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Research Article

Dexamethasone enhances CTLA-4 expression during T cell activation

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Abstract. T cell activation is enhanced by the costimulatory interaction of B7 on antigen-presenting cells and CD28 on T cells, resulting in long-term T cell proliferation, differentiation and production of large amounts of cytokines, such as interleukin (IL)-2. CTLA-4 is a co-stimulation receptor that shares 31% homology with CD28 and binds B7 family members with higher affinity. CTLA-4 is transiently expressed intracellularly and on the cell surface following activation of T cells. We have studied the kinetics of CTLA-4 expression and the effects of dexamethasone on CTLA-4 expression during T cell activation in cultures of mouse spleen cells stimulated by a mixture of immobilized anti-CD3 and anti-CD28 monoclonal antibodies (anti-CD3/CD28 mAb) or concanavalin A (ConA). CTLA-4 expression peaked on day 2 and returned to background levels after 7 days. Dexamethasone was found to potentiate CTLA-4 expression in a dose-dependent manner with an EC_{50} effective concentration 50%) of about 10^{-8} M. In contrast, other immunosuppressive agents, such as rapamycin or cyclosporin A had no or an inhibitory effect on CTLA-4 expression, respectively. Dexamethasone also stimulated CD28 expression, but inhibited IL-2R expression during anti-CD3/CD28 mAb-induced mouse splenic T cell activation. Western blot analyses of lysates of activated mouse T cells showed that dexamethasone increased CTLA-4 protein levels twofold during anti-CD3/CD28 mAb-induced activation. Dexamethasone also enhanced CTLA-4 messenger RNA twofold as quantified by ribonuclease protection assay. The effects of dexamethasone on CTLA-4 expression were glucocorticoid-specific and completely inhibited by the glucocorticoid receptor antagonist mifepristone (RU486), indicating that the effect of dexamethasone on CTLA-4 expression is mediated through the glucocorticoid receptor. In conclusion, the immunosuppressive agent dexamethasone actually stimulates CTLA-4 expression, which is involved in downregulation of T cell activation.

Key words. T cell; CTLA-4; dexamethasone; costimulation; CD28.

Activation of T lymphocytes requires two signals, antigen recognition by the T cell receptor and costimulatory interaction of CD28 on T cells with B7 on antigen-presenting cells. There are two B7 family members, B7-1 and B7-2. Signaling through CD28 with anti-CD28 monoclonal antibody (mAb) dramatically enhances T lymphocyte activation and proliferation as well as increases expression and secretion of IL-2, tumor necrosis factor (TNF)-α, lymphotoxin, interferon (IFN)-γ, and granulocyte/macrophage-colony stimulating factor (GM-CSF) [1, 2]. CTLA-4 is expressed during activation of T cells and was originally cloned from a cDNA library derived from activated cytolytic T (CTL) cells [3]. CTLA-4 shares 31% similarity at the amino acid

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level with CD28 [3–5] and is expressed on T cells during activation of T cells [6, 7]. In contrast to the costimulatory interaction of CD28 with B7, binding of CTLA-4 to B7s delivers an inhibitory signal to T cells [8, 9]. Lack of this inhibitory signal in CTLA-4-deficient mice resulted in lymphoproliferative disease with massive lymphocytic infiltration and tissue destruction [10, 11]. CTLA-4 appears to play an essential role to prevent uncontrolled T cell activation.

Dexamethasone, a synthetic glucocorticoid, has both antiinflammatory and immunosuppressive effects. The effects of dexamethasone are due to interaction with a cytoplasmic glucocorticoid receptor (GR) complex. When dexamethasone binds to GR, the complex translocates into the nucleus and binds to promotor regions (glucocorticoid receptor response elements, GREs), resulting in either induction or repression of transcription of steroid-responsive target genes [12, 13]. Although the inhibitory effect of dexamethasone on expression of cytokines and cytokine receptors such as IL-2 and IL-2 receptor as well as T cell proliferation has been well documented [14, 15], little is known about the effect of dexamethasone on CTLA-4 expression during T cell activation. Here, we present evidence that dexamethasone affects expression of CTLA-4 in mouse splenic T cells activated by various stimuli. It is demonstrated by using fluorescence activated cell sorter (FACS) analysis, ribonuclease protection assay (RPA) and Western blot analysis that dexamethasone upregulates intracellular and cell surface expression of CTLA-4 in activated T cells. This effect was mediated through GR because the glucocorticoid antagonist RU486 [15a] inhibited the dexamethasone effect on CTLA-4 expression completely. The immunosuppressive effects of dexamethasone may be enhanced by its upregulation of expression of CTLA-4 in T cells.

Materials and methods

Animals. Female C57BL/6 mice of 10–14 weeks of age (Charles River Labs, Wilmington, MA) were used in these studies.

