



Explore what's possible with innovative research tools

Discover the difference >



Corticosteroids Enhance the Capacity of Macrophages to Induce Th2 Cytokine Synthesis in CD4⁺ Lymphocytes by Inhibiting IL-12 Production

This information is current as of June 15, 2021.

Rosemarie H. DeKruyff, Yu Fang and Dale T. Umetsu

J Immunol 1998; 160:2231-2237; ;
<http://www.jimmunol.org/content/160/5/2231>

References This article **cites 32 articles**, 19 of which you can access for free at:
<http://www.jimmunol.org/content/160/5/2231.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
<http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at:
<http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
<http://jimmunol.org/alerts>



Corticosteroids Enhance the Capacity of Macrophages to Induce Th2 Cytokine Synthesis in CD4⁺ Lymphocytes by Inhibiting IL-12 Production¹

Rosemarie H. DeKruyff,² Yu Fang, and Dale T. Umetsu

We investigated the effects of corticosteroids on IL-12 production by mouse splenic adherent cells and the subsequent capacity of these cells to induce cytokine production by CD4⁺ T cells. To distinguish the effects of corticosteroids on APCs from those on T cells, only the APCs and not the T cells were exposed to corticosteroids. Treatment of splenic adherent cells with dexamethasone greatly inhibited production of IL-12, a cytokine known to enhance IFN- γ synthesis and decrease IL-4 synthesis by CD4⁺ T cells. The reduction in IL-12 production by corticosteroid-treated macrophages decreased their ability to induce IFN- γ and increased their ability to induce IL-4 synthesis in Ag-primed CD4⁺ T cells. Splenic adherent cells from mice treated in vivo with dexamethasone also displayed a reduced capacity to produce IL-12. These results help to resolve previous conflicting observations regarding the effects of corticosteroids on cytokine production by T cells, and indicate that while corticosteroids may directly inhibit Th1 and Th2 cytokine production in T cells, corticosteroids, by reducing IL-12 production in APCs, have the potential to indirectly enhance Th2 cytokine synthesis. Therefore, treatment of diseases such as allergy with chronic corticosteroids may indirectly exacerbate the course of the disease, which is caused primarily by the overproduction of Th2 cytokines in allergen-specific CD4⁺ T cells. *The Journal of Immunology*, 1998, 160: 2231–2237.

Corticosteroids are potent immunosuppressive and anti-inflammatory agents and are used therapeutically for a broad spectrum of diseases, including autoimmune and allergic inflammatory diseases and organ transplant rejection. Corticosteroids affect the immune system by modulating cytokine production in lymphocytes and altering the trafficking and function of neutrophils, eosinophils, mast cells, and endothelial cells (1–4). Corticosteroids inhibit the production of most ILs in activated T cells by increasing the production of I- κ B, which binds to the transcription factor NF- κ B and inhibits its capacity to translocate to the nucleus (5–7). However, the effect of corticosteroids on IL-4 production in lymphocytes is controversial. Some investigators have demonstrated that corticosteroids inhibit IL-4 production in lymphocytes (8), whereas other investigators have demonstrated that corticosteroids enhance IL-4 synthesis in T cells both in vivo and in vitro (9–11). Determination of the precise effect of corticosteroids on IL-4 synthesis is important, since enhancement of IL-4 synthesis by corticosteroids may be clinically undesirable, for example in the setting of allergic disease and asthma, and may reduce enthusiastic use of corticosteroid therapy in asthma, as recommended by the National Institutes of Health (12).

Since many cell types respond to corticosteroids, conflicting results regarding cytokine synthesis in different studies may be due to a neglect in some studies of evaluating the effects of corticosteroids on the APCs used to activate T cells. We therefore wished to distinguish the effects of corticosteroids on the APC used to

activate the T cell from direct effects of corticosteroids on the T cell, and performed experiments in which only the APC, and not the T cell, was exposed to corticosteroids. Here we show that corticosteroids affect murine macrophages by inhibiting the production of IL-12, a cytokine that is extremely potent in enhancing IFN- γ and inhibiting IL-4 synthesis in T cells (13, 14). The reduced production of IL-12 by corticosteroid-treated macrophages resulted in a decreased ability to induce IFN- γ and an increased ability to induce IL-4.

These results strongly suggest that corticosteroids have the potential to enhance Th2 cytokine synthesis. Since Th2 cytokines inhibit inflammatory conditions such as diabetes and transplant rejection, such an effect may be beneficial for such inflammatory problems. However, although administration of corticosteroids may benefit acute asthma or allergic disease by directly inhibiting cytokine synthesis in T cells, enhanced production of Th2 cytokines by corticosteroid therapy may indirectly exacerbate allergic disease, which is caused by the overproduction of Th2 cytokines in allergen-specific CD4⁺ T cells. This deleterious effect would result from a reduction in IL-12 synthesis by corticosteroids, thereby enhancing the production of Th2 cytokines, and limiting the production of Th1 cytokines (which attenuate allergic disease).

Materials and Methods

Animals

DBA/2 mice or BALB/c \times DBA/2 (CByD2)F₁ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were used at 6 to 8 wk of age.

Reagents

9-Fluoro-16-methyl-prednisolone (dexamethasone, or DXM)³ was purchased from Sigma Chemical Company (St. Louis, MO).

Department of Pediatrics, Stanford University, Stanford, CA 94305

Received for publication May 16, 1997. Accepted for publication November 17, 1997.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by Grants RO1AI26322, RO1AI24571, and KO7AI01026 from the U. S. Public Health Services, National Institutes of Health.

² Address correspondence and reprint requests to Dr. R. H. DeKruyff, Department of Pediatrics, Room S303, Stanford University, Stanford, CA 94305-5119.

