

# Positive Effects of Glucocorticoids on T Cell Function by Up-Regulation of IL-7 Receptor $\alpha^1$

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Despite the effects of glucocorticoids on immune function, relatively little is known about glucocorticoid-inducible genes and how their products may regulate lymphocyte function. Using DNA microarray technology to analyze gene expression in PBMC from healthy donors, we identified IL-7R $\alpha$  as a glucocorticoid-inducible gene. This observation was confirmed at the mRNA and protein levels. Conversely, TCR signaling decreased IL-7R $\alpha$  expression, and the relative strength of signaling between these two receptors determined the final IL-7R $\alpha$  levels. The up-regulation of IL-7R $\alpha$  by glucocorticoids was associated with enhanced IL-7-mediated signaling and function. Moreover, IL-7-mediated inhibition of apoptosis at increasing concentrations of glucocorticoids is consistent with enhanced cell sensitivity to IL-7 following glucocorticoid exposure. These observations provide a mechanism by which glucocorticoids may have a positive influence on T cell survival and function. *The Journal of Immunology*, 2002, 168: 2212–2218.

Elicitation of a T cell response to a specific self or foreign Ag requires the presentation of an Ag/MHC complex by APCs to the TCR (1). This process takes place in secondary lymphoid and nonlymphoid organs, where it maintains naive and mature T cell responses to foreign Ags, peripheral T cell homeostasis, and self-tolerance (2–4). Alternative concurrent signals regulate TCR-mediated expansion or deletion of peripheral T cells. In fact, glucocorticoids may modulate the nature and the intensity of the immune response (5–7).

The most recognized biologic effect of glucocorticoids at high concentrations (exogenously administered or endogenously produced in response to stress) is immunosuppression, and these hormones are potent inducers of T cell apoptosis (8). Because the glucocorticoid receptor (GR)<sup>4</sup> is a transcriptional regulator, the simplest means to account for its effects on cell viability is to postulate that glucocorticoids induce the expression of gene products that directly or indirectly cause cell death. To date, however, there are no clear candidates for glucocorticoid-induced genes that cause cell death. Alternatively, glucocorticoids also have positive effects on immune development and function (5). The GR and the TCR independently induce apoptosis, yet together they promote T cell survival (9). Indeed, glucocorticoids antagonize and repress

TCR-mediated signals for cell death (10–12). This mutual regulation for survival not only occurs in the thymus but also exists in the peripheral immune system.

This context-dependent action of the GR led us to identify genes whose expression is modified by glucocorticoids and ask how they might influence T cell function. In this work we report that screening of almost 10,000 genes with DNA microarrays revealed that IL-7R  $\alpha$ -chain (IL-7R $\alpha$ ) is one of the most prominent gene induced by glucocorticoids. Furthermore, the positive regulation of IL-7R $\alpha$  by glucocorticoids was associated with enhanced IL-7-mediated signaling and function. These observations provide a mechanism by which glucocorticoids may influence T cell function and immune responsiveness.