Reagents. Concanavalin A, ConA (# 27648, Serva, Heidelberg, Germany), dexamethasone (1 mg/ml, NDC0641-2273-41, Elkins-Sinn, Cherry Hill, NJ, USA), RU-486 (mifepristone, M-8046), testosterone (T-6147), progesterone (P-8783) and cyclosporin A (C-3662) all from Sigma, St. Louis, MO, and rapamycin (553210, Calbiochem, La Jolla, CA) were used. Hamster-anti-mouse-CD3e (clone 145-2C11), hamster-anti-mouse-CD28 (clone 37.51), hamster-anti-mouse-CTLA-4 (clone UC10-4F10-11), phycoerythrin (PE)-labeled anti-mouse-IL-2 receptor α-chain, PE-labeled anti-mouse-CD28, fluorescein isothiocyanate (FITC)-labeled hamster-anti-mouse TCR (T cell receptor clone H57-597), and PE-labeled hamster IgG isotype standard (anti-TNP (tri-nitro-phenyl), clone G235-2356) as a control all were purchased from Pharmingen, San Diego, CA.

Splenocyte preparation, culture and treatment. Spleens were harvested from C57BL/6 mice and prepared as single-cell suspensions by grinding tissue through sterile wire mesh. Red blood cells were disrupted by hypotonic solution. Mononuclear cells were resuspended in RPMI1640 medium containing 5% fetal bovine serum, 2 mM L-glutamine, 100 units/ml of penicillin G, 100 μg/ml of streptomycin sulfate, 50 μM of 3-mercapto-1,2-propanediol, 1 mM MEM sodium pyruvate solution, 10 mM Hepes, 0.1 mM MEM nonessential amino acid solution, and 1% MEM vitamin solution. Cells were plated at a concentration of 0.5×10^6/200 μl in 96-well plates, and stimulated with immobilized anti-CD3 mAb (2.5–20 μg/ml) and anti-CD28 mAb (5–20 μg/ml) or ConA (1–2.5 μg/ml). Dexamethasone (10^{-10}–10^{-6} M), testosterone (10^{-10}–10^{-6} M), progesterone (10^{-10}–10^{-6} M), cyclosporin A (10^{-10}–10^{-6} M), rapamycin (10^{-10}–10^{-6} M) or RU486 (10^{-10}–10^{-6} M) were added 24 h later as a 20 × concentrated solution in 10 μl of medium. The cells were incubated at 37 °C in 5% CO_2 for various intervals as indicated before being harvested and analyzed by flow cytometry, as described below.

Flow cytometry analysis. Flow cytometry was used to measure expression of CTLA-4 and CD28 on splenic T cells. Briefly, splenocytes were double-stained with FITC-conjugated anti-TCR mAb, and either a hamster IgG isotype-matched control, PE-conjugated anti-CTLA-4 mAb, or anti-CD28 mAb for 30 min at 4 °C followed by two 2-ml washes in ice-cold 0.5% bovine serum albumin–phosphate-buffered saline (BSA-PBS), and resuspended in a final volume of 500 μl of BSA-PBS. For total, intracellular and cell surface, detection of CTLA-4, splenocytes were first fixed in fix solution and then in permeabilizing solution (# BUF09, batch 190697, Harlan Bioproducts for Science, IN) with FITC and PE-conjugated mAb, as described above. Flow cytometric analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA). Data were electronically gated for TCR-positive cells, and mean channel fluorescence was calculated using CELLQuest software.

RNase protection assay. Total cellular RNA was isolated from untreated or treated mouse splenocytes using RNA STAT-60 reagent (TEL-TEST, TX). The RNA pellet was precipitated with isopropanol and washed with 70% ethanol, then redissolved in diethyl pyrocarbonate water. To construct the riboprobe for mouse CTLA-4 messenger RNA (mRNA), a 318-bp polymerase chain reaction (PCR) fragment (bases 118–436)

of mouse CTLA-4 complementary DNA (cDNA) (accession number x05719, GenBank) was amplified by PCR with the following sets of sense and antisense primers: 5′-CCTTTTGTAGCCCTGCTC-3′ (bases 118–135) and 5′-ACAGTCCCGTGTCAACAG-3′ (bases 436–419). The PCR product of the CTLA-4