³ Abbreviations used in this paper: DXM, dexamethasone; KLH, keyhole limpet hemocyanin; SpAC, splenic adherent cells; HKL, heat killed *Listeria monocytogenes*; cDMEM, complete DMEM.

Culture medium

Cells were cultured in DMEM (HyClone Laboratories, Logan, UT) supplemented as described previously (15) and containing 10% FCS (HyClone Laboratories, Logan, UT) cDMEM.

Immunizations

To generate primed CD4⁺ T cells, mice were primed with 150 µg of keyhole limpet hemocyanin (KLH, Calbiochem, San Diego, CA) in CFA in the footpads 9 days before use.

Monoclonal Abs

Monoclonal anti-Ly 2.1 (HB129), anti-I-A^d (MKD6), anti-CD24 (TIB183), and anti-I-E^d (14.4.4s) Ab-secreting hybridomas were obtained from ATCC (American Type Culture Collection, Rockville, MD) and purified from ascites fluid by ammonium sulfate precipitation. Anti-IL-12 p40 mAbs C17.8 and C15.6 (provided by Dr. Giggio Trinchieri, Wistar Institute of Anatomy and Biology, Philadelphia, PA), monoclonal anti-IFN-γ Abs R4-6A2 (ATCC) and XMG1.2 (obtained from Dr. Tim Mosmann, University of Manitoba, Edmonton, Canada), monoclonal anti-IL-10 Abs JES-2A5 and SXC.1 (Dr. M. Howard, DNAX Research Institute, Palo Alto, CA), and monoclonal anti-IL-4 Abs BVD4-1D11 and BVD6-24G2 (Dr. M. Howard, DNAX) were purified from ascites fluid by ammonium sulfate precipitation followed by DEAE-Sephacel chromatography (Sigma).

Lymphokines

Recombinant murine IL-12 was generously provided by Dr. Stanley Wolf (Genetics Institute, Cambridge, MA). Recombinant murine IL-4 was a generous gift of Dr. M. Howard (DNAX). Recombinant murine IFN-γ was provided by Dr. M. Palladino, Genentech (San Francisco, CA).

Preparation of splenic adherent cells (SpAC)

Spleen cells were cultured at 10⁶/ml in cDMEM medium in tissue culture dishes (Falcon 3003; Becton Dickinson, Mountain View, CA) for 2 to 3 h at 37°C. The nonadherent cells were removed by washing with warm cDMEM until visual inspection revealed a lack of lymphocytes (>98% of the cell population). The adherent cells were removed from plates by incubating for 15 min with ice-cold PBS and rinsing repeatedly.

Stimulation of SpAC with heat-killed *Listeria monocytogenes* (HKL) or LPS

The isolated SpAC population was stimulated in the presence or absence of DXM at 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻⁹ M at 1 × 10⁵ per well in 96-well culture plates with either 50 µg/ml of LPS or with HKL at 2 × 10⁶ bacteria per well, prepared as previously described (16). Supernatants were collected after 18 h and analyzed for IL-12 content. In some experiments, the SpAC were incubated overnight in media in the presence or absence of DXM, then washed with warm cDMEM three times to remove DXM before addition of HKL, LPS, or CD4⁺ T cells. Supernatants were collected after 18 h and assayed for cytokine content by ELISA.

Purification of CD4⁺ T cells

Draining axillary, popliteal, and inguinal lymph nodes were removed from mice 9 days after priming with KLH in CFA in the footpads. Lymphocytes were depleted of B cells by adherence to goat anti-mouse Ig-coated dishes for 1 h at 4°C. Nonadherent cells were depleted of CD8⁺ T cells, residual B cells, and other accessory cells by treating the cells with a mixture of anti-CD8, anti-class II, anti-CD24 mAbs on ice for 10 min, followed by addition of baby rabbit C' (Pel Freeze, Rogers, AR) and incubation at 37°C for 45 min.

Induction of cytokine synthesis in CD4⁺ T cells

Purified CD4⁺ T cells were incubated in 96-well plates at 4 × 10⁵/well with SpAC and KLH (10 µg/ml) in 0.15 ml of cDMEM in quadruplicate. Culture supernatants were harvested on day 2 or 4 as indicated and assayed for levels of IL-4, IL-10, IL-12, and IFN-γ by ELISA. In some experiments, T cells were recovered after 4 days of culture and stimulated at 4 × 10⁵/well with fresh normal SpAc (10⁵/well) and Ag (KLH, 10 µg/ml). Supernatants were harvested at 24 h after restimulation.

Cytokine ELISA

Cytokine content in supernatants was determined by ELISA. In brief, 96-well plates were coated overnight with primary anti-cytokine capture Ab.

Plates were washed, blocked, and dilutions of supernatants or standards were added. Dilutions of culture supernatant were incubated overnight at 4°C, and after washing, the wells were incubated with biotin-conjugated anti-cytokine-detecting mAb. After a 2-h incubation, the plates were washed and a horseradish peroxidase-streptavidin conjugate (Southern Biotechnology Associates, Inc., Birmingham, AL) was added. The plates were incubated for an additional hour, and after washing, O-phenyldiamine (OPD) substrate was added. After developing, the OD was determined at 492 nm. The amount of cytokine in each supernatant was extrapolated from the standard curve. The Ab pairs used were as follows, listed by capture/biotinylated detection: IL-4, BVD4-1D11/BVD6-24G2; IFN-γ, R4-6A2/XMG1.2; IL-12, C17.8/C15.6. The standards were cytokine curves generated in 1:2 dilutions from 500 to 39 pg/ml for IL-4, 20 to 0.156 ng/ml for IFN-γ, and 4000 to 30 pg/ml for IL-12. Standards for IL-4, IL-10, and IFN-γ are culture supernatants of Th2 or Th1 clones generated in our lab, which were calibrated against recombinant cytokines. The IL-12 standard used was a culture supernatant of SpAC stimulated with HKL, which was calibrated against rIL-12.

