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Glucocorticoids down-regulate dendritic cell function *in vitro* and *in vivo*

Exogenous glucocorticoid hormones are widely used as therapeutical agents, whereas endogenous glucocorticoids may act as physiological immunosuppressants involved in the control of immune and inflammatory responses. The optimal activation of T lymphocytes requires two distinct signals: the major histocompatibility complex-restricted presentation of the antigen and an additional co-stimulatory signal provided by the antigen-presenting cells. There is ample evidence that, among the cells able to present the antigen, the dendritic cells (DC) have the unique property to activate antigen-specific, naive T cells *in vitro* and *in vivo*, and are therefore required for the induction of primary immune responses. In this work, we tested whether glucocorticoids affected the capacity of DC to sensitize naive T cells. Our data show that, *in vitro*, the steroid hormone analog dexamethasone (Dex) affects the viability of DC, selectively down-regulates the expression of co-stimulatory molecules on viable DC, and strongly reduces their immunostimulatory properties. *In vivo*, a single injection of Dex results in impaired antigen presenting function, a finding which correlates with reduced numbers of splenic DC. These results show that glucocorticoids regulate DC maturation and immune function *in vitro* and *in vivo* and suggest that this mechanism may play a role in preventing overstimulation of the immune system.

1 Introduction

Glucocorticoids (GC) are widely used as anti-inflammatory and immunosuppressive agents to prevent graft rejection and to treat autoimmune and allergic diseases. The inhibitory effect of GC on the immune system is still poorly understood, although several reports have shown that GC inhibit the transcription of many interleukins [1–3] as well as the cell surface expression of MHC-encoded restricting elements [4, 5], and down-regulate signal transduction through IL-2 receptor [6].

The discovery of MHC restriction has focused attention on the main role of the antigen presenting-cells (APC) on the induction of immune responses. More recently, it has been shown that APC not only display processed antigen in the polymorphic MHC peptide-binding groove, but also express a second co-stimulatory signal which determines the outcome (*i.e.* activation versus the induction of anergy) of T cell receptor occupancy [7].

Although B cells, macrophages and dendritic cells (DC) can present antigens to T cells, only DC have the capacity to activate naive T cells fully *in vitro* and *in vivo* (for review, see [8]). For this report, we investigated whether DC function was affected by GC *in vitro* and *in vivo*. Our data show that dexamethasone (Dex), a potent steroid hormone analog, affects the viability of splenic DC and down-regulates the immunostimulatory properties of remaining DC *in vitro*. Furthermore, the injection of Dex results in decreased DC numbers and impaired DC function, suggesting that, *in vivo*, GC may control the immune response at the level of antigen presentation.

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Abbreviations: **DC:** Dendritic cell **Dex:** Dexamethasone **SEB:** Staphylococcal enterotoxin B **GC:** Glucocorticoid

Key words: Glucocorticoids / Dendritic cells / Co-stimulatory molecules / Antigen presentation

2 Materials and methods

2.1 Mice

Female DBA/2 (H-2^d) and CBA (H-2^k), 6–8 weeks old, were purchased from Charles River Wiga (Sulzfeld, Germany), and maintained in a pathogen-free environment.

2.2 Reagents and antibodies

Ethanol-soluble Dex for studies *in vitro* (9a-fluoro-16a-methyl-prednisolone) and water-soluble Dex for injection *in vivo* were purchased from Sigma (Bornem, Belgium). RU486 was obtained from Roussel UCLAF (Paris, France).

The hamster antibody to mouse CD3ε, 145-2C11 [9], was used to stimulate T cells. Purified exotoxin staphylococcal enterotoxin B (SEB) was purchased from Toxin Technology (Sarasota, FL). Recombinant GM-CSF was a kind gift from Innogenetics (Gent) and from Dr. Kris Thielemans (VUB, Brussels, Belgium).

2.3 Cell line

The I-E^d-restricted, myoglobin-specific T cell hybridoma 13-26-8-HG.1 [10] was derived by Dr. A. Livingstone (Basel Institute of Immunology, Basel, Switzerland).

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2.4 T cell activation *in vitro*

The complete medium used in all experiments was RPMI 1640 (Seromed Biochem KG, Berlin, Germany) supplemented with 2% HY ultroser (a serum-free medium supplement purchased from Gibco BRL, Merelbeke, Belgium) or with 5% FCS (Gibco BRL), penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 2-ME and L-glutamine (Flow ICN Biomedicals, Bucks, GB).