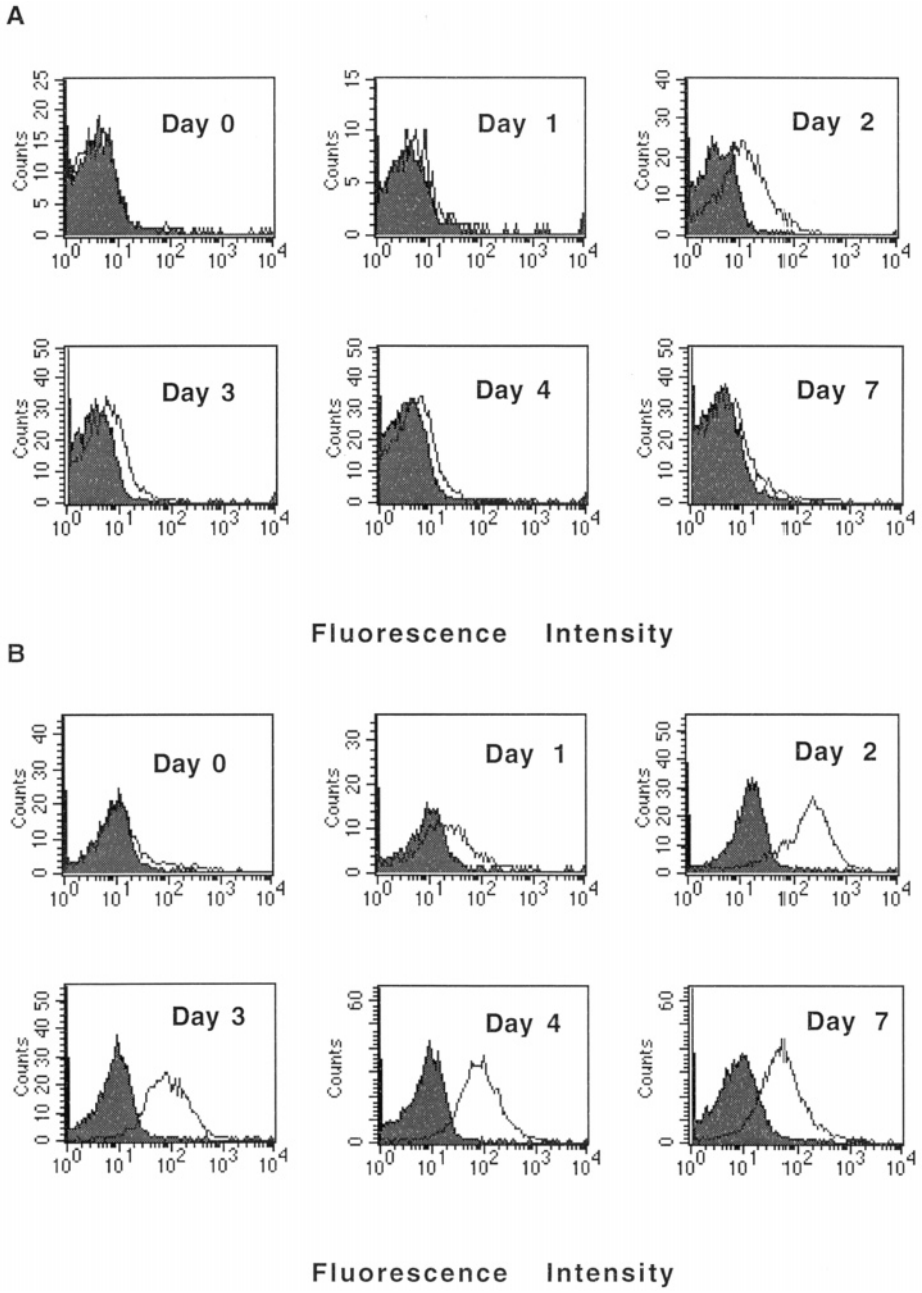

Figure 1. Kinetics of cell surface and total expression of CTLA-4 after anti-CD3/CD28-induced T cell activation. (*A*) Cell surface expression of CTLA-4. Splenocytes of C57BL/6 mice were incubated with anti-CD3 (5 μg/ml) and anti-CD28 (5 μg/ml) mAb for 0 to 7 days. Cells were double-stained with anti-TCR FITC and either hamster IgG isotype-matched control (filled peak) or anti-CTLA-4 PE (blank peak). Data were electronically gated for TCR-positive cells and analyzed on a FACScan. (Data shown are representative of three independent experiments.) (*B*) Total expression of CTLA-4. Splenocytes of C57BL/6 mice were incubated with anti-CD3 (5 μg/ml) and anti-CD28 (5 μg/ml) mAb for 0 to 7 days. The cells were then permeabilized using a cell permeabilization kit (Harlan Bioproducts for Science, IN), and double-stained with anti-TCR FITC and either hamster IgG isotype-matched control (filled peak) or anti-CTLA-4 PE (blank peak). Data were electronically gated for TCR-positive cells and analyzed on a FACScan. (Data shown are representative of three independent experiments.)