Lymphokine assays

IL-2 was assayed by using the T cell growth factor-dependent line HT2 (generously provided by Dr. Sam Strober, Stanford University, Palo Alto, CA). HT2 cells (5 × 10³/well) were added to dilutions of test samples in 96-well plates. Differential blocking of IL-4 or IL-2 was achieved by adding the anti-IL-4 mAb 11B11, or the anti-IL-2 mAb S4B6, or both. Test samples were diluted such that the mAbs were present in excess, as demonstrated by complete inhibition of HT2 proliferation in the presence of both mAbs. After 18 h, cells were pulsed with 1 mCi of [³H]thymidine for 4 h. Cultures were harvested with a PHD harvester (Cambridge Technology, Cambridge, MA) and [³H]thymidine was measured using standard liquid scintillation counting techniques. Units of IL-2 present in culture supernatants were calculated using recombinant murine IL-2 as a standard.

Results

Treatment of adherent cells with DXM inhibits IL-12 production

Previous studies demonstrated that DXM acts directly on T cells to inhibit cytokine production. We wished to determine whether DXM could also influence T cell cytokine synthesis indirectly via an effect on IL-12 production by APCs. SpAC were isolated from naive DBA/2 mice and incubated with HKL or LPS for 24 or 48 h in the presence or absence of DXM. Figure 1 shows that SpAC produced large quantities of IL-12 when cultured with HKL or LPS, and that such IL-12 production was greatly inhibited by the presence of DXM. The inhibition of IL-12 synthesis was dose dependent over a wide range of DXM concentrations, and occurred when the adherent cells were stimulated with either HKL or LPS. Furthermore, the reduction in IL-12 synthesis occurred both in cultures in which DXM remained and in those in which DXM was washed out after 6 to 8 h of pretreatment of monocytes with DXM before stimulation with *Listeria* (Fig. 2).

Pretreatment of adherent cells with DXM inhibits their capacity to induce IFN-γ and enhances their capacity to induce IL-4 production by T cells

Since IL-12 has been shown to have potent effects on cytokine production by CD4⁺ T cells, we asked if the cytokine profiles of CD4⁺ T cells responding to Ag presented by DXM-pretreated APC would be altered. Direct effects of DXM on T cells were eliminated by pretreatment of SpAC from naive DBA/2 in vitro with DXM for 18 h and washing them to remove DXM, before culture with syngeneic CD4⁺ T cells purified from lymph nodes of KLH-primed mice and the Ag KLH. Figure 3A shows that IL-12 production in cultures of DXM-pretreated SpAC was significantly decreased in comparison with untreated SpAC. In the absence of DXM treatment, stimulation with KLH resulted in the development of T cells producing high levels of IFN-γ. However, pretreatment of SpAC with DXM for 18 h greatly inhibited their capacity to induce the development of IFN-γ production by T cells (Fig. 3B) and significantly increased production of IL-4 (Fig. 3C).

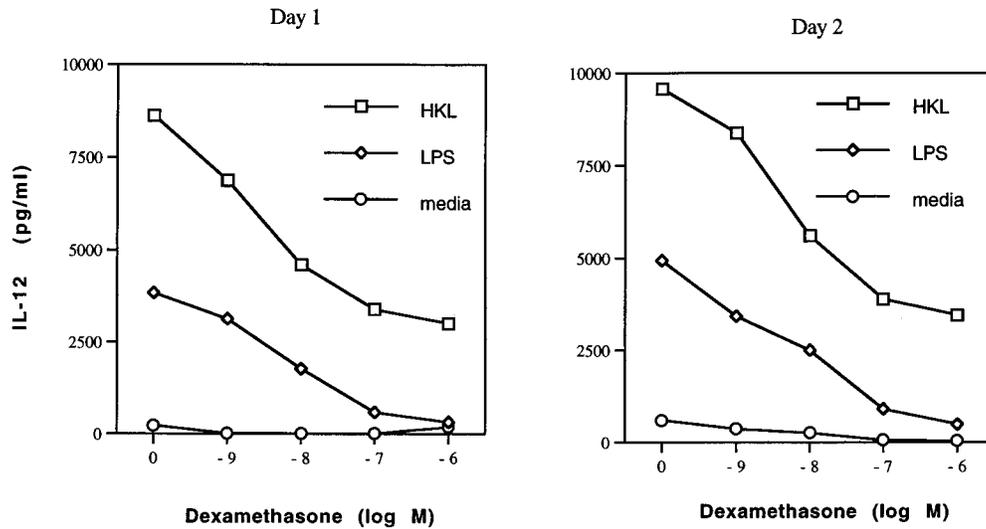


FIGURE 1. DXM inhibits IL-12 production by SpAC stimulated with LPS or HKL. SpACs (1×10^5 /well) were activated by HKL (2×10^6 bacteria/well) or LPS ($50 \mu\text{g/ml}$) in the absence or presence of increasing amounts of DXM. Supernatants were harvested 24 or 48 h later and IL-12 content was assayed by ELISA. This experiment is representative of four experiments.

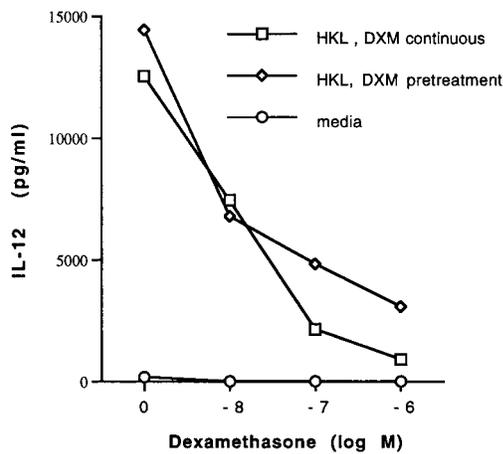


FIGURE 2. Pretreatment of SpAC with DXM inhibits subsequent IL-12 production. SpAC (1×10^5 /well) were preincubated with media or DXM at the indicated concentration. Cells were washed after 18 h and incubated with HKL (2×10^6 bacteria/well). Supernatants were harvested 24 h later and IL-12 content was assayed by ELISA. This experiment is representative of four experiments.