Whole spleen cells, G-10-nonadherent spleen cells or purified T lymphocytes (see figure legends) were stimulated with serial dilutions of SEB or anti-CD3 mAb 145-2C11 in round-bottom 96-well plates, or were cultured in the presence of various numbers of irradiated allogeneic spleen cells. The proliferation was measured by incorporation of 0.4 μCi/well of tritiated thymidine (specific activity: 50 Ci/mmole) during the last 16 h of 2–4-day culture, as indicated in the figure legends. The supernatants were collected after 24–48 h of culture, frozen and assayed for IL-2 content by a bioassay using an IL-2 dependent, IL-4 insensitive subclone of the CTL.L line.

2.5 Purification of DC and treatment with Dex

Spleens were digested with collagenase (CLSIII; Worthington, Freehold, NJ) and separated into low and high density fractions on a BSA gradient (Bovuminar Cohn fraction V powder; Armour, Tarrytown, NY). The splenic DC were purified according to a procedure described by Crowley et al. [11]. Low-density cells were cultured for 2 h in complete medium containing 5×10^{-8} M Dex or solvent (ethanol) and the nonadherent cells were removed by vigorous pipetting. The adherent cells were cultured for 1 h in serum-free medium and the nonadherent cells were removed by gentle pipetting. The remaining cells were incubated overnight in complete medium containing Dex or solvent and the nonadherent cells (containing the DC-enriched fraction) were washed, counted and assayed for their antigen presenting function. In some experiments (see figure legends), 10 ng/ml recombinant granulocyte/macrophage (GM)-CSF was added in the 2 h culture (first adherence) and during overnight culture.

2.6 Treatment with Dex *in vivo*

Water-soluble Dex, solubilized in pyrogen-free NaCl 0.9%, was injected intraperitoneally into mice 24 h before testing.

2.7 FACS analysis

The cells were incubated with 2.4G2 (a rat anti-mouse Fcγ RII/III mAb) for 10 min prior to staining to prevent antibody binding to FcR, and incubated with FITC-coupled mAb: 14.4.4 (murine IgG2a anti I-E^d), N418 (hamster anti-murine CD11c, 12), 16-10A1 (rat IgG2a anti-B7.1, 13), GL1 (hamster mAb anti B7.2, 14), anti-ICAM-1 (Pharmingen-San Diego, CA), and 145-2C11 (hamster mAb anti CD3ε, 9). The cells were stained for CD11b/Mac-1α expression using biotinylated anti-CD11b (Pharmingen), which was revealed by FITC-conjugated streptavidin (Amersham, Gent, Belgium). To detect the presence of ligands of CTLA4, the cells were incubated with the human fusion protein CTLA4-Ig (CTLA4 human IgG fusion protein, [15]), and bound CTLA4 was revealed by fluorescent anti-human IgG reagent (Biosource International, Camarillo, CA). For two-color staining, the cells were incubated simultaneously either with CD45R/B220-specific mAb (rat IgG2a, Pharmingen) coupled to phycoerythrin, or with biotinylated N418 followed by PE-avidin and a fluoresceinated mAb (see above). The cells were gated for size and side scatter to eliminate dead cells and debris, and analyzed on a FACScan (Becton Dickinson, CA). To enumerate DC accurately, which only represent 1–2% of unseparated spleen cells, we used combinations of antibodies which include a positive (N418) and a negative (B220) marker, an approach which enabled us to distinguish DC from autofluorescent, double-positive cells.

3 Results

3.1 Dex selectively down-regulates B7 expression on DC in culture

It has been shown that the immunostimulatory properties of purified DC correlated with the expression of molecules of the B7 family [16]. Since DC have been shown to undergo maturation *in vitro* [17–19], we analyzed the expression of CTLA-4 ligand, B7-1 and B7-2 molecules on fresh versus 24 h-cultured DC, and found that B7 expression was strongly up-regulated during overnight culture. Indeed, fresh (0 h) low-density spleen cells and purified (after 24 h of culture) DC were double stained with N418 (mAb specific for CD11c, selectively expressed on murine DC) and either CTLA-4-Ig followed by anti-human mAb, anti-B7-1, anti-B7-2 or anti-I-E. The results in Fig. 1, in agreement with similar data recently published by Inaba et al. [20], indicate that fresh, immature DC, which have been shown actively to process antigen but only weakly to