gene (bases 118–436) was subcloned into the pGEM-T vector (Promega, WI). The vector was linearized with Sph I, and radiolabeled antisense transcripts were synthesized using ^{32}P-UTP (uridine triphosphate, 3000 Ci/mol, Amersham) and cyclophilin probes as internal controls. RNase protection assay (RPA) was determined by using the RPA II kit (Ambion, Austin, TX) according to the manufacturer's instructions. In brief, hybridization was performed at 55 °C for 16 h and with 20 μg of total RNA and 10^4 counts of the ^{32}P-labeled RNA probe. After hybridization, the unhybridized RNA (ribonucleic acid) was digested by the addition of diluted (1:100) RNase A-T1 mixture at 37 °C for 40 min. Digestion was terminated by the addition of an RNase inactivation and precipitation mixture. The protected fragment (318 bp for CTLA-4 and 103 bp for cyclophilin) was separated on a 6% TBE/urea gel (Novex, CA), followed by Storm 840 Phosphorimager system analysis (Molecular Dynamics, CA). Data were analyzed with ImageQuat image analysis software.

Western blot. Splenocytes were washed once with PBS and homogenized using a Dounce homogenizer in ice-cold RIPA buffer (1 × PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS (sodium dodecyl sulfate), 1 mM PMSF (phenylmethylsulfonyl fluoride), 30 μl/ml aprotinin and 1 mM sodium-p-vanadate). After addition of 10 μl of a 100 mM PMSF stock solution, homogenates were incubated on ice for 30 min and centrifuged at 15,000*g* for 20 min at 4 °C. Replicate aliquots of 15,000*g* supernatants were electrophoresed in 1-mm-thick SDS-10% polyacrylamide gel at 140 V for 1.5 h at room temperature. Proteins resolved were transferred by electroblotting to nitrocellulose membranes (Hybond, Novex, CA) at 100 V for 1.5 h. The membranes were developed with mouse anti-CTLA-4 mAb (Pharmingen, CA), followed by horseradish peroxidase-conjugated goat anti-hamster IgG (Southern Biotech Associates, AL) and luminescence analysis (Santa Cruz Biotec, CA). The intensity of bands was quantified with a Personal Densitometer SI (Molecular Dynamics, CA). Data were analyzed with imageQuant software.

Statistical analysis. All experimental results are presented as the mean ± SEM. The statistical significance of differences was evaluated by Student's *t* test. Values of $P < 0.05$ were considered to be significant.

Results

Kinetics of CTLA-4 expression in mouse spleen T cells. First the optimal concentrations for anti-CD3/CD28 mAb induction of CTLA-4 in T cells were established. To restrict the analysis of CTLA-4 expression to T cells, double-staining methods including anti-TCR staining to identify T cells were used. Anti-CD28 mAb augments anti-CD3 mAb-induced cell surface expression of CTLA-4 (data not shown, [16, 17]). Five μg/ml of both anti-CD3 and anti-CD28 mAb were found to be optimal concentrations for maximal induction of CTLA-4 expression in T cells, and therefore subsequent experiments were conducted at these concentrations. No CTLA-4 expression was found in freshly isolated T cells on day 0 or in unstimulated T cells (fig. 1A, see also figs 5 and 6). Stimulation of splenocytes from C57BL/6 mice with anti-CD3/CD28 mAb resulted in high cell surface expression of CTLA-4. Maximal expression of CTLA-4 was found after 2 days of stimulation (fig.fig. 1A and [9]). CTLA-4 expression returned to near background levels by day 7 (fig. 1A). To study total, cell surface plus intracellular, CTLA-4 expression, anti-CD3/CD28 mAb-stimulated T cells were permeabilized before the staining. Total CTLA-4 expression was induced at an even higher magnitude but with similar