DXM-pretreated APC induced robust amounts of IL-4 and IL-5 in purified T cells, indicating that these cells are viable. No cytokine production by T cells was detected in the absence of APC, demonstrating that the DXM-treated APC are inducing the cytokine production. Thus, pretreatment of monocytes with DXM enhances their capacity to preferentially induce Th2 and inhibit Th1 cytokine synthesis.

Pretreatment of adherent cells with DXM enhances their capacity to induce IL-5 and reduces their capacity to induce IFN- γ production by T cells

SpACs pretreated with DXM developed an increased capacity to induce another Th2 cytokine, IL-5 (Fig. 4A), and a reduced capacity to induce IL-2 (Fig. 4B). In these experiments, CD4⁺ T cells were purified from lymph nodes of KLH-primed mice and cultured with DXM-pretreated SpAC or control SpAC in the presence of the Ag KLH. T cells stimulated with control APC produced very low levels of IL-5 in the initial culture, while T cells stimulated with DXM-pretreated SpAC produced much greater quantities of IL-5. Moreover, the change in cytokine profile induced by DXM-pretreated SpAC was made more pronounced when the T cells in the initial culture were restimulated after 4 days with normal APC and KLH (Fig. 4). These results indicate that T

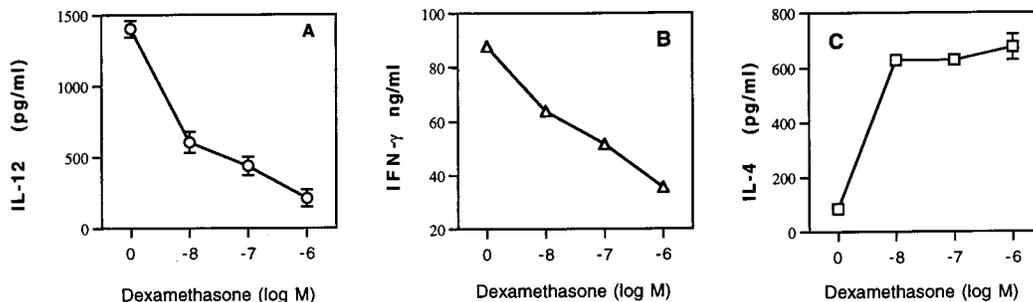


FIGURE 3. Adherent cells pretreated with DXM induce the production of high levels of IL-4 and low levels of IFN- γ production by CD4⁺ T cells. SpAC (1×10^5 /well) were pretreated with media or DXM. After 18 h, cells were washed and incubated with Ag (KLH, $10 \mu\text{g/ml}$) and Ag-primed CD4⁺ T cells (5×10^5 /well). Supernatants were harvested after 2 days (for IL-12) or after 4 days and assayed for their IL-12, IL-4, and IFN- γ content by ELISA. This experiment is representative of three other experiments.

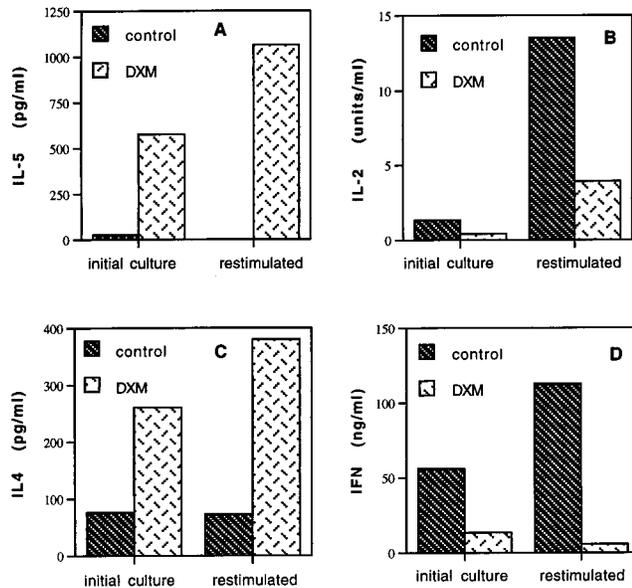


FIGURE 4. Pretreatment of SpAC with DXM enhances their capacity to induce IL-5. SpAC (1×10^5 /well) were pretreated with media or DXM. After 18 h cells were washed and incubated with Ag (KLH, $10 \mu\text{g/ml}$) and Ag-primed CD4^+ T cells (5×10^5 /well). Culture supernatants were harvested after 24 h (for IL-2), 96 h (for IL-4 and IFN- γ), and 120 h (for IL-5). T cells were recovered from some cultures after 96 h, and restimulated at 4×10^5 /well with fresh SpAC (10^5 /well) and Ag (KLH, $10 \mu\text{g/ml}$). Supernatants were harvested at 24 h after restimulation.

cells stimulated with DXM-treated SpAC and Ag become committed to a Th2 pattern of cytokine synthesis.