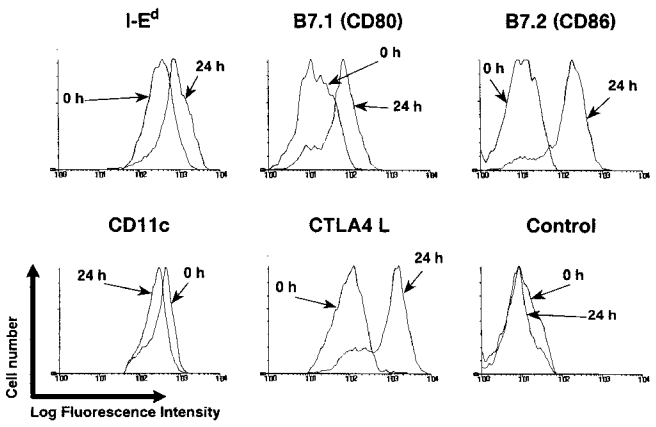

Figure 1. The expression of the B7 family is up-regulated on DC during culture. Fresh low-density spleen cells (0 h) and purified DC (24 h) from DBA/2 mice were stained for red fluorescence with N418-biotin followed by phycoerythrin-conjugated streptavidin and for green fluorescence with CTLA4-Ig followed by anti-human-FITC, with anti-human-FITC alone (negative control), with anti-B7-1-FITC, with anti-B7-2-FITC or with anti-I-E^d-FITC. The data represent the expression of various markers on cells gated for N418 expression.

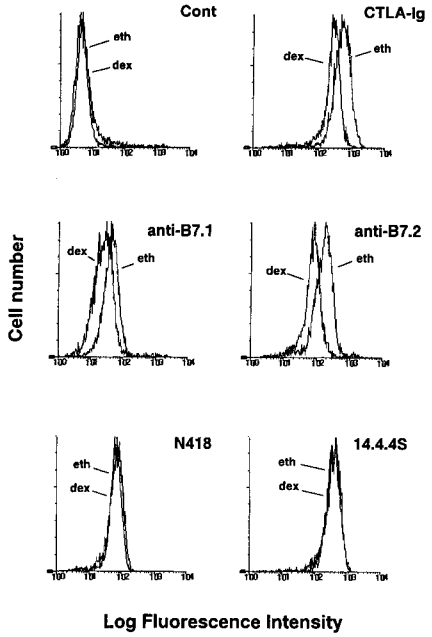

Figure 2. Dex selectively down-regulates CTLA-4 ligand expression on DC. DC were purified from spleens of DBA/2 mice in the presence of 5×10^{-8} M Dex or solvent only (eth), washed and stained using reagents described in Sect. 2.7. As negative control, cells were stained with anti-human antibodies coupled to FITC (cont).

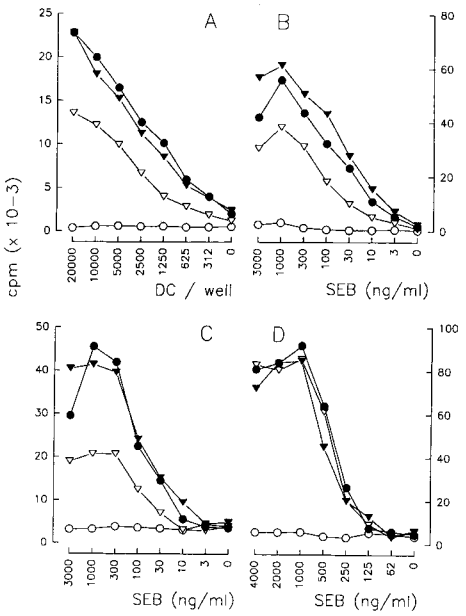

Figure 3. Dex-treated DC have a decreased capacity to sensitize naive T cells, but fully activate T-T hybridoma cells. (A) Various numbers of ethanol-, Dex-treated DC or both, isolated from DBA/2 mice, were cultured with 3×10^5 G10-nonadherent spleen cells from CBA mice, and proliferation was measured by thymidine incorporation after 3 days of culture. (B, C) Control and/or Dex-treated DC (10^4) from DBA/2 mice were cultured with 3×10^5 G10-nonadherent syngeneic spleen cells in the presence of serial dilutions of SEB. The proliferation was measured by thymidine incorporation after 2 days of culture (B) and the IL-2 production was quantitated from the 24 h culture supernatants (C). (D) Control DC, Dex-treated DC (10^4), or 10^4 of both were cultured with T-T hybridoma cells 13-26-8 and serial dilutions of SEB. After 24 h, culture supernatants were assayed for IL-2 production in response to SEB. Closed circles: ethanol-treated DC; open triangles: Dex-treated DC; closed triangles: same numbers of ethanol- and Dex-treated DC were cultured with responder cells; open circles: responder cells alone.

