FITC-DX, which is mainly taken up via the MR. Efficient accumulation of FITC-DX has been shown to be characteristic of immature DC, and neither monocytes nor macrophages share this property (7). Dex-DC showed a vigorous endocytosis of FITC-DX, higher than Ctr-DC (2.3-fold increase at 1 h, 2.4-fold increase at 2 h, n = 13) (Fig. 6A). The same behavior was seen when we used LY as a marker of fluid-phase pinocytosis (1.8-fold increase at 1 h, 1.6-fold increase at 2 h, n = 13) (Fig. 6B). We investigated the expression of two receptors involved in Ag capture in DC: MR and IgG FcRII (CD32). Dex up-regulates MR expression on DC (CFII = 1.61, n = 18, p < 0.05 vs Ctr). Similar up-regulation was observed for IgG FcRII CD32 (CFII = 2.16, n = 18, p < 0.05 vs Ctr). The cells cultured in the presence or absence of Dex were both negative for IgG FcRI (CD64).

**Dex impairs DC maturation after stimulation by TNF-α or CD40L.**

DC obtained with GM-CSF and IL-4 show functional and phenotypic characteristics of immature DC and can be further differentiated in vitro into mature DC. TNF-α 10 ng/ml was added to induce maturation of DC for 48 h. Alternatively, J558L cells transfected with CD40L (J558LmCD40L) were used to induce CD40 triggering on DC. Ctr-DC exposed to maturation-inducing stimuli showed an increase of MHC I, MHC II, CD80, CD86, CD40, CD54, CD58, and CD83 expression and a reduction of CD1a and CD83 expression was quantitatively less evident in Dex-DC than in Ctr-DC (Table I). Dex-DC were less sensitive to maturation-inducing stimuli. In fact, after TNF-α exposure the up-regulation of MHC I, MHC II, CD80, CD86, CD40, and CD83 expression was quantitatively less evident in Dex-DC than in Ctr-DC (Table I). The same behavior was seen after the exposure to the more powerful stimulus CD40L. Inhibition of DC maturation by Dex was confirmed also in endocytosis assay. The endocytic activity of FITC-DX and LY, despite a reduction induced by maturation, remained higher in Dex-DC than in Ctr-DC (Table I).

**Dex down-regulates the immunostimulatory capacity of DC**

DC are potent stimulators of allogeneic T cells. We tested if Dex-DC were able to stimulate allogeneic T lymphocytes in MLR. Dex-DC showed an impaired capacity to induce MLR (Fig. 7). In vitro exposure of DC to TNF-α or CD40L (Fig. 7) increased T cells proliferation in Ctr-DC and in Dex-DC but the ability of Dex-DC was always lower than control DC. The down-regulation
of APC function by Dex was noted even when DC were formalin-fixed before setting-up the MLR (data not shown).

**Dex down-regulates the presentation of soluble Ag by DC**

In view of the fact that Ag capture was increased in cells cultured with Dex but the stimulatory capacity was impaired in MLR, we evaluated the ability to present soluble Ag that need to be uptaken and processed. Cells differentiated in the presence of Dex showed much lower efficiency in presenting TT to specific autologous T cell lines (Fig. 8). In vitro exposure of DC to TNF-α or CD40L (Fig. 8) for 48 h after Ag pulsing increased T cell proliferation in Ctr-DC and in Dex-DC, but the latter were less potent than Ctr-DC.

**Dex affects cytokine production by DC**

To investigate the capacity of Dex to interfere in cytokines production, after 7 days of culture with GM-CSF and IL-4 with or without Dex, DC were washed, seeded in the presence of maturation-inducing stimuli, and cultured for 3 days. Supernatants were quantified for IL-1β, IL-6, IL-10, IL-12 p70, and TNF-α. Dex-DC showed a reduction of TNF-α and IL-1β production in response to the maturation-inducing stimuli CD40L, LPS, and TNF-α. IL-12 p70 production was decreased when Dex-DC were exposed to TNF-α or LPS, while with CD40L, a more powerful stimulus, the secretion of IL-12 p70 was comparable in Dex-DC and in Ctr-DC (Fig. 9). IL-6 and IL-10 production was not affected.