Addition of IL-12 to cultures of DXM-pretreated SPAC restores the induction of IFN and inhibits induction of IL-4 synthesis by T cells

To determine whether the diminished capacity of DXM-pretreated SpAC to induce IFN- γ synthesis in T cells was a result of their diminished production of IL-12, we reconstituted cultures of DXM-pretreated monocytes and T cells with rIL-12. In cultures of primed T cells and untreated SpAC, IFN- γ production is stimulated by endogenous production of IL-12 since addition of anti-IL-12 Ab blocks production of IL-12 (Ref. 17, and data not shown). Figure 5 shows that addition of 5 or 1 pg/ml of IL-12 to cultures of DXM-pretreated SpAC and T cells greatly reduced IL-4 production and enhanced IFN- γ production in these cultures to levels seen in control cultures. These results suggest that reduc-

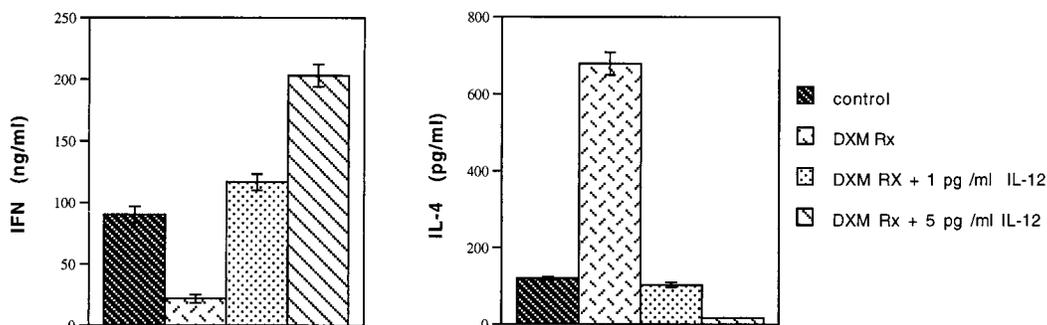


FIGURE 5. Addition of rIL-12 to cultures of DXM-pretreated monocytes restores the production of IFN- γ in cultures with CD4^+ -primed T cells. CD4^+ T cells were cultured with DXM-pretreated monocytes as described in Figure 3, in the presence of rIL-12 (1.0 or 5.0 pg/ml). Supernatants were harvested 4 days later and assayed for their IL-4 and IFN- γ content by ELISA. This experiment is representative of three other experiments.

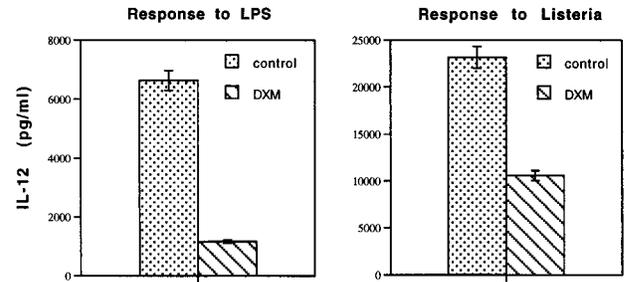


FIGURE 6. In vivo exposure of SpAC to DXM reduces IL-12 production. Mice were treated in vivo with DXM ($100 \mu\text{g}$ i.p.) in DMSO ($40 \mu\text{l}$) or DMSO only. After 24 h, SpAC were purified and stimulated with HKL or LPS as described in the legend to Figure 1. Supernatants were harvested 24 h later and IL-12 content was assayed by ELISA. This experiment is representative of four experiments.

tion of IL-12 synthesis by DXM-treated monocytes was a major effect that determined the capacity of monocytes to regulate cytokine synthesis in T cells.

APCs from mice treated in vivo with DXM

To demonstrate that DXM had a consequential effect on APCs in an in vivo setting, mice were injected with a therapeutic dose of DXM ($100 \mu\text{g}$ i.p.). After 24 h, SpAC were purified from the DXM-pretreated mice, or from control injected mice. Figure 6 shows that SpAC from DXM-pretreated mice produced much lower amounts of IL-12 in response to either HKL or LPS than SpAC from control mice. To determine whether proliferation or cytokine production by Ag-primed T cells would differ in the presence of Ag presented by SpAC from mice treated in vivo with DXM, SpAC were purified from DBA/2 mice 24 h following i.p. injection of DXM ($100 \mu\text{g}$), and cultured with CD4^+ T cells from KLH-primed mice, and KLH. Figure 7 demonstrates that proliferation of KLH-primed T cells was greatly reduced when stimulated with Ag and SpAC from DXM-treated animals compared with SpAC from control mice. Production of IL-12 in cultures containing T cells and SpAC from DXM-treated mice was also reduced. Moreover, SpAC from animals injected with DXM induced significantly lower amounts of IFN- γ and greater amounts of IL-4 than SpAC from control mice. These results show that in vivo treatment with DXM alters the capacity of APC to induce IFN- γ and IL-4.

We next examined the duration of the effect of DXM on the SpAC. (CByD2) F_1 mice were injected with DXM 1 to 7 days before sacrifice. SpAC were purified from the DXM-treated mice

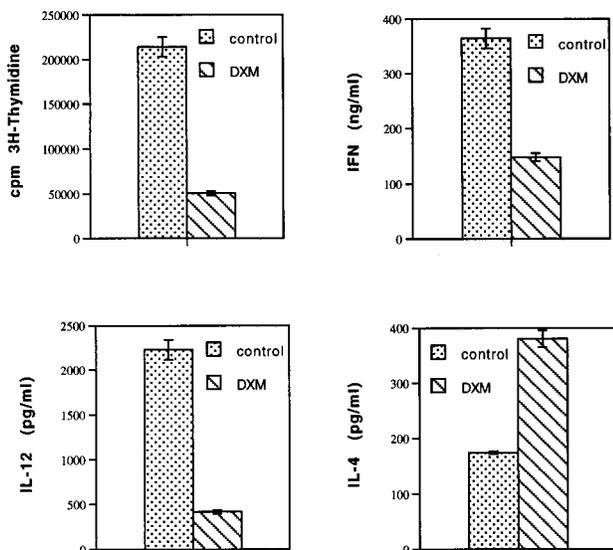


FIGURE 7. SpAC purified from mice treated in vivo with DXM are altered in their capacity to induce cytokine production by primed CD4⁺ T cells. DBA/2 mice were treated in vivo with DXM (100 μg i.p.) in DMSO (40 μl) or DMSO only. After 24 h, SpAC were purified and incubated with KLH-primed CD4⁺ T cells and KLH (10 μg/ml). Proliferation was measured on day 3 of culture. Supernatants were harvested 4 days after initiation of culture and assayed for IL-4, IL-12, and IFN-γ content by ELISA. This experiment is representative of three experiments.