activate resting T cells [17], express detectable, albeit low levels of co-stimulatory molecules. Mature DC, by contrast, which have lost the capacity of processing, while acquiring the unique ability to activate naive T cells, display high levels of CTLA4 ligands, B7-1 and B7-2, and MHC class II molecules. The N418$^-$ fraction, which is composed of low-density T and B cells, did not display significant increases in B7 expression after overnight culture, showing that the up-regulation of B7 was restricted to DC (data not shown).

To evaluate the effect of GC on DC maturation *in vitro*, low-density spleen cells were cultured in the presence or absence of Dex during the purification procedure. The DC-enriched fraction was stained with N418 mAb or with antibodies specific for class II expression and for B7-1 and B7-2 molecules. Fig. 2 shows that DC isolated in the presence of 5×10^{-8} M Dex had reduced surface expression of CTLA-4 ligand, as compared to control DC. By contrast, the expression of CD11c and class II determinants remained unchanged. In addition, Dex-treated DC had lower expression of B7-2 and, to a lesser extent, of B7-1, as assessed by staining using specific mAb (Fig. 2). It should be noted that Dex also affected the viability of DC: the number of DC isolated in culture containing 5×10^{-8} M Dex was reduced by 10 to 50% compared to ethanol-treated DC.

3.2 Dex down-regulates the immunostimulatory capacity of DC *in vitro*

To test whether the decrease in B7 expression correlated with diminished APC function, untreated and Dex-treated DC were tested for their capacity to activate naive T cells *in vitro*. Allogeneic T cells (CBA, H-2^k) were stimulated in the presence of DC purified from DBA/2 mice (H-2^d) (Fig. 3A). In addition, DBA/2 naive T cells were stimulated in the presence of syngeneic DC with various doses of the bacterial superantigen SEB (Fig. 3B, C). The data show that in both assays, the accessory activity was impaired when the DC were purified in the presence of Dex, as assessed by diminished T cell proliferation (Fig. 3A, B) and IL-2 production (Fig. 3C). The addition to the same culture of ethanol-treated DC restored the T cell activation to control level, showing that Dex inhibits the APC function but does not affect the responder T cell population.

We next compared the ability of control and Dex-treated DC to stimulate naive T cells and a CD28-negative T cell hybridoma (known to respond optimally to T cell receptor ligation in the absence of B7-related co-stimulatory functions, [21]), in the presence of the bacterial superantigen SEB. The data in Fig. 3D demonstrate that both control and Dex-treated DC were able efficiently to stimulate IL-2 production by a T cell hybrid. These observations strongly suggest that DC recovered from Dex-supplemented cultures retain the ability to generate a TcR ligand, but display defective co-stimulatory properties.