## Materials and Methods

### *Cytokines, Abs, and cells*

The following reagents were purchased: recombinant human IL-7 (R&D Systems, Minneapolis, MN), anti-phosphotyrosine Ab (4G10; Upstate Biotechnology, Lake Placid, NY), polyclonal rabbit anti-IL-7R $\alpha$  Ab (Santa Cruz Biotechnology, Santa Cruz, CA), and monoclonal mouse anti-human CD3 and human CD28 (BD PharMingen, San Diego, CA). The following Abs used for flow cytometry were purchased: PE-conjugated anti-human IL-7R $\alpha$  (CD127) mAb (Immunotech, Marseilles, France); FITC-conjugated anti-human IL-2R $\alpha$  (CD25), anti-CD3, anti-CD4, anti-CD8, anti-CD19, and anti-CD14 mAb (BD Biosciences, San Jose, CA); and isotype-matched IgG controls (BD PharMingen). Polyclonal rabbit anti-Stat5a Ab was produced as previously described (13). Polyclonal rabbit anti-IL-7 and anti-IL-7R $\alpha$  neutralizing Abs were obtained from R&D Systems. Anti- $\beta$ -actin mAb was purchased from Sigma-Aldrich (St. Louis, MO). Streptavidin-PE was purchased from Caltag Laboratories (Burlingame, CA). Metyrapone was obtained from ICN Biochemicals (Costa Mesa, CA). Human IL-2 was provided by Dr. C. Reynolds (National Cancer Institute, Frederick, MD). NK3.3 cells were provided by Dr. J. Kornbluth (St. Louis University, St. Louis, MO). PBMC from healthy donors were isolated by Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ) gradient centrifugation. When peripheral T cells were used, PBMC were activated with PHA (2  $\mu$ g/ml) for 72 h and cultured for an additional day in presence of IL-2 (40 IU/ml), as described previously (14). Typically, this resulted in >95% CD3<sup>+</sup> cell purity, and before stimulation cells were washed with acidified medium (pH 6.4) and rested in RPMI 1640 containing 1% BSA. For TCR cross-linking experiments, PBMC were activated with coated anti-CD3/anti-CD28 (10  $\mu$ g/ml) for 3 days and isolated by Ficoll-Paque

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<sup>4</sup> Abbreviations used in this paper: GR, glucocorticoid receptor; Dex, dexamethasone;  $\gamma_c$ , common  $\gamma$  subunit; GILZ, glucocorticoid-induced leucine zipper.

gradient centrifugation. Purified human cord blood CD4<sup>+</sup> T cells and purified human bone marrow stromal cells were purchased from Poietic Technologies (Gaithersburg, MD). Naive CD4<sup>+</sup> T cells, which express CD45RA (>95%), were isolated from cord blood from umbilical blood collected from scheduled cesarean deliveries. Cells were isolated and re-suspended in HBSS containing 0.5% BSA and 5 mM EDTA, and CD4<sup>+</sup> T cells were from cord blood mononuclear cells using negative immunomagnetic selection, and purity was >95%.

#### FACS analysis

Expression of IL-7R $\alpha$  was detected using PE-conjugated anti-human IL-7R $\alpha$  mAb or PE-conjugated anti-mouse IL-7R $\alpha$  Ab. Expression of IL-2R $\alpha$  was detected using FITC-conjugated anti-human IL-2R $\alpha$  mAb or FITC-conjugated anti-mouse IL-2R $\alpha$  (CD25) Ab. The cells were washed, incubated with conjugated Abs and isotype-matched IgG-PE/isotype-matched FITC-IgG as controls for 30 min at 4°C, washed three times with PBS containing 0.5% BSA, and then analyzed by flow cytometry. Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Apoptosis was evaluated using propidium iodide/annexin V (Immunotech) staining.

#### Microarrays

PBMC were prepared as described and stimulated with dexamethasone (Dex; 10<sup>-7</sup> M) for 16 h. RNA extraction was performed (RNAagents; Promega, Madison, WI), and mRNA was purified with oligoTex mRNA isolation columns (Qiagen, Valencia CA). Microarray analysis was performed using the GEM microarray (Incyte, St. Louis, MO). The two fluorescent cDNA probes were mixed and simultaneously hybridized to a microarray containing 9182 human expressed genes. The microarray was scanned, and the intensity of the fluorescence at each array element was proportional to the expression level of that gene in the sample. The ratio of the two fluorescence intensities provided a quantitative measurement of the relative gene expression level in the two cell samples.