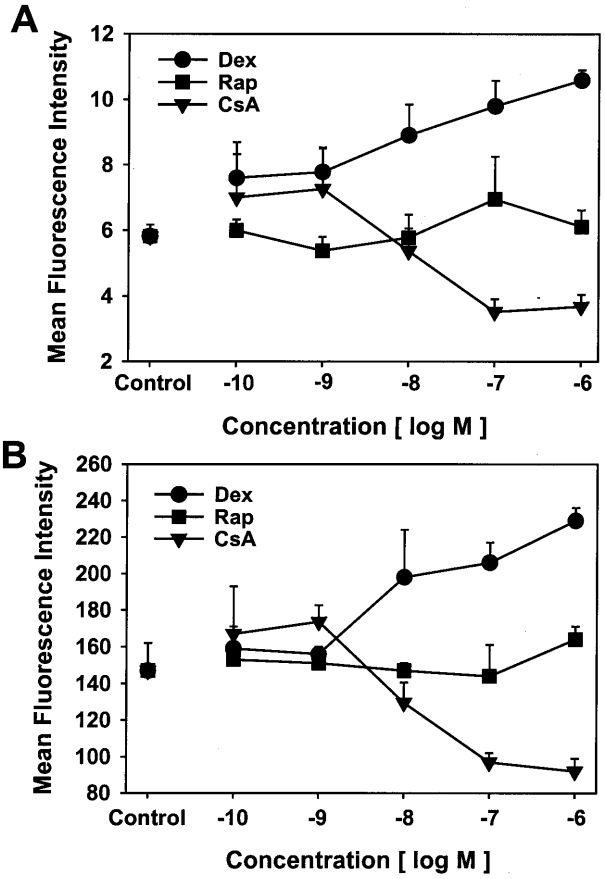

Figure 2. Concentration dependence of the effect of dexamethasone on cell surface (*A*) or total (*B*) CTLA-4 expression on mouse spleen T cells. Murine splenocytes were incubated for 2 days with anti-CD3/CD28 mAb (controls), or with anti-CD3/CD28 mAb, and on day 1 various doses of dexamethasone (circles), rapamycin (squares) or cyclosporin A (triangles) were added. Samples were processed and analyzed as described in figure 1. Each point is the mean ± SEM of the results of three independent experiments.

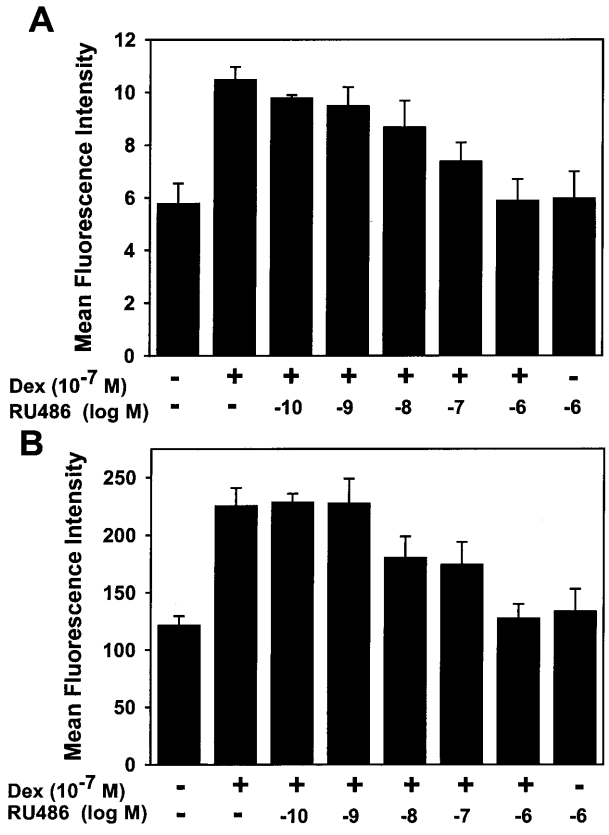

Figure 3. RU486 inhibition of dexamethasone-induced increase of cell surface (*A*) or total (*B*) CTLA-4 expression on splenic T cells. Murine splenocytes were incubated for 2 days with anti-CD3/CD28 mAb, and on day 1 dexamethasone (10^{-7} M) in the presence or absence of various concentrations of RU486 (10^{-10}–10^{-6} M) was added. Samples were processed and analyzed as described in figure 1. Each point is the mean ± SEM of the results of three to five independent studies.

kinetics as cell surface expression (fig. 1B). Interestingly, most CTLA-4 is intracellular, and only a small percentage of CTLA-4 is found on the cell surface at all times as indicated by a mean fluorescence intensity of about 7 for extracellular and of about 150 for total CTLA-4 (see figs 1 and 2). Other T cell stimulants such as ConA have similar effects with identical kinetics as anti-CD3/CD28 mAb on CTLA-4 expression (data not shown).

Effect of dexamethasone on CTLA-4 expression in mouse T cells. We studied the effects of dexamethasone on CTLA-4 expression in mouse T cells following activation with anti-CD3/anti-CD28 mAb. We chose to add dexamethasone after 1 day to cultures of mouse spleen cells stimulated by ConA or anti-CD3/anti-CD28 mAb. Thus, T cell activation was under way when we added dexamethasone. Interestingly, dexamethasone dose-dependently with EC_{50} values of 10^{-8} M enhanced CTLA-4 expression both of cell surface and intracellular levels following anti-CD3/CD28 mAb (fig. 2A, B) or ConA (data not shown) stimulation. Dexamethasone did not induce CTLA-4 expression in unstimulated T cells (data not shown). Other steroids such as testosterone and progesterone at concentrations from 10^{-10} to 10^{-6} M had no effect on CTLA-4 expression in T cells induced by anti-CD3/CD28 mAb (data not shown).

In contrast to dexamethasone, other immunosuppressive drugs had no stimulatory effect on CTLA-4 expression. CTLA-4 expression induced by anti-CD3/CD28 mAb was not affected by rapamycin, but inhibited by cyclosporin A (fig. 2).