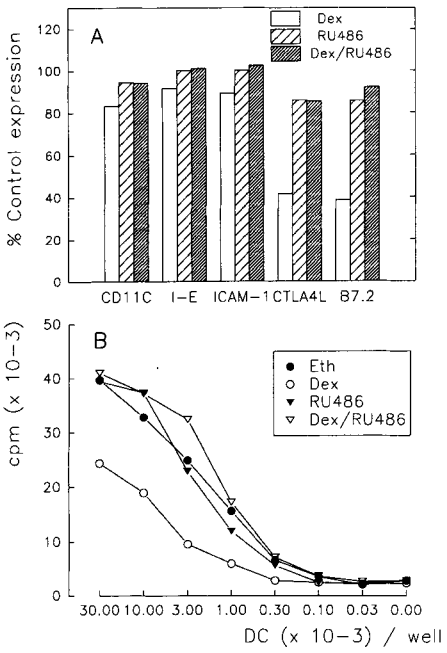

Figure 4. The inhibitory effect of Dex on DC phenotype and function requires binding to steroid receptor. DC were purified from DBA/2 mice in the presence of 5×10^{-8} M Dex or solvent only (ethanol). In some groups, 5×10^{-7} M RU486 was added to the culture 15 min prior to addition of ethanol (RU486) or Dex (Dex/RU486). (A) DC were stained for expression of CD11c, I-E, ICAM-1, CTLA4-ligand and B7-2 using reagents described in Sect. 2.7. Data are expressed as mean fluorescence intensity relative to control DC. (B) G10-nonadherent spleen cells (3×10^5) from CBA mice were stimulated with decreased numbers of DC purified under different conditions. The proliferation was measured by thymidine incorporation after 2 days of culture.

3.3 RU486 prevents Dex-induced down-regulation of accessory cell function *in vitro*

To test whether the inhibition of DC function by Dex required binding to steroid receptors, we evaluated the effect of the potent steroid hormone antagonist RU486. Fig. 4 shows that addition of 5×10^{-7} M RU486 15 min before Dex (5×10^{-8} M) largely prevented the decrease in B7 expression (Fig. 4A) as well as the inhibition of the immunostimulatory capacity of DC (Fig. 4B).

3.4 The addition of GM-CSF prevents the inhibitory effect of Dex

Since GM-CSF was found to sustain DC viability and promote their maturation [17], we tested whether the addition of GM-CSF would restore the phenotype and APC function of Dex-treated DC. Fig. 5 shows that DC cultured with Dex and 10 ng/ml recombinant GM-CSF expressed increased levels of B7-1 and B7-2 co-stimulatory molecules compared to DC treated with Dex only (Fig. 5A) and exhibited the immunostimulatory capacities of control DC in an allogeneic MLR assay (Fig. 5B).

3.5 Injection of Dex *in vivo* results in impaired DC function

Mice were injected with various doses of Dex intraperitoneally and their splenic cells were tested *in vitro* in DC-independent and DC-dependent activation assays. Fig. 6 shows that stimulation of spleen cells with the T cell mitogen anti-CD3 mAb (which requires FcR^+ B cells or macrophages, [22]) resulted in strong proliferation in all groups, suggesting that T cell function was not affected by Dex at the doses used. By contrast, the proliferation induced by SEB, a superantigen which has been shown to be best presented by DC [23, 24], was impaired in animals injected with Dex. The inhibition was dose-dependent and could be overcome by addition of DC to the culture (data not shown). The spleen cells from control and Dex-injected mice were irradiated and used as APC for unprimed allogeneic T cells. Fig. 7 shows that spleen cells from mice injected with Dex had a decreased capacity to activate the proliferation of allogeneic T cells in culture.

B and T lymphocyte, DC and macrophage populations were analyzed in the spleens of control versus Dex-injected animals. Unseparated spleen cells were double-stained for CD45R/B220 in red and class II, CD3, CD11c or CD11b/Mac-1α in green. The results in Table 1 show