**Effects of Dex on the maturation of DC**

To evaluate the effects of Dex on the maturation of DC induced by LPS or ligation of CD40, Dex $10^{-8}$ M was added to 7-day culture DC for 48–72 h, together with the maturation stimulus. Dex partially inhibited the LPS-induced up-regulation of costimulatory molecules (e.g., CD40, CD80, CD83, and MHC II) and their accessory cell function in MLR. In addition, DC exposed to LPS and Dex had higher levels of MR and CD32 and higher endocytic activity of FITC-DX than LPS-treated control cells (data not shown). These results indicate that Dex interferes with process of DC maturation induced by LPS and freezes the cells at an immature stage. Similar result were obtained when CD40L-transfected cells where used as maturation stimulus, though inhibition of DC maturation was less striking, as ligation of CD40 is a more potent stimulus than LPS (data not shown).

**Discussion**

GCs are physiological inhibitors of inflammatory responses and are widely used as immunosuppressive and antiinflammatory agents. The present study demonstrates that one of the mechanisms...
by which corticosteroids can suppress the immune response in humans is by inhibiting differentiation, terminal maturation and function of DC. Because DC has the unique property to activate naive T cells and are required for the induction of a primary response, suppression of DC function may very efficiently control the specific immune response. Dex, a potent synthetic steroid, showed complex effects on DC. Dex partially blocked the GM-CSF plus IL-4-driven differentiation of monocytes into DC. In fact, in Dex-treated cells the expression of CD1a was inhibited and CD14 and CD16 expression was increased, two markers of mature macrophages normally not present in DC. Interestingly, previous findings showed that in mice a single injection of Dex resulted in a dose-dependent loss of splenic DC and a reciprocal increase in the macrophage population (45); moreover both topical and systemic administrations of GCs resulted in a decreased density of Langerhans cells as assessed by staining for la Ag (46). In addition to a partial inhibition of differentiation, Dex-differentiated DC showed important modifications in membrane phenotype. During DC differentiation from precursors, two major stages can be identified (6, 7, 47, 48): 1) an immature stage characterized by a high efficiency in taking up and processing Ags; and 2) a mature stage characterized by the loss of Ag uptake capacity and migration to regional lymph nodes where DC exert their function of potent APC (49, 50). When added at the beginning of the culture, Dex induced some paradoxical modifications. In fact, Dex simultaneously increased the expression of molecules involved in Ag capture (CD32, MR, CD16, CD11b) and molecules involved in Ag presentation and T lymphocytes stimulation (MHCI-I, MHCIII, CD80, CD54). Moreover, some important costimulatory molecules (CD86, CD40, CD58) were inhibited. Overall this phenotype correlates with impaired Ag-presenting function to T lymphocytes and a higher endocytic activity. Our data showed also that Dex partially inhibits DC maturation after exposure to TNF-α or CD40L. In the literature, two reports have shown that, in mice, corticosteroids inhibit DC maturation. Moser have reported that Dex affects culture-induced maturation of mouse DC by selectively inhibiting CD80 and CD86 expression (45). Kitajima have shown that Dex inhibits the T cell-mediated terminal maturation of a murine epidermal-derived dendritic cell line by diminishing CD86 expression and cytokine secretion (51). In our system, Dex inhibited the MHCI-II, CD86, CD40, and CD83 increase induced by maturation and the secretion of some cytokines. The observation that Dex inhibits cytokine secretion by DC is not unexpected. In fact GCs are known to down-regulate the capacity of monocytes and macrophages to secrete IL-1 (18–21), IL-6 (22), TNF-α (24), and IL-12 (52). The inhibition of secretion of those cytokines that play a relevant role in the induction of T cell responses demonstrates a relevant mechanism of GC action in the first phase of the human immune response.

The effects of Dex on immature DC, which have already been differentiated for 7 days in the presence of GM-CSF and IL-4, appear to be similar but not identical to the effects of Dex included at the beginning of the culture. Overall the effect of GCs on immature DC can be summarized as follows: a partial conversion to a monocyte/macrophage phenotype, an impaired capacity to reach maturation, and a decreased ability to stimulate T cells (data not shown). These results confirm the in vitro instability of immature DC generated with GM-CSF and IL-4 (53). Palucha et al. (54) showed that upon removal of both GM-CSF and IL-4 and/or re-culture with M-CSF, immature CD1a/CD14+ DC easily converted to a macrophage phenotype-expressing CD14 with a decreased ability to stimulate allogeneic T cells. Dex showed a similar action even in the presence of GM-CSF and IL-4. It is tempting to speculate that GCs may act at two different steps of DC life: 1) by inhibiting the differentiation from blood precursors, thus impairing the normal turnover of DC in tissues, and 2) by inhibiting the terminal maturation of DC into a potent APC.

