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*J Immunol* 1998; 161:2723-2730; [http://www.jimmunol.org/content/161/6/2723](http://www.jimmunol.org/content/161/6/2723)

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Prostaglandin E$_2$ and Dexamethasone Inhibit IL-12 Receptor Expression and IL-12 Responsiveness

Chang-You Wu,* Kening Wang,† John F. McDyer,* and Robert A. Seder1*

Regulation of the factors governing IL-12 expression and IL-12 responsiveness has been shown to be important in the generation and stability of Th1- and Th2-type responses. In this regard, cytokines have been shown to have a prominent role in regulating IL-12R expression. In this study, the role that PGE$_2$ and dexamethasone (DXM) have in regulating IL-12R expression was evaluated. Addition of PGE$_2$ or DXM to human PBMCs stimulated with immobilized anti-CD3 plus IL-12 inhibited the production of IFN-$\gamma$ in a dose-responsive manner. Moreover, PBMCs stimulated with immobilized anti-CD3 in the presence of PGE$_2$ or DXM for 3 days, washed extensively, and restimulated in the presence of IL-12 did not produce IFN-$\gamma$. This lack of IL-12 responsiveness from cells cultured in either PGE$_2$ or DXM was correlated with diminished surface expression of IL-12R$_{b1}$, IL-12R$_{b2}$ mRNA expression, and IL-12 binding. Finally, the PGE$_2$- and DXM-mediated inhibition of IL-12R expression was not affected significantly by addition of neutralizing Abs against either IL-4, IL-10, or TGF-$\beta$. By contrast, addition of dibutyryl cAMP, 8-bromoadenosine 3:5 cAMP (8-Br-cAMP), or cholera toxin substantially reduced IL-12R expression, suggesting that PGE$_2$ may be mediating its effects through enhancement of cAMP. The Journal of Immunology, 1998, 161: 2723–2730.

Interleukin-12 is a potent immunoregulatory cytokine important for protective immunity to a variety of infectious pathogens. The biologic activities of IL-12 are mediated through specific, high affinity receptors composed of IL-12R$_{b1}$ and IL-12R$_{b2}$ chains that exist primarily on T and NK cells (1–3). In transfection experiments using COS cells, each respective subunit of the IL-12R binds IL-12 with an apparent dissociation constant of 2 to 5 nM (3). Cotransfection of both IL-12R$_{b1}$ and IL-12R$_{b2}$ subunits in COS cells, however, resulted in two classes of IL-12 binding sites (high and low) with affinities of 50 pM and 5 nM, respectively (3). In addition, stable transfection of IL-3-dependent BaF3 cells with both IL-12R subunits also revealed high and low affinity IL-12 binding sites. These cells proliferated in response to IL-12, indicating that the two IL-12R chains are required to generate a functional receptor (3). It also has been shown that activated human T cells express both high and low affinity binding sites (4). Consistent with these observations, in vitro studies using mAbs to human IL-12R$_{b1}$ markedly block IL-12-induced biologic activities on T cells and NK cells (5), and IL-12R$_{b1}$ chain-deficient mice are impaired both in IFN-$\gamma$ production and in Th1 differentiation (6).

Due to the importance of IL-12 responsiveness to biologic function, there has been interest in understanding the factors involved in regulating IL-12. Human in vitro studies have shown that IL-12R$_{b1}$ is expressed at low levels on freshly isolated PBMCs (7). Activation of PBMCs with mitogens or anti-CD3 mAb leads to up-regulation of IL-12R$_{b1}$ expression and IL-12 binding (7). The maximum expression of IL-12R$_{b1}$ and IL-12 binding in PBMCs or mRNAs for IL-12R$_{b1}$ and IL-12R$_{b2}$ in CD4 T cells required stimulation with anti-CD3 plus costimulation with either anti-CD28 mAb (7) or B7-2-transfected Chinese hamster ovary cells (8). Additional positive regulators of IL-12R$_{b1}$ expression and IL-12 binding include IL-2, IL-7, and IL-15 (7). By contrast, negative regulators of IL-12R$_{b1}$ expression and IL-12 binding include TGF-$\beta$, IL-4, and IL-10 (7, 9). Of interest, regulation of IL-12R$_{b2}$ mRNA transcripts in mouse and humans appears to be different. Thus, IFN-$\gamma$ in mice (10) and IFN-$\alpha$ in humans (11) appear to regulate IL-12R$_{b2}$ mRNA expression using in vitro priming cultures.