In order to determine that the dexamethasone-induced increase in CTLA-4 expression is due to specific glucocorticoid-GR interaction, splenocytes were stimulated in the presence of dexamethasone alone, dexamethasone plus various doses of the GR antagonist RU486, or RU486 alone. The results show that only dexamethasone resulted in an augmentation of both intracellular and cell surface CTLA-4 expression following stimulation with anti-CD3/CD28 mAb. The augmentation of CTLA-4 expression by dexamethasone was dose-dependently inhibited by RU486 (fig. 3). RU486

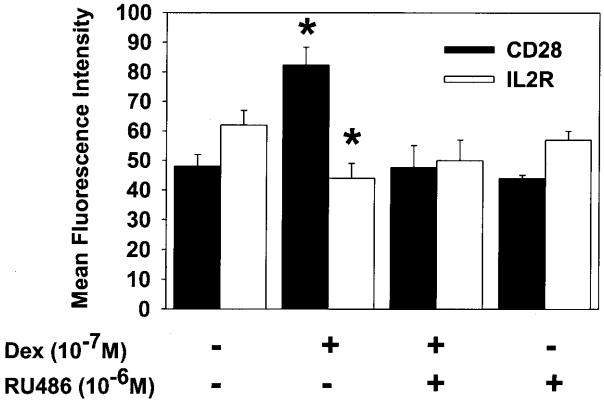

Figure 4. Effect of dexamethasone on CD28 and IL-2R expression on mouse spleen T cells. Mouse splenocytes were incubated for 2 days with anti-CD3/CD28 mAb. Dexamethasone and RU486 were added on day 1. Cells were double-stained with FITC-labeled anti-TCR antibody and with either PE-labeled hamster IgG isotype-matched negative control or anti-CD28 or anti-IL-2R antibodies. Data were electronically gated for TCR-positive cells and analyzed on a FACScan. Mean fluorescence intensity was calculated by subtracting the baseline of isotype-matched control staining for each experiment from that of anti-CD28 or IL-2R antibody staining. Each point is the mean ± SEM of the results of three independent experiments. $^*P < 0.05$ compared with control group.

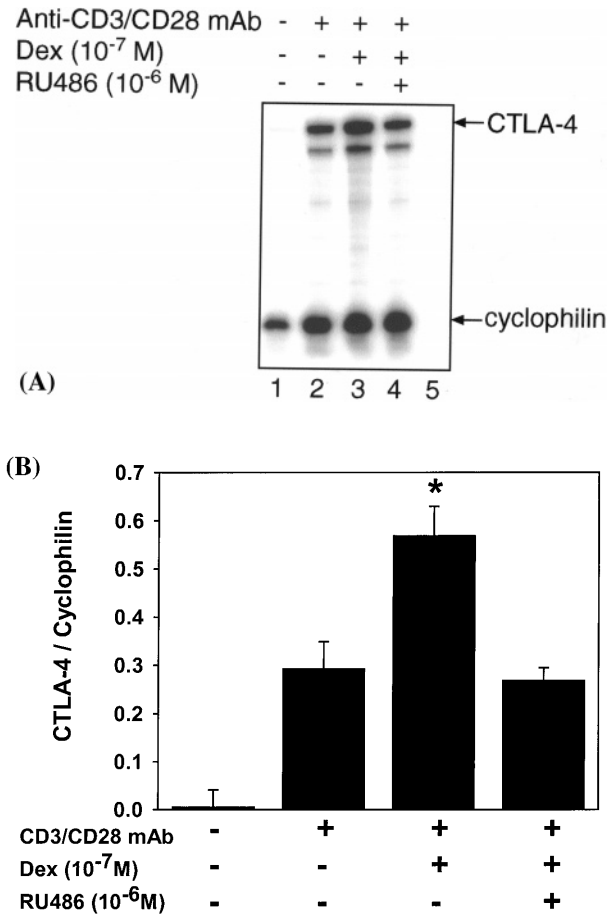

Figure 5. Measurement of CTLA-4 mRNA by RNA protection assay. (*A*) mRNA was extracted from mouse spleen cells activated for 2 days with anti-CD3/CD28 mAb in the presence or absence of dexamethasone added on day 1. RPA analysis (see 'Materials and methods') shows the effects of 10^{-7} M dexamethasone (lane 3) or dexamethasone plus 10^{-6} M of RU486 (lane 4) on CTLA-4 mRNA-activated splenocytes (lane 2) relative to that in unstimulated cells (lane 1) at 2 days. Lane 5 is yeast RNA. The 318-bp fragment is derived from the protection of CTLA-4 mRNA. A cyclophilin probe was used as an internal control (103 bp of protected portion). (*B*) Densitometric analysis of radiolabeled gels by storage phosphor screens is shown in the histogram. Absorbance values were normalized to cyclophilin and the mean values ± SEM of three independent experiments are shown. $*P < 0.05$ compared with anti-CD3/CD28 mAb treatment group.

alone did not affect total or cell surface expression of CTLA-4 (fig. 3).