and stimulated with LPS or HKL. Figure 8A shows that IL-12 production by SpAC was still significantly reduced from that of control 3 days after injection. However, by 7 days after injection of

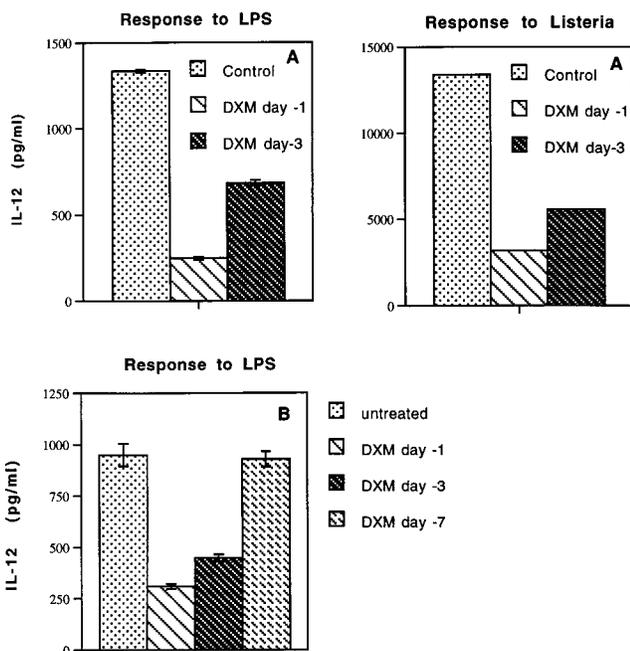


FIGURE 8. Duration of effect of in vivo treatment with DXM on APC. (CByD2)F₁ mice were treated in vivo with DXM (100 μg i.p.) dissolved in DMSO and diluted in PBS (5 μl DXM in DMSO + 195 μl PBS/mouse) or DMSO + PBS (control). SpAC were purified after 1 and 3 days (A, upper panel) or 1, 3, and 7 days (B, lower panel) and stimulated with HKL or LPS as described in the legend to Figure 1. Supernatants were harvested 24 h later and IL-12 content was assayed by ELISA.

DXM, IL-12 production by SpAC returned to levels comparable to that seen in untreated mice (Fig. 8B).

Discussion

We demonstrated that DXM treatment of SpACs inhibits their subsequent capacity to produce IL-12. The in vitro pretreatment of SpAC with DXM resulted in an increased capacity of the SpAC to induce IL-4 and IL-5 and a reduced capacity to induce IFN-γ and IL-2 in CD4⁺ T cells. Moreover, SpAC pretreated in vivo with DXM, by i.p. injection of mice with DXM, were similar to in vitro DXM-pretreated SpAC in producing significantly reduced levels of IL-12. These results demonstrate that corticosteroids can enhance the production of IL-4 in T cells indirectly via effects on APC, and help to resolve conflicting data generated in different laboratories regarding the effects of corticosteroids on cytokine production, particularly of IL-4 in CD4⁺ T cells.

Previous studies have shown that corticosteroids could either inhibit or enhance IL-4 synthesis in T cells. Corticosteroids inhibited the transcription of multiple cytokines, including IL-4 and IFN-γ (18, 19), as well as TNF-α, granulocyte macrophage-CSF, IL-3, and IL-5 (2, 20, 21). The inhibition of transcription and synthesis of IL-4, IL-5, and IFN-γ by corticosteroids was demonstrated in both in vivo and in vitro studies, using both resting T cells as well as effector T cell clones (18, 19). On the other hand, several studies showed that corticosteroids enhance IL-4 synthesis (9, 10) and may enhance IFN-γ synthesis in vivo (22). That corticosteroids have the capacity to enhance IL-4 synthesis in T cells is consistent with the observation that administration of corticosteroids in vivo increases IgE synthesis (11).

The contrasting past results regarding the effects of glucocorticoids on cytokine synthesis in different systems may be due to the fact that glucocorticoids have a wide range of effects on intracellular biochemistry in a variety of different cell types, including T cells and APC. Glucocorticoids affect cytokine synthesis in T cells by binding to and activating cytoplasmic glucocorticoid receptors. The receptor-corticosteroid complex then translocates to the nucleus, where it regulates the transcription of target genes through several mechanisms (23). First, the activated receptor complex inhibits transcription of cytokine genes by binding to the transcription factor AP-1 (composed of c-Fos and c-Jun), and preventing AP-1 from binding to the promoter regions of cytokines (24–26). In addition, the activated glucocorticoid receptor complex functions as a positive transcription factor that increases transcription of the I-κBα, a protein that blocks the translocation into the nucleus of NF-κB, another potent positive regulator of many cytokine and cell adhesion genes (5, 6). Inhibition of transcription of cytokine genes by corticosteroids occurs in both resting and in activated effector T cells, and therefore corticosteroids are important in inhibiting acute disease in which cytokine production is ongoing, as well as in functioning to prevent the actual development of allergy-inducing effector cells.