In addition to cytokines, certain biochemical mediators and hormones have been shown to have potent immunoregulatory functions. With regard to Th cell differentiation, PGE$_2$, an arachidonic acid metabolite released from various cells including APCs, has been shown to suppress T cell proliferation (12–14) and to inhibit the differentiation of Th1 cells (15–20). These latter effects have been supported by the observation that PGE$_2$ can suppress IL-12 production by monocytes and dendritic cells (21); however, in some studies, it should be noted that PGE$_2$ can also enhance IL-12 production from human dendritic cells (22). Similarly, corticosteroids, commonly used immunosuppressive agents, have also been shown to exert potent inhibitory function on developing Th1 responses through inhibition of IL-12 (23, 24).

In this study, PGE$_2$ and dexamethasone (DXM) are shown to inhibit responsiveness of activated human PBMCs and T cells to IL-12 through the suppression of IL-12R$_{b1}$ expression and IL-12R$_{b2}$ mRNA transcription. These inhibitory effects of PGE$_2$ and DXM on IL-12R expression were not affected substantially by addition of neutralizing Abs to IL-4, IL-10, or TGF-$\beta$; however, cAMP analogues were found to inhibit IL-12R$_{b1}$ expression. Taken together, these data are consistent with the striking anti-inflammatory properties of DXM, and also suggest that PGE$_2$ may, in fact, suppress inflammation through increasing cAMP.

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Received for publication April 1, 1998. Accepted for publication May 12, 1998.

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2 Abbreviations used in this paper: DXM, dexamethasone; PGE$_2$, prostaglandin E$_2$; 8-Br-cAMP, 8-bromoadenosine 3:5 cyclic monophosphate; MFI, mean fluorescence intensity; PE, phycoerythrin.
Materials and Methods

Reagents

Complete cell culture medium consists of RPMI 1640 supplemented with 10% heat-inactivated human AB serum (Sigma, St. Louis, MO), penicillin (100 U/ml), streptomycin (100 U/ml), and l-glutamine (2 mM). All were purchased from Biofluids (Rockville, MD). Lymphocyte separation medium was purchased from Organon Teknika (Durham, NC). PGE, and DXM were purchased from Sigma. Anti-CD3 (OKT3) mAb was purified from ascites. Neutralizing mAbs to human IL-4, TGF-β, and IL-10 were purchased from PharMingen (Torrance, CA). Purified human IL-12, bio-
tinylated anti-human IL-12 mAb 4D6 (B-4D6), biotinylated anti-human IL-12Rβ1 mAb 2.4E6 (B-2.4E6), and IL-12Rβ2 cDNA probe for Northern blot analysis were kindly provided by Drs. M. Gately and U. Gubler, re-
spectively (Department of Inflammation/Autoimmune Diseases, Hoff-
mann-La Roche, Nutley, NJ). cAMP analogues 8-bromoadenosine 3:5 cy-
clonophosphate (8-BrcAMP), N6, and 2-o-dibutyrylclonomophosphate 3:5 cyclic monophosphate (dibutyl cAMP) were purchased from Sigma and used at concentrations of 1 mM. Cholera toxin was purchased from Sigma and used at 100 ng/ml.

Cell isolation and culture conditions

PBMCs were isolated from buffy coats by density-gradient centrifugation over lymphocyte separation medium. T cells were isolated by rosetting with neuraminidase-treated SRBCs. Rosette-forming cells were lysed with ACK lysing buffer (Biofluids), washed in HBSS, and resuspended in com-
plete cell culture medium. For activation, PBMCs at 106 cells/ml in cell culture medium were stimulated with anti-CD3 mAb at concentrations of 5 µg/ml. In addition, purified T cells (106 cells/ml) were stimulated with immobilized anti-CD3 mAb in 24-well tissue culture plates (Costar, Cambridge, MA) that had been coated overnight at 4°C with 5 µg/ml anti-CD3 mAb in coating buffer (0.05 M sodium carbonate buffer, pH 9.6) and washed three times with PBS before use. After incubation for 3 days, cells were harvested, washed, and assayed for the expression of IL-12Rβ1 and IL-12 binding by flow cytometry.