Next we examined effects of dexamethasone on the expression of CD28 and IL-2R on T cells activated by anti-CD3/CD28 mAb stimulation. Dexamethasone significantly stimulated CD28 expression, but inhibited IL-2R expression after anti-CD3/CD28 mAb stimulation (fig. 4). RU486 (10^{-6} M) also completely abrogated the effects of dexamethasone on CD28 or IL-2R expression. Again, RU486 alone had no effect on CD28 and IL-2R expression (fig. 4).

Effect of dexamethasone on CTLA-4 mRNA and protein level. In agreement with previous studies [17], we did not detect CTLA-4 mRNA, which was analyzed by RPA in unstimulated murine splenocytes (fig. 5A, lane 1). CTLA-4 mRNA was expressed after splenocytes were stimulated with anti-CD3/CD28 mAb (fig. 5A, lane 2), which confirms previous studies that CTLA-4 mRNA is only expressed in activated T cells [17]. We did not separate T cells from splenocytes to measure mRNA and protein because there was no detectable CTLA-4 expression in B cells by FACScan analyses (data not shown). To determine whether dexamethasone affected CTLA-4 expression at the mRNA level, mouse splenocytes were stimulated with anti-CD3/CD28 mAb for 2 days, and dexamethasone and/or RU486 was added at day 1. CTLA-4 mRNA from splenocytes activated by anti-CD3/CD28 mAb was increased significantly by dexamethasone (fig. 5A, lane 3). The mean augmentation of CTLA-4 mRNA by dexamethasone was determined by a phosphorimager to be twofold over that of anti-CD3/CD28 mAb stimulation alone (fig. 5B). Dexamethasone-induced augmentation of CTLA-4 mRNA expression was completely inhibited in the presence of RU486 and thus appears to be mediated through interaction with GR (fig. 5A, lane 4). Western blot analysis of extracted cytosolic content of CTLA-4 protein in murine splenocytes showed that there was no detectable CTLA-4 protein in unstimulated splenocytes (fig. 6, lane 1). A major protein band of approximately 32 kDa was observed for anti-CD3/CD28 mAb-activated T cells (fig. 6, lane 2). Addition of dexamethasone to activated T cells resulted in a twofold increase of CTLA-4 protein (fig. 6, lane 3). RU486 completely inhibited dexamethasone-induced augmentation of CTLA-4 protein expression (fig. 6, lane 4). These data show that dexamethasone-induced augmentation of CTLA-4 expression by FACScan analysis correlates with an augmentation of both CTLA-4 mRNA and CTLA-4 protein.

Discussion

CD28 is a costimulatory molecule constitutively expressed on T cells. CD28 enhances T cell activation when interacting with B7 on antigen-presenting cells. The CD28 homolog CTLA-4 is not constitutively expressed on T cells. Following T cell activation, both intracellular and cell surface levels of CTLA-4 rise, peak by day 2 and return to background levels by day 7 (fig.1). CTLA–4 binds to B7 with higher affinity than CD28 and results in a downregulatory signal in T