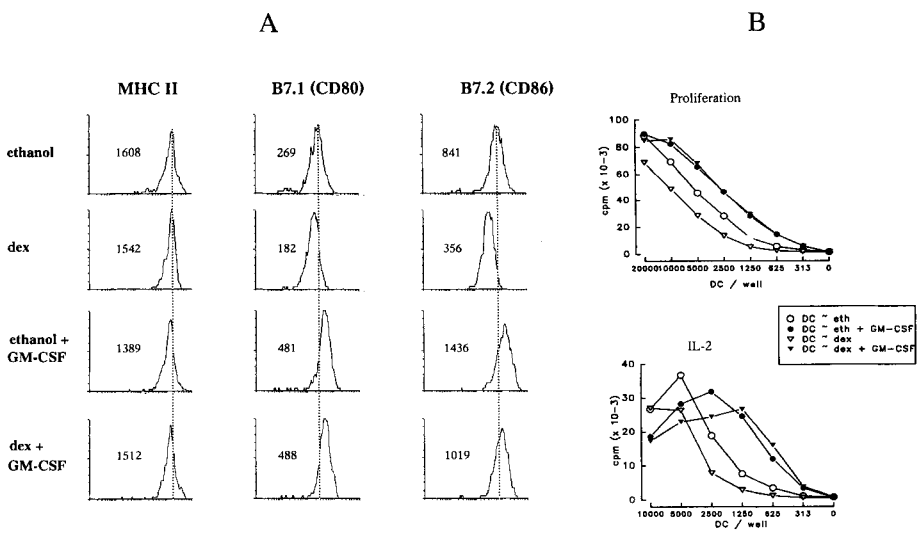

Figure 5. The addition of GM-CSF prevents the inhibitory effects of Dex. DC were isolated from DBA/2 mice in the presence of ethanol or Dex. In some groups, GM-CSF (10 ng/ml) was added in the cultures. (A) DC were stained for MHC class II, B7-1 or B7-2 expression using reagents described in Sect. 2.7. The numbers included in the figure represent the mean fluorescence intensity. (B) Purified T cells from CBA mice were stimulated with various numbers of DC isolated under different conditions. Proliferation was measured by thymidine incorporation after 96 h of culture. IL-2 production was measured in the supernatants after 48 h of culture.

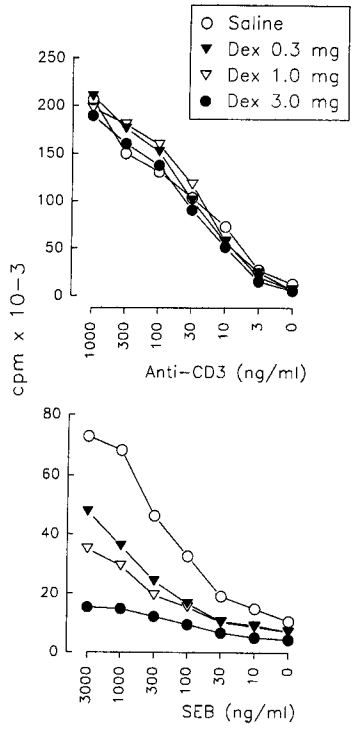

Figure 6. Mice injected with Dex are hyporesponsive to SEB. DBA/2 mice were injected with 3, 1 or 0.3 mg of Dex intraperitoneally and their spleens harvested 1 day later. Spleen cells (5×10^5) were cultured in the presence of serial dilutions of anti-CD3 mAb or SEB. The proliferation was measured by thymidine incorporation 24 h later.

that mice injected with Dex displayed a dose-dependent reduction in the relative number of splenic DC (identified as $CD11c^+$, $CD45R/B220^-$) compared to mice injected with saline only. By contrast, the percentages of B and T lymphocytes remained unchanged, whereas the relative numbers of splenic macrophages ($CD11b/Mac\text{-}1^+$, $CD45R/B220^-$) increased in the same mice. The expression of I-E decreased in all mice injected with Dex in a dose-dependent manner. Thus, although a single injection of GC appears to have pleiotropic effects, the lowest doses of GC used in this study provoke a selective decrease in DC numbers in mice, which correlates with impaired APC function.

4 Discussion

The present study demonstrates that one of the mechanisms by which corticosteroids can suppress immune responses *in vitro* and *in vivo* is by inhibiting the ability of splenic DC to present antigen to responding T lymphocytes. Since DC have the unique property to activate naive T cells and are required for the induction of a primary response, the suppression of DC function may very efficiently control specific immune responses.

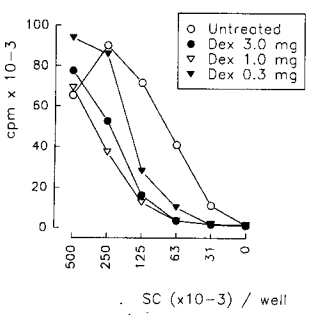

Figure 7. Injection of Dex *in vivo* results in decreased capacity to activate naive T cells *in vitro*. DBA/2 mice were injected with 3, 1 or 0.3 mg of Dex intraperitoneally and their spleens harvested 24 h later. Various numbers of irradiated spleen cells were cultured with 5×10^5 allogeneic T cells. The proliferation was measured by thymidine incorporation after 96 h of culture.