Flow-cytometric analysis

The methods used for the detection of IL-12Rβ1 and IL-12 binding cells by flow cytometry have been described previously (5, 7). Briefly, to evaluate expression of IL-12Rβ1, cells were incubated with biotinylated anti-
IL-12Rβ1 mAb 2.4E6 (B-2.4E6), followed by streptavidin conjugated to phycoerythrin (S-PE; PharMingen). For detection of IL-12 binding, cells were incubated with unlabeled IL-12, followed by biotinylated, nonneu-
tralizing anti-IL-12 mAb 4D6 (B-4D6), and finally with S-PE. Stained cell populations were analyzed on a FACScan (Becton Dickinson, Mountain View, CA). The mean fluorescence intensity (MFI) difference was calcu-
lated by subtracting the MFI for cells stained with isotype control Ab or

Proliferation and IFN-γ production assays

Cell cultures were performed as previously described (7). PBMCs were incubated at a final density of 105/ml in complete culture medium with 5 µg/ml anti-CD3 mAb in the presence of absence of concentrations of PGE2, or DXM. All cultures were conducted in six-well plates in a total volume of 4 ml/well. After incubation for 3 days at 37°C, the lymphoblasts were collected and extensively washed. For measurement of cytokine-in-
duced proliferation, anti-CD3-activated lymphoblasts were incubated in 96-well plates (Costar) at a final cell density of 2 × 103 cells/0.2 ml with either IL-12 or IL-2. After incubation for 2 days at 37°C, the cultures were pulsed with 3H-thymidine (New England Nuclear, Boston, MA) for 6 to 8 h. 3H-Thymidine incorporation into cellular DNA was measured in trip-
licate samples. For production of IFN-γ, anti-CD3-activated lymphoblasts were incubated at a final density of 2 × 103 cells/0.2 ml in complete culture medium in the presence or absence of varying concentrations of IL-12. All cultures were conducted in triplicate in 96-well plates. After incubation for 48 h at 37°C, the supernatant fluids were harvested and assayed in triplicate for IFN-γ.

Measurement of IFN-γ production

The levels of IFN-γ in the culture fluids were determined by a two-step specific ELISA, according to the manufacturer’s suggestions (Endogen, Woburn, MA; lower limit of detection at 50–100 pg/ml). Results for all cytokines represent the mean of triplicate wells. The SEM was <10% for all experiments.

Results

PGE2 and DXM inhibit IL-12R expression on T cells

PBMCs were cultured with medium alone or with anti-CD3 mAb in the presence or absence of PGE2 and DXM. After incubation for 48 h, cells were harvested and total RNA was isolated using the RNA STAT-60 re-
agent (TEL-TEST, Friendswood, TX) following the instructions of the manufacturer. For Northern blot analysis, 25 µg of total RNA per sample was denatured and electrophoresed on formaldehyde-denaturing gel and transferred to a nylon membrane (Schleicher & Schuell, Keene, NH). An IL-12Rβ2 cDNA fragment encompassing the coding region was labeled with [32P]dCTP using the Multiprime DNA labeling system (Amersham, Arlington Heights, IL). Hybridization was conducted overnight at 42°C in a buffer containing 50% formamide, 0.5% SDS, 10% dextran sulfate, 6× SSC, 100 µg/ml denatured sheared DNA, and 5 × 106 cpm/ml probe. Northern blot was washed three times at room temperature in a buffer containing 2× SSC/0.1% SDS, then washed twice at 65°C for 20 min in 0.2× SSC/0.1% SDS, and finally washed twice at 70°C for 20 min in 0.1× SSC/0.5% SDS. The membrane was subjected to autoradiography and PhosphorImager quantitation. The probe was then stripped by washing with 0.1× SSC/0.5% SDS for 20 min twice at 90 to 100°C. The membrane was rehybridized as above with a probe of human 28S rRNA for the as-
essment of equal loading.

RNA preparation and Northern blot analysis

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essment of equal loading.