In our cultures, DXM decreased the production of IL-12 by SpAC, and altered their subsequent capacity to induce IL-4 and IFN-γ production in T cells. The effect of corticosteroids on cytokine production in T cells was indirect, since the T cells in these cultures were never directly exposed to the corticosteroids. It is very unlikely that even small amounts of contaminating DXM were present when the T cells were added, since the pretreated adherent cells were washed extensively, and since contaminating DXM would directly affect the T cells by inhibiting rather than enhancing IL-4 synthesis. These results indicate that a short incubation of SpAC with DXM can have persistent effects on the ability of SpAC to regulate cytokine synthesis in T cells. Since we and

others have shown that the cytokine profiles of activated effector T cells are difficult to modify (14, 27, 28), we believe that corticosteroid-pretreated SpAC altered the cytokine profiles of uncommitted but memory CD4⁺ T cells, rather than differentially expanding a subset of T cells already committed toward the Th2 profile (29).

Although corticosteroids may affect cytokine production in T cells in several ways, we believe that inhibition of IL-12 production in SpAC is a major mechanism by which corticosteroids affect cytokine synthesis (and enhance IL-4 synthesis) in T cells, particularly since IL-12 is extremely potent in inhibiting IL-4 and enhancing IFN- γ synthesis in both unprimed as well as resting memory T cells (13, 14, 28). Although the IL-12 determinations performed in our experiments utilized an IL-12 p40 ELISA, which does not measure the presence of the IL-12 p35 chain, in studies in which both IL-12 p40 and IL-12 p70 were measured, IL-12 p40 levels correlated directly with those of IL-12 p70, though levels of IL-12 p70 were lower (17). The levels of IL-12 p40 correlated directly with that of IFN- γ , consistent with the idea that the major biologic effects of IL-12 are to enhance Th1 cytokine synthesis (30).

Corticosteroids might also enhance IL-4 production in T cells by increasing the production of IL-10 in SpAC. We have recently observed that corticosteroids enhance IL-10 production (but decrease IL-12 production) in human peripheral blood monocytes (31), but corticosteroid treatment did not affect IL-10 production in the murine APC that we tested. Since reconstitution of our DXM-treated cultures with rIL-12 reversed the effects of corticosteroids on IL-4 and IFN- γ synthesis, we believe that reduction of IL-12 production in SpAC by corticosteroids was the major immunoregulatory mechanism that occurred in our system. IL-12 has been reported to increase IL-10 production by T cells in systems using established T cell lines rather than resting T cells (32), or in systems using IL-12 in the presence of anti-IL-4 mAb (33), but these effects appear to occur mainly when very high concentrations of IL-12 (10 ng/ml, or 1000 times what was placed in our cultures) are used.

Our observations demonstrating that pretreatment of APC with corticosteroids inhibit IL-12 production and enhance their capacity to induce IL-4 but not IFN- γ have significant clinical implications. For example, current therapy for asthma focuses on early intervention with inhaled corticosteroids, which eliminate pulmonary eosinophilia and reduce Th2 cytokine synthesis by activated T cells in the lung. During subsequent immune responses, circulating T cells that become activated against Ag presented by resident APC that produce reduced quantities of IL-12. Activation of such T cells in the absence of IL-12 would drive the production of Th2 cytokines in these newly arriving T cells, but limit synthesis of IFN- γ . Similar situations could arise with systemic corticosteroid therapy, in particular because of the very short half-life of most synthetic corticosteroids. Since we demonstrated that in vivo exposure (as well as in vitro exposure) to corticosteroids clearly results in a reduction of IL-12 production (Fig. 5), which persists for at least several days, a short burst of systemic corticosteroid therapy is likely to reduce the capacity of APC to produce IL-12 (and greatly reduce cytokine synthesis in activated but not resting T cells). If, however, resting (cytokine-uncommitted) T cells are subsequently activated by APC preexposed to corticosteroids, enhanced IL-4 production but limited IFN- γ synthesis would be induced. Although therapy for autoimmune inflammatory disease or solid organ transplant rejection may benefit by such enhanced IL-4 production in CD4⁺ T cells, the long-term course of allergic diseases may be negatively affected by intermittent or chronic inhaled corticosteroid therapy that enhances Th2 cytokine synthesis. In the

allergic patient with chronic disease, the effect would be insidious and difficult to detect, since T cells in allergic individuals are already predisposed toward the production of Th2 cytokines, and since the immediate inhibitory effects of corticosteroids on eosinophilic inflammation are so dramatic.

Acknowledgments

We thank Dr. Giorgio Trinchieri, Wistar Institute; Dr. Maureen Howard, DNAX Research Institute; and Dr. Lucy Tompkins, Stanford University for invaluable reagents and Dr. Randy Giени, Stanford University, for providing heat-killed *Listeria* and for helpful discussions.