Table 1. Effect of Dex on splenic cell populations

	PBS[a)]	Dex 0.03 mg	Dex 0.1 mg	Dex 0.3 mg	Dex 1 mg
T cells[b)]	21.0[c)]	24.9	26.3	24.7	29.2
B cells	57.3 (172)[d)]	56.5 (112)	55.8 (94)	54.9 (49)	49.7 (28)
DC	1.4	0.6	0.4	0.3	0.2
Macrophages	2.0	3.1	4.2	5.2	6.2

a) The numbers of spleen cells were: 5.6×10^7 (PBS); 4.4×10^7 (0.03 mg); 4.4×10^7 (0.1 mg); 2.8×10^7 (0.3 mg); 2.2×10^7 (1 mg).
b) Splenic cell populations were identified as follows: T cells, CD3; B lymphocytes, $CD45R/B220^+$; DC: $CD45R/B220^-$, $CD11c^+$; macrophages: $CD45R/B220^-$, $CD11b/Mac\text{-}1\alpha^+$.
c) % splenic cells.
d) The numbers in parentheses represent the mean channel fluorescence for MHC class II-(I-E) staining of B lymphocytes.

In vitro, we show that Dex, a potent synthetic steroid, selectively down-regulates the expression on DC of B7 molecules which are required for optimal activation of specific T cells. All other markers tested (CD11c, class II, ICAM-1) remained unchanged. The decrease in the expression of the co-stimulatory signal correlates with impaired antigen-presenting function to naive T lymphocytes (the inhibition of function in over 50 experiments ranged between 30% and 90%). Of note, no such effect was observed when Dex was added to the medium later, *i.e.* after the first adherence *in vitro* (data not shown), suggesting that the maturation of splenic DC is induced early in culture and may not be reversible.

Our data show that the addition of Dex during the purification procedure does not impair the ability of DC to present the superantigen SEB to the class II-restricted T cell hybridoma, but strongly inhibits their capacity to present the same antigen to naive cells. These results suggest that the ability of DC to generate a TcR ligand is not affected by Dex, a finding which correlates with the intact cell surface expression of class II antigens by GC-treated DC, but that their capacity to sensitize naive T lymphocytes is impaired. Therefore, the immunosuppressive effect of GC is probably due to a reduced ability to deliver the co-stimulatory signal, which has been shown to determine the outcome (activation versus anergy) of T cell receptor occupancy. In particular, it would be of interest to analyze whether T lymphocytes cultured with DC purified in the presence of Dex are rendered anergic, *i.e.* become unable to respond to a subsequent stimulation by APC expressing both signals. The dramatic effect in APC activity could be due to limiting expression of molecules of B7 family, to the down-regulation of yet undefined co-stimulatory ligands important to the unique function of these cells, or both. Of note, a recent report by Cassel and Schwartz [25] shows that a slightly diminished expression of B7 on APC correlates with a lower immunostimulatory capacity.

It should be noted that Dex affects the DC yield in culture, as assessed by N418 staining: depending on the experi-

ment, the number of DC isolated in the presence of 5×10^{-8} M Dex is reduced by 10 to 50% as compared to the control population. Experiments are underway to test whether the mechanism affecting DC viability is apoptosis, as recent data by Kämpgen et al. [26] have shown that DC may undergo apoptosis in the presence of Dex *in vitro*.

Thus, the addition of Dex to DC cultures appears to have two distinct effects: it results in reduced numbers of viable DC recovered following overnight culture, and furthermore, viable cells recovered in these cultures display diminished levels of B7-1 and B7-2 molecules, but unchanged levels of MHC class II, CD11c and CD54. Of note, the viable, Dex-treated DC have the same light scattering properties as control DC and exclude propidium iodide, indicating that down-regulation of B7 molecules is not merely due to toxicity.

The mechanism by which GC down-regulate co-stimulatory function of DC remains to be determined. However, the observation that GM-CSF prevents or reverses the phenomenon suggests that Dex may indirectly inhibit DC function by blocking a soluble factor which is required for DC maturation. It is possible that GM-CSF released by splenic macrophages in culture is essential for inducing the maturation and may be the target of Dex. Experiments are in progress to quantify the GM-CSF present during overnight culture of DC in the presence or absence of Dex. Alternatively, the addition of GM-CSF with Dex in the culture could promote the maturation of a subset of DC which is less sensitive to GC. Spleen DC cultured in GM-CSF-containing medium expressed increased levels of both B7-1 and B7-2 (Fig. 5), as previously shown by Larsen et al. [27]. Of note, the addition of GM-CSF prevents the decrease in DC number, suggesting that GC do not have a direct cytotoxic effect on DC, but rather indirectly affect their viability. It should be noted that neither IL-1α nor TNFα could restore the steroid-mediated decreased B7 expression of DC (our unpublished data).