PGE2 and DXM inhibit IL-12R expression on T cells

PBMCs were stimulated with plate-bound anti-CD3 and IL-12 in the presence of varying concentrations of PGE2 or DXM, and IFN-γ production was assessed. As shown in Figure 1, A and B, addition of PGE2 or DXM, respectively, resulted in a striking decrease in production of IFN-γ in a dose-dependent manner. To further determine whether the effects of PGE2 and DXM impaired the subsequent ability of the cells to respond to IL-12, PBMCs were first stimulated for 3 days with anti-CD3 in the presence of PGE2 or DXM, washed extensively, and restimulated with varying concentra-
tions of IL-12. As shown in Figure 2, the presence of PGE2 or DXM in primary cultures abrogated the ability of IL-12 to induce production of IFN-γ (Fig. 2A) or enhance proliferation (Fig. 2B) in secondary cultures. As a control, cells cultured in PGE2 or DXM maintained IL-2 responsiveness in secondary cultures (Fig. 2C). It should be noted, however, that PGE2 did inhibit IL-2R chain expression (data not shown). Implications for these data are dis-
cussed below.

PGE2 and DXM inhibit IL-12Rβ1 expression and IL-12 binding in PBMCs stimulated with anti-CD3

Since the data above showed that PGE2 and DXM impaired IL-12 responsiveness, the effect they had on IL-12R expression was as-
se ssed. As shown in Figure 3, PBMCs stimulated with plate-bound anti-CD3 and varying concentrations of PGE2 (Fig. 3A) or DXM (Fig. 3B) had a dose-dependent decrease in IL-12Rβ1 expression. Moreover, this correlated with a decrease in IL-12 binding (Fig. 3, C and D).

PGE2 and DXM inhibit IL-12R expression on T cells

As the previous experiments were done using PBMCs, it was of interest to see whether the effects of PGE2 and DXM on IL-12 responsiveness were on T cells. As shown in Figure 4, both PGE2 and DXM inhibited IL-12Rβ1 expression and IL-12 binding on purified T cells. In addition, in experiments not shown, PBMCs stimulated with anti-CD3 in the presence of PGE2 and DXM had
a reduction in IL-12Rβ1 expression on cells double stained with CD3 or CD16/CD56 and IL-12Rβ1. Taken together, these data show that PGE2 and DXM inhibit IL-12Rβ1 expression on T cells and on NK cells.

**PGE2 and DXM inhibit IL-12Rβ2 mRNA expression**

IL-12R is composed of at least two chains (3). The previous experiments focused on expression of the IL-12Rβ1 protein. To determine whether PGE2 or DXM also inhibited the IL-12Rβ2 subunit, PBMCs were stimulated with or without plate-bound anti-CD3 in the presence or absence of DXM or PGE2 for 48 h, and mRNA expression for IL-12Rβ2 was assessed by Northern blot analysis. As shown in Figure 5, there was no IL-12Rβ2 mRNA expression from cells cultured in medium alone, while there was an approximately 4-kb band noted from cells stimulated with anti-CD3 alone, consistent with previous data showing that activation is required to induce this receptor subunit (3, 10, 11). Addition of PGE2 or DXM strikingly diminished the mRNA expression for IL-12Rβ2 (Fig. 5A). Moreover, additional quantitative analysis using a PhosphorImager shows that the ratio of IL-12Rβ2:28S rRNA was diminished three- to fourfold in cells stimulated in the presence of PGE2 and DXM (Fig. 5B). Thus, taken together with the previous figures, these data confirm that PGE2 or DXM down-regulates both chains of the IL-12R.

**PGE2 and DXM inhibit IL-12Rβ1 expression independent of IL-4, IL-10, and TGF-β**

Finally, it was of interest to determine the mechanism by which PGE2 and DXM inhibited IL-12R expression. In this regard, previous work in both mouse and human studies has shown that cytokines such as IL-10, IL-4, and TGF-β all inhibit IL-12Rβ1 expression (7). Moreover, PGE2 has been shown to increase production of IL-10 (18, 21, 25). To examine whether PGE2 and DXM were acting directly or indirectly via induction of inhibitory cytokines, PBMCs were stimulated in the presence of PGE2 or DXM with or without anti-IL-4, anti-IL-10, or anti-TGF-β. As shown in Figure 6, there appeared to be a modest effect on reversing the inhibition of IL-12Rβ1 induced by PGE2 when all three Abs were added; however, the effects of these cytokines on DXM inhibition were more modest.