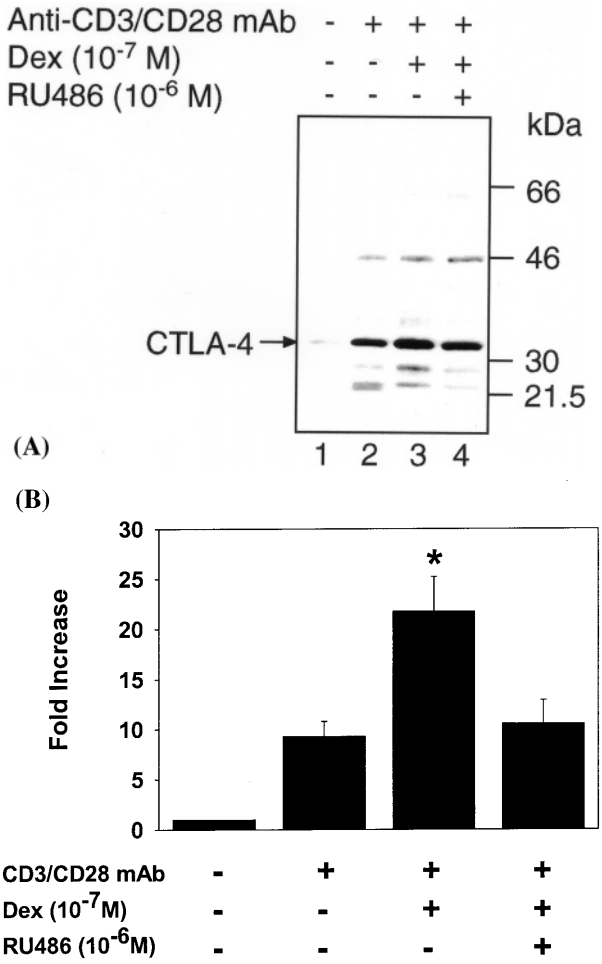

Figure 6. Measurement of CTLA-4 protein expression. (*A*) Western blot of CTLA-4 protein in mouse T cell extracts following 2 days in culture without (lane 1) or activation with anti-CD3/CD28 mAb in absence (lane 2) or presence of 10^{-7} M dexamethasone (lane 3) or 10^{-7} M dexamethasone plus 10^{-6} M of RU486 (lane 4) added on day 1. Western blots were developed as described in 'Materials and methods,' and the films were exposed for 5 s. (*B*) Films of Western blots were analyzed by densitometry. Mean values $\pm$ SEM of three independent experiments are shown. $^*P < 0.05$ compared with anti-CD3/CD28 mAb treatment group.

cells. This is illustrated by the uncontrolled T cell activation seen in CTLA-4 knockout mice.

Glucocorticoids are widely used for their anti-inflammatory and immunosuppressive activity. They have profound effects on T cells such as inhibiting cytokine production and T cell activation. For this study we have used dexamethasone as a model glucocorticoid. In addition, RU486 has been described as a glucocorticoid antagonist and was used to block activities of glucocorticoids mediated through the glucocorticoid receptor. The inhibitory effect of dexamethasone on T cell activation and proliferation is associated with decreased IL-2 production [18] and expression of the IL-2 receptor (fig. 4). Interestingly, we observed upregulation of CTLA-4 expression during activation of T cells in the presence of dexamethasone (fig. 2). Dexamethasone concentrations at which the CTLA-4 stimulatory effects were seen are comparable to the concentrations which inhibit T cell proliferation in vitro (data not shown).

The upregulation of CTLA-4 expression by dexamethasone was dose-dependent (fig. 2), mediated through interaction with GR (fig. 3), counteracted by the competitive antagonist RU486 and correlated with an increase of the CTLA-4 protein and the specific mRNA (figs 5 and 6). However, CTLA-4 expression was not affected by rapamycin treatment and was inhibited by cyclosporin A treatment (fig. 2). Cyclosporin A binds to an intracellular receptor, cyclophilin, and forms inhibitory complexes that block the phosphatase activity of calcineurin. Calcineurin is an essential component of the T cell activation pathway [19, 20]. Rapamycin suppresses T cell activation mainly through inhibition of growth-promoting lymphokines and inhibition of the costimulatory signal through the CD28 receptor [20, 21]. The findings presented here underline that the immunosuppressive effects of glucocorticoids are unique when compared with other T cell-directed immunosuppressive agents, such as cyclosporin A and rapamycin (fig. 2).

Glucocorticoids inhibit IL-2 gene expression by activating a GRE in the promotor region of the IL-2 gene [22]. Modulation of IL-2 transcription or production by dexamethasone in stimulated T cells results in downregulation of immune responses. The ability of glucocorticoids to suppress already activated T cells provides a basis for their efficacy in the treatment of various diseases whereby tissue injury is mediated by activated T cells.

CD28 and CTLA-4 interact with B7-1 (CD80) and B7-2 (CD86) as counterparts. CTLA-4 interaction with B7 is a down-regulatory signal during T cell activation. This can be demonstrated in vivo by blocking CTLA-4 from interacting with B7. For example, treatment of mice with an anti-CTLA-4 antibody at the time of disease induction resulted in more severe experimental allergic encephalomyelitis (EAE) [23]. Freshly isolated human monocytes express B7-2 but not B7-1 [24]. In in vitro culture of monocytes stimulated with PPD (purified protein derivative of tuberculin) B7-1 is induced and B7-2 expression is enhanced [24]. It is interesting to note that dexamethasone downregulates the expression of B7-1 but not B7-2 [25]. B7-2 seems to be more important for downregulation of T cell responses, at least in some cases [for review see [26]]. For example, in studies with anti-B7 antibodies in EAE (experimental allergic encephalitis) in mice it was found that blocking B7-1or B7-2 resulted in opposite effects on disease. Treatment with anti-B7-1 ameliorated disease whereas treatment

with anti-B7-2 exacerbated disease [27]. Based on such results one could speculate that the downregulation of B7-1 but not B7-2 [25] and upregulation of CTLA-4 (shown here) by dexamethasone could be one facet of the strong immunosuppressive effects of glucocorticoids.

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