References

- Goulding, N. J., and P. M. Guyre. 1993. Glucocorticoids, lipocortins and the immune response. *Curr. Opin. Immunol.* 5:108.
- Gillis, S., G. R. Crabtree, and K. A. Smith. 1979. Glucocorticoid-induced inhibition of T cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation. *J. Immunol.* 123:1624.
- Arya, S. K., F. Wong-Staal, and R. C. Gallo. 1984. Dexamethasone-mediated inhibition of human T cell growth factor and γ -interferon messenger RNA. *J. Immunol.* 133:273.
- Schleimer, R. P., S. A. Sterbinsky, J. Kaiser, C. A. Bickel, D. A. Klunk, K. Tomioka, W. Newman, F. W. Lusinskas, M. A. Gimbrone, Jr., B. W. McIntyre, and B. S. Bochner. 1992. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J. Immunol.* 148:1086.
- Scheinman, R. I., P. C. Cogswell, A. K. Lofquist, and A. S. Baldwin, Jr. 1995. Role of transcriptional activation of I κ B α in mediation of immunosuppression by glucocorticoids. *Science* 270:283.
- Auphan, N., J. A. DiDonato, C. Rosette, A. Helmborg, and M. Karin. 1995. Immunosuppression by glucocorticoids: inhibition of NF κ B activity through induction of I κ B synthesis. *Science* 270:286.
- Barnes, P. J. 1996. Molecular mechanisms of steroid action in asthma. *J. Allergy Clin. Immunol.* 97:159.
- Wu, C. Y., C. Fargeas, T. Nakajima, and G. Delespesse. 1991. Glucocorticoids suppress the production of interleukin 4 by human lymphocytes. *Eur. J. Immunol.* 21:2645.
- Ramirez, F., D. J. Fowell, M. Puklavec, S. Simmonds, and D. Mason. 1996. Glucocorticoids promote a Th2 cytokine response by CD4⁺ T cells in vitro. *J. Immunol.* 156:2406.
- Daynes, R. A., and B. A. Araneo. 1989. Contrasting effects of glucocorticoids on the capacity of T cells to produce the growth factors interleukin 2 and interleukin 4. *Eur. J. Immunol.* 19:2319.
- Zieg, G., G. Lack, R. J. Harbeck, E. W. Gelfand, and D. Y. Leung. 1994. In vivo effects of glucocorticoids on IgE production. *J. Allergy Clin. Immunol.* 94:222.
- International Consensus Report on Diagnosis and Management of Asthma*. 1992. U. S. Department of Health and Human Services, Public Health Service, Washington, DC.
- Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251.
- Marshall, J. D., H. Secrist, R. H. DeKruyff, S. F. Wolf, and D. T. Umetsu. 1995. IL-12 inhibits the production of IL-4 and IL-10 in allergen-specific human CD4⁺ T lymphocytes. *J. Immunol.* 155:111.
- DeKruyff, R. H., P. H. Brown, G. J. Thorbecke, and N. M. Ponzio. 1985. Characterization of SJL T cell clones responsive to syngeneic lymphoma (RCS): RCS-specific clones are stimulated by activated B cells. *J. Immunol.* 135:3581.
- DeKruyff, R. H., R. S. Giени, and D. T. Umetsu. 1997. Antigen-driven but not lipopolysaccharide-driven IL-12 production in macrophages requires triggering of CD40. *J. Immunol.* 158:359.
- Giени, R. S., Y. Fang, G. Trinchieri, D. T. Umetsu, and R. H. DeKruyff. 1996. Differential production of IL-12 in BALB/c and DBA/2 mice controls IL-4 versus IFN- γ synthesis in primed CD4 lymphocytes. *Int. Immunol.* 8:1511.
- Snijderwint, F. G., M. L. Kapsenberg, P. J. Wauben-Penris, and J. D. Bos. 1995. Corticosteroids class-dependently inhibit in vitro Th1- and Th2-type cytokine production. *Immunopharmacology* 29:93.
- Masuyama, K., M. R. Jacobson, S. Rak, Q. Meng, R. M. Sudderick, A. Kay, and O. Lowhagen. 1994. Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 82:192.
- Culpepper, J. A., and F. Lee. 1985. Regulation of IL-3 expression by glucocorticoids in cloned murine T lymphocytes. *J. Immunol.* 135:3191.
- Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin synthesis: mechanisms of endotoxin resistance. *Science* 232:977.
- Leung, D. Y., R. J. Martin, S. J. Szefer, E. R. Sher, S. Ying, A. B. Kay, and Q. Hamid. 1995. Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. *J. Exp. Med.* 181:33.
- Truss, M., and M. Beato. 1993. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocr. Rev.* 14:459.

24. Yang-Yen, H. F., J. C. Chambard, and Y. S. Sun. 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62:1205.
25. Adcock, I. M., C. R. Brown, C. M. Gelder, H. Shirasaki, M. J. Peters, and P. J. Barnes. 1995. The effects of glucocorticoids on transcription factor activation in human peripheral blood mononuclear cells. *Am. J. Physiol.* 37: C331.
26. Ray, A., and K. E. Prefontaine. 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF- κ B and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* 91:752.
27. Manetti, R., F. Gerosa, M. G. Giudizi, R. Biagiotti, P. Parronchi, M. Piccinni, S. Sampognaro, E. Maggi, S. Romagnani, and G. Trinchieri. 1994. Interleukin 12 induces stable priming for interferon γ (IFN- γ) production during differentiation of human T helper (Th) cells and transient IFN- γ production in established Th2 cell clones. *J. Exp. Med.* 179:1273.
28. DeKruyff, R. H., Y. Fang, S. F. Wolf, and D. T. Umetsu. 1995. IL-12 inhibits IL-4 synthesis in keyhole limpet hemocyanin-primed CD4⁺ T cells through an effect on antigen-presenting cells. *J. Immunol.* 154:2578.
29. Murphy, E., K. Shibuya, N. Hosken, P. Openshaw, V. Maino, K. Davis, K. Murphy, and A. O'Garra. 1996. Reversibility of T helper 1 and 2 populations is lost after long-term stimulation. *J. Exp. Med.* 183:901.
30. Magram, J., S. E. Connaughton, R. R. Warrier, D. M. Carvajal, C. Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty, and M. K. Gately. 1996. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. *Immunity* 4:471.
31. Blotta, M. H., R. H. DeKruyff, and D. T. Umetsu. 1997. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4⁺ lymphocytes. *J. Immunol.* 158:5589.
32. Jeannin, P., Y. Delneste, M. Seveso, P. Life, and J. Y. Bonnefoy. 1996. IL-12 synergizes with IL-2 and other stimuli in inducing IL-10 production by human T cells. *J. Immunol.* 156:3159.
33. Windhagen, A., D. E. Anderson, A. Carrizosa, R. E. Williams, and D. A. Hafler. 1996. IL-12 induces human T cells secreting IL-10 with IFN- γ . *J. Immunol.* 157:1127.