**Increased cAMP leads to down-regulation of IL-12Rβ1 expression in PBMCs and T cells**

As cytokines did not appear to be the mechanism by which PGE2 down-regulates IL-12Rβ1 expression, it was possible that enhancement in cAMP by PGE2 was mediating the inhibition (26). As cAMP has been shown to affect Th1-type cytokine production (27, 28), PBMCs or purified T cells were stimulated with anti-CD3 in the presence of dibutyryl cAMP, 8-Br-cAMP, or cholera toxin (an inducer of cAMP). As shown in Table I, addition of PGE2 resulted in a 50% reduction in IL-12Rβ1 expression in both PBMCs and T cells. Moreover, dibutyryl cAMP, 8-Br-cAMP, and cholera toxin all resulted in a striking four- to fivefold reduction in IL-12Rβ1 expression. These results suggest that cAMP has a profound effect on regulating IL-12Rβ1 expression and that PGE2 may be mediating its effects through this pathway.

**Discussion**

IL-12 is a central regulator in controlling immune responses. Thus, understanding the factors involved in regulating its induction as well as its responsiveness may have important clinical application. In the study reported in this work, PGE2 and DXM were evaluated for their ability to regulate IL-12R expression on human PBMCs and T cells. Both PGE2 and DXM inhibited IL-12Rβ1 expression and mRNA for IL-12Rβ2. Moreover, PGE2 and DXM inhibited binding for IL-12, correlating with reduced IL-12 responsiveness as assessed by proliferation and induction of IFN-γ. The potential importance of these findings and the mechanism by which these mediators exerted their effects are discussed below.
PGE₂ inhibits the induction of type 1 cytokine responses (IFN-γ and IL-12) and IL-12 responsiveness

PGE₂, an arachidonic acid metabolite produced in a variety of inflammatory and infectious diseases, may have a role in affecting the immune response and biologic outcome in those diseases. With regard to the effects of PGE₂ on cytokine production, there are several reports showing that PGE₂ inhibits type 1 cytokine responses and enhances Th2 responses (15–20). This effect appears to be indirect through inhibition of IL-12 (21) and/or through direct inhibition of IL-2/IFN-γ (18). In this study, we show that in primary short-term stimulation, PGE₂ inhibits the ability of IL-12 to augment production of IFN-γ on PBMCs through inhibition of IL-12R expression. In addition, cells cultured for a brief period in the presence of PGE₂ were still unresponsive to subsequent stimulation with IL-12, suggesting that these effects are sustained at least in a short-term secondary stimulation. It should be noted that in a recent report (18), the presence of PGE₂ in priming cultures led to an increase in IL-4 production. In this study, we were not able to detect IL-4 following stimulation in the presence of PGE₂ (data not shown). It is likely that differences in the experimental conditions (e.g., type of cells used, duration and type of stimulation) are important in regulating IL-4 production. The mechanism by which PGE₂ could be inhibiting IL-12R could be due to several factors. First, if PGE₂ caused enhanced production of IL-4 and diminished production of IL-12 (cytokines that negatively and positively regulate IL-12Rβ1 expression, respectively), then an indirect effect might be operative. Evidence against this was the fact that, in these short-term culture conditions, IL-4 was not detected (data not shown). Moreover, the fact that anti-IL-4, anti-IL-10, and anti-TGF-β alone did not substantially reverse the inhibition seen with PGE₂ mitigates this as a mechanism. Second, the data showing that other inducers of cAMP such as dibutyryl cAMP, 8-BrcAMP, and cholera toxin (Table I) inhibited IL-12Rβ1 expression and IL-12 binding make this the more likely mechanism, since this is how PGE₂ mediates its effects. Whether PGE₂, through cAMP, directly inhibits IL-12R expression or indirectly mediates this effect through another mechanism (i.e., cytokines) remains an open question. In this regard, the ability of PGE₂ to inhibit IL-2 production (18) may diminish IL-12 responsiveness, since IL-2 enhances IL-12Rβ1 expression (7). Moreover, the fact that addition of exogenous IL-2 to cells stimulated in the presence of PGE₂ restored IL-12Rβ1 expression supports this as an important mechanism (data not shown).