In vivo, a single injection of Dex results in a dose-dependent loss of splenic DC and a corresponding inability of splenic cells to sensitize naive T cells optimally. The mechanism of DC loss remains to be determined and could be the result of a migration out of the spleen, or alternatively, due to the induction of programmed cell death. Studies *in vitro* show that some DC do not survive Dex treatment, and that the remaining DC have impaired APC function. It is possible that, upon sustained GC secretion *in vivo*, following stress or infection, some DC would migrate or undergo apoptosis, whereas a subset of DC would express decreased levels of B7 co-stimulatory molecules. Additional experiments, including immunostaining *in situ*, will be required to clarify the effect of Dex on DC *in vivo*.

The reciprocal increase in the macrophage population was unexpected, suggesting that Dex does not similarly affect all APC populations. The relative numbers of T and B lymphocytes remained unchanged in Dex-treated animals, although the percentage of T cells appears slightly increased in some mice injected with higher doses. As previously reported by others (see below), class II expression on B lymphocytes was decreased even in mice injected with 0.03 mg Dex (see Table 1). Taken together, these data indicate that DC function and class II expression on B lymphocytes are down-regulated at doses of Dex which do not affect the production of cytokines by T cells (about 1–2 mg/kg). Kunicka et al. [1] have indeed shown that inhibition of anti-CD3-induced cytokine release was only observed following the injection of high doses of Dex (30 to 200 mg/kg).

A number of reports in the literature have shown that, *in vivo*, corticosteroids induced changes in the phenotypic expression and function of various cell types. Dex administration down-regulates class II expression on B cells and causes rapid depletion of developing B-lineage cells in the marrow by reducing the number of cycling precursor B cells and inducing apoptosis [28–30]. Similarly, corticosteroids have been shown to inhibit macrophage Ia expression, production of IL-1 and the presentation of antigen for T cell proliferation [5, 31, 32]. The finding that Dex did not alter the MHC class II expression on DC suggests that the mechanism of inhibition may be different on the various populations of APC.

Our data extend previous findings showing that both topical and systemic administration of GC resulted in a decreased density of Langerhans cells as assessed by staining for ATPase and Ia antigen [33]. Taken together, these data as well as our observations suggest that the injection of GC could be a useful approach for depleting cells of the dendritic family from solid organs before transplantation.

Of note, Daynes and Araneo [34] have recently reported that GC promoted, both *in vitro* and *in vivo*, the production of IL-4 and suppressed the production of IL-2 [34]. Although the authors clearly show that steroids directly affect a T cell clone *in vitro*, our data suggest that GC may indirectly tip the balance in favor of Th2 by affecting the population of APC. Indeed, we have evidence that the DC are required for optimal activation of Th1 cells *in vivo* [35].

Whether the changes in expression of surface antigens that occur during maturation *in vitro* (see Fig. 1) are of physiological relevance remains to be determined. Inaba et al. [17] recently reported that DC expressed high levels of B7-2 in several sites *in vivo*. In a skin explant culture system, Larsen et al. [27] have found that Langerhans cells migrated out of skin and concomitantly up-regulated both B7-1 and B7-2. *In vivo*, Austyn et al. [36, 37] have shown that DC migrated from the allogeneic graft to the central organs. The analysis of B7 expression on DC undergoing migration may help to clarify this issue.

In our system, the increase in B7 expression is T cell-independent, since it occurs in DC isolated in athymic mice, as well as in DC purified in the presence of the T cell inhibitor cyclosporin A (T. Sornasse, unpublished observations). Recent reports have shown that surface expression of B7 on B cells [16, 38] and macrophages [39, 40] is only inducible by external stimuli or interaction with activated T lymphocytes. The distinct regulation of B7 in DC (which does not require intentional stimulation or T cell interaction) versus that of B cells or macrophages may therefore explain why DC are the only APC able to stimulate resting T cells.

Endogenous and exogenous GC have been shown to regulate T cell responses and to protect mice against the lethal

effects of acute T cell activation *in vivo* [41]. Our data suggest that the modulation of DC numbers, function, or both could be part of the dynamic regulatory interactions between the immune and neuroendocrine systems [42, 43]. A major locus of action of corticosteroids may be at the very first step of the immune response, to inhibit maturation of DC into potent APC, thereby preventing the activation of new, antigen-specific, naive T cells.

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