On the molecular mechanisms by which GCs modulate maturation and differentiation of DC, we can speculate a possible role of the NF-κB-Rel transcription factor family. NF-κB-Rel protein’s family plays an important role in the expression of genes involved in the immune response or acute-phase reaction. DC contain high levels of all known Rel family members and express strong activity for NF-κB in DNA binding (55–57). The expression of Rel B correlates with DC differentiation, and disruption of Rel B expression in Rel B−/− animals blocked the development of DC (57–59). Recently, it was shown that GCs are potent inhibitors of NF-κB in mice and cultured cells. The activation of NK-κB involves the targeted degradation of its cytoplasmic inhibitor IκBα and the translocation of NF-κB to the nucleus. GCs induce the transcription of the IκBα gene, which results in an increased rate of IκBα protein synthesis and in a reduction of the amount of NF-κB that translocates to the nucleus (60, 61). Therefore inhibition of NF-κB activity by GCs is likely to have a role in the impaired DC differentiation. Interestingly the effects of Dex on DC maturation and differentiation are very similar to those observed with IL-10, an antiinflammatory cytokine. IL-10 was shown to prevent differentiation of monocytes to DC (14, 62), to promote their maturation to macrophages (63), to impair DC’s capacity to induce a Th1 response, to inhibit IL-12 secretion (64, 65), to increase DC’s capacity to capture Ags (66), and to inhibit DC’s maturation (67, 68). IL-10 also inhibits NF-κB activity via an effect on IκBα (69). However, we can exclude that GC’s effects on DC are mediated by IL-10 as we show that Dex-DC did not secrete augmented levels of IL-10.

Another important point to clarify is whether the effects described in this study are relevant only at pharmacological doses of GCs or have relevance also for physiological concentrations. In humans, the main GC in plasma is cortisol. Cortisol is secreted episodically with 8–10 bursts per day, especially in the morning, leading to a diurnal fluctuation of plasmatic concentration ranging between 0.80 and 6.90 × 10−7 M (70). However only 3%–10% of circulating cortisol is in free state. In our experiments, we used a standard concentration of 10−8 M of Dex. Because Dex has a GC potency of 30 and cortisol is arbitrarily assigned a value of 1 (71), in terms of GC potency, 10−8 M of Dex corresponds to 3 × 10−7 M of cortisol. This value is about 4- to 125-fold higher than the normal free plasma cortisol, and so we can assume that the effects described on DC differentiation and function by GCs are not relevant in normal conditions. However, in situations of acute stress or in some pathological conditions, endogenous GCs may reach levels that are in the range of the concentration used in this study. In fact, cortisol secretion increases up to 10-fold in acute stress situations (72) like trauma, surgeries, burns, etc. DC may be an important target of the immune system-CNS regulatory loop (72). Inflammatory cytokines (e.g., IL-1, IL-2, IL-3, IL-6, and TNF) and inflammatory mediators (e.g., PGF2α and platelet-activating factor) stimulate the hypothalamic-pituitary-adrenal axis to increase cortisol plasma levels (72). These, in turn, suppress the inflammatory and immune response via a variety of mechanisms, and this study shows that this suppression may occur also by inhibiting DC differentiation and function. Several lines of evidence suggest that high cortisol levels are associated with immunosuppression in several pathological conditions, including depression, chronic alcoholism, anorexia nervosa, and bulimia (71). Moreover, very high cortisol levels were seen in some malignant neoplasms like bronchial carcinoids, medullary thyroid carcinoma, and metastatic prostatic carcinoma due to the capacity of these tumors to produce
corticotropic-releasing hormone (71). Also, patients with Cushing syndrome, characterized by hypercortisolemia, are highly susceptible to opportunistic infections (73), and there is a direct correlation between the risk of infection and the degree of hypercortisolism (73). Moreover, patients receiving corticosteroid therapy for long periods show a defect of T-cell-mediated immunity and susceptibility to opportunistic infections. In conclusion, our data suggest that GCs modulate DC differentiation, maturation, and function and these effects could be part of the dynamic regulatory interactions between the immune and neuroendocrine system. GCs may act at the very first step of the immune response by inhibiting maturation of DC into potent APC, thereby preventing the activation of naive T cell.

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