The role of DXM in regulating Th1/Th2 responses and IL-12 responsiveness

Due to the importance of IFN-γ and IL-4/IL-5 in inflammatory and allergic diseases, respectively, the ability of corticosteroids to affect Th cell differentiation has been of great interest. Studies examining the effect of corticosteroid Th1- and Th2-type cells have

**FIGURE 2.** PGE₂ and Dexamethasone Inhibit IL-12R Expression

Fresh human PBMCs were plated at \(1 \times 10^6\) cells/ml in six-well plates previously coated with anti-CD3 (5 µg/ml) in the presence or absence of PGE₂ (10⁻⁶ M) or DXM (10⁻⁷ M) and stimulated for 3 days. Cells were then harvested, washed extensively, and plated at 2 \(\times 10^5\) cells/200 µl in 96-well plates with varying concentrations of rIL-12. Two days later, supernatants were harvested, and IFN-γ production was assessed by ELISA. Cells were also restimulated in a similar manner as above with varying concentrations of IL-12 (B) or IL-2 (C), and [³H]thymidine was added for 6 h. Plates were subsequently harvested, and cpm was determined. Results are representative of three separate experiments.
FIGURE 3. PGE₂ and DXM inhibit IL-12Rβ1 expression and IL-12 binding in PBMCs stimulated with anti-CD3. Fresh human PBMCs were plated at 1 × 10⁶ cells/ml in 24-well plates previously coated with anti-CD3 (5 µg/ml) in the presence or absence of varying concentrations of PGE₂ (A) or DXM (B) and stimulated for 3 days. Cells were then harvested, washed, and stained with biotinylated anti-IL-12Rβ1 Ab, followed by streptavidin PE. MFI correlating with IL-12Rβ1 expression was determined by FACS analysis. In the same experiment, following stimulation in the presence or absence of varying concentrations of PGE₂ (C) or DXM (D), IL-12 binding was assessed by incubating cells with IL-12 (20 ng) for 30 min at 4°C, followed by washing and staining with biotinylated (nonneutralizing) Ab, followed by streptavidin PE. Results are presented as MFI. Results are representative of three separate experiments.
yielded inconsistent results due to several variables. These include the dose of steroids used in the cultures, whether T cells are stimulated in the presence or absence of APCs, and the stage of differentiation at which the T cells are tested. Thus, an early report using mouse cells stimulated in vitro showed that low-dose corticosteroids led to increased production of IL-4 (29), while human PBMCs stimulated with mitogens in vitro had decreased production of IL-4 (30). In this latter report, however, IFN-γ was not assessed, so specificity for IL-4 cannot be assured. In subsequent studies, the presence of corticosteroids inhibited transcription of both IL-4 and IFN-γ (31, 32); however, in one of these studies, low doses of corticosteroids appeared to increase IL-4 (31). In a rat model of in vitro priming, mRNA levels for IL-4, IL-10, and IL-13 were increased, but IFN-γ decreased, by culturing cells in DXM (33). Finally, using an APC-independent in vitro priming model, the presence of low-dose corticosteroids decreased production of IL-4, IL-5, and IFN-γ, but increased IL-10 using naive CD4+ / CD45RO+ cells (34). Of interest, the presence of corticosteroids in the restimulation cultures enhanced IL-4, but diminished IL-5 and IFN-γ (34).

More recently, the role of DXM in affecting Th cell differentiation via its effects on IL-12 has been elucidated in two reports using mouse (24) and human (23) in vitro culture systems. These studies show that DXM inhibits IL-12 production from macrophages, leading to a reduction of IFN-γ and increase in production of IL-4. Furthermore, in both of these studies, addition of exogenous IL-12 was able to restore the defect caused by DXM, suggesting that IL-12 responsiveness was maintained. In the studies reported in this work, human PBMCs cultured in the presence of DXM and IL-12 had a marked reduction in IFN-γ production. Moreover, cells cultured with DXM alone during primary stimulation were unresponsive to subsequent stimulation with exogenous IL-12. Differences among these studies may be due to different in vitro culture conditions. In our studies, PBMCs or total T cells were stimulated with anti-CD3, and receptor expression and cytokine production were assessed within 3 to 5 days following stimulation. By contrast, the other studies used CD4+ T cells stimulated with adherent cells plus PHA and PMA for a longer time.

**FIGURE 4.** PGE₂ and DXM inhibit IL-12R expression and IL-12 binding on T cells. T cells were isolated from fresh human PBMCs, as described in Materials and Methods. T cells were then plated at 1 × 10⁶ cells/ml in 24-well plates previously coated with anti-CD3 (5 μg/ml) in the presence or absence of PGE₂ (10⁻⁶ M) or DXM (10⁻⁷ M) and stimulated for 3 days. Cells were then harvested, washed, and stained with biotinylated anti-IL-12Rβ1 Ab, followed by streptavidin PE. IL-12 binding was done in a similar manner as described in Figure 3. Results are representative of three separate experiments.

**FIGURE 5.** PGE₂ and DXM inhibit IL-12Rβ2 mRNA expression. Fresh human PBMCs were plated at 1 × 10⁶ cells/ml in six-well plates previously coated with anti-CD3 (5 μg/ml) in the presence or absence of PGE₂ (10⁻⁶ M) or DXM (10⁻⁷ M) and stimulated for 2 days. Cells were then harvested, and total RNA was prepared as described in Materials and Methods. A, mRNA expression for IL-12Rβ2 and 28S rRNA was assessed by Northern blot analysis. B, The ratio of IL-12Rβ2:28S rRNA was determined by PhosphorImager. Results are representative of two separate experiments.
expression in PBMCs and T cells previously coated with anti-CD3 (5 μg/ml) plus PGE2 (10−6 M) or DXM (10−7 M) in the presence or absence of anti-IL-4 (10 μg/ml), anti-IL-10 (10 μg/ml), anti-TGF-β (10 μg/ml), or all three for 3 days. IL-12Rβ1 expression was assessed as described in Figure 3. Results are representative of two separate experiments.

(12 days) or in multiple rounds of stimulation. Despite these differences, the functional result is that DXM inhibits the induction of IFN-γ. Finally, with regard to mechanism, addition of anti-IL-4, IL-10, and TGF-β did not significantly affect the inhibition of IL-12Rβ1 expression or IL-12 binding by DXM.

Clinical implications of DXM affecting IL-12 responsiveness

In conclusion, since corticosteroids are among the most potent drugs for treatment of inflammatory and allergic diseases, understanding the mechanism by which they regulate immune responses may allow for more targeted use depending on the stage and type of disease. It has recently been speculated that the ability of corticosteroids to selectively inhibit type 1 cytokine responses would lead to enhancement of Th2 responses (24). Thus, in the case of allergic or asthmatic disease, it is possible that prolonged treatment could potentiate ongoing Th2 responses (35). This concept, however, must be viewed in light of the fact that steroids prevent eosinophil accumulation. Moreover, in a study of BAL cells from asthmatic patients, it was shown that steroid-sensitive asthmatics had a decrease in the number of BAL cells expressing IL-4 or IL-5 mRNA with a rise in the number of cells expressing IFN-γ (36). In the same study, steroid-resistant patients had a reduction in the number of BAL cells expressing IFN-γ following steroid treatment. Overall, while the mechanism by which corticosteroids affect Th1 and Th2 responses is not entirely clear, they remain the best treatment for treating the symptoms of acute allergic and asthmatic disease. The real question will be whether corticosteroids can be reserved for treatment for acute disease, while various immune manipulations (i.e., cytokine treatment, altered T cell ligands, DNA) can alter established Th2 responses, leading to a change in the underlying cause of the disease. Finally, the ability of steroids to inhibit both IL-12 production and responsiveness may be related to some of their effectiveness in treating autoimmune diseases in which IL-12 has a role.

Acknowledgments

We thank Drs. Maurice Gately and Ueli Gubler (Hoffmann-La Roche) for reagents and helpful advice, and Brenda Rae Marshall for editorial assistance.

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Table I. Increased cAMP leads to down-regulation of IL-12Rβ1 expression in PBMCs and T cells

<table>
<thead>
<tr>
<th>Addition to Cultures</th>
<th>IL-12Rβ1 (MFI)</th>
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<tr>
<td></td>
<td>PBMCs</td>
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<tr>
<td>Medium</td>
<td>12.3</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>63.3</td>
</tr>
<tr>
<td>Anti-CD3 + PGE2</td>
<td>39.5</td>
</tr>
<tr>
<td>Anti-CD3 + cholera toxin</td>
<td>15.1</td>
</tr>
<tr>
<td>Anti-CD3 + dibutyryl cAMP</td>
<td>11.6</td>
</tr>
<tr>
<td>Anti-CD3 + 8-Br-cAMP</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* Fresh human PBMCs or T cells were plated at 1 × 10⁶ cells/ml in 24-well plates previously coated with anti-CD3 (5 μg/ml) in the presence or absence of PGE2 (10−6 M), cholera toxin (100 ng/ml), dibutyryl cAMP (1 mM), or 8-Br-cAMP (1 mM) for 3 days. IL-12Rβ1 expression and IL-12 binding was assessed as described in Figure 3.