#### RNase protection assay

Human thymocytes and mature T lymphocytes were rested for 4 h, pretreated with Dex at different concentrations for various times, and stimulated with IL-7 (10 ng/ml) for various times. RNA extraction was performed, and mRNA expression was evaluated by RNase protection assay. RNase protection assay was performed as follows: <sup>32</sup>P-labeled RNA probes were synthesized using SP6 RNA polymerase or T7 RNA polymerase for the multiprobe template set (Riboquant; BD PharMingen). DNA was digested with DNase I (Roche, Indianapolis, IN), and RNA probes were extracted with phenol/chloroform and precipitated with ethanol. Labeled RNA probes were hybridized overnight with target RNA (5  $\mu$ g) at 56°C and were digested with T1 RNase (Life Technologies, Gaithersburg, MD). The protected mRNA fragment was extracted with phenol and chloroform, precipitated with ethanol, resolved on a 6% denaturing polyacrylamide gel, and subjected to autoradiography. Gene transcripts were identified by the length of the protected fragments. Equal loading of RNA was estimated from the amounts of protected fragments of two housekeeping genes, L32 and GAPDH.

#### Immunoprecipitation and immunoblotting

NK3.3 cells and peripheral T lymphocytes were pretreated with different concentrations of Dex for various times, resuspended in 1 ml of serum-free medium (2  $\times$  10<sup>6</sup> NK3.3 cells), and stimulated with IL-7 (20 ng/ml) for 15 min. Following stimulation cells were washed once in PBS and lysed on ice in a buffer containing 1% Triton X-100, 50 mM Tris-HCl (pH 7.5), 300 mM NaCl, 2 mM EDTA, 200  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>, 10  $\mu$ g/ml aprotinin, 10  $\mu$ g/ml leupeptin, and 2.5  $\mu$ M *p*-nitrophenyl *p*-guanidinobenzoate on ice for 30 min. Immunoprecipitation with anti-Stat Ab and subsequent SDS-PAGE were performed as described previously (13) with detection by ECL (LumiGLO; Kirkegaard & Perry Laboratories, Gaithersburg, MD).

Table I. Glucocorticoid-regulated genes of activated PBMC<sup>a</sup>

	GB	0	Dex	Rank
Gc up-regulated genes				
Cell life and death				
$\delta$ sleep-inducing peptide, GILZ homolog	W63717	546	3357	1
Cell surface receptors/transporters				
IL-7R $\alpha$ chain	AF043129	122	546	5
Scavenger receptor superfamily	Z22968	216	1052	3
IL-1R, type II	U64094	729	2336	13
Redox and oxidation damage control				
Indolamine-pyrrole 2,3 dioxygenase	M34455	486	2118	4
Metabolism				
Diamine acetyltransferase	AA336592	372	1264	10
Dihydroxyvitamin D <sub>3</sub> -induced protein	S73591	370	2300	2
Cell division/proliferation				
Metallothionein 1L	F26137	1672	6322	6
Metallothionein 1E	Y12653	1672	6126	8
Metallothionein 1B	H72532	1343	4801	9
Metallothionein 3 (growth inhibitory factor)	AI418147	2680	8937	12
B cell translocation gene 1, antiproliferative	AI925293	163	558	11
Diubiquitin	Y12653	193	704	7
Gc down-regulated gene				
Cell division/proliferation				
Epiregulin	D30783	146	40	1
Extracellular matrix/cell adhesion				
Integrin, $\alpha_1$	X68742	132	38	3
Carcinoembryonic Ag (member 6)	X52378	116	34	4
Cell surface receptors/transporters				
Potassium inwardly rectifying channel	U65406	145	46	5
Solute carrier family 28 (sodium transporter)	U84392	118	37	8
Solute carrier family 26 (sulfate transporter)	U14528	170	57	10
Miscellaneous				
Histatin 3	AA37671	811	533	2
Ser-Thr protein kinase	U59305	131	41	7
Cerebellar degeneration-related protein	M16965	123	43	12
Unknown function				
mRNA from chromosome 5q21-22	AB002438	133	41	6
Expressed sequence tag	U79293	132	44	9
Expressed sequence tag	AA400569	156	52	11

<sup>a</sup> Relative expression in untreated (0) and Dex-treated cells (Dex). GB, gene bank accession number; Gc, glucocorticoid.

## Results

### Gene expression in glucocorticoid-activated PBMC

The molecular response of human PBMC to glucocorticoids was determined with cDNA microarrays representing ~10,000 human genes. Cells were stimulated for 16 h with a high concentration ( $10^{-7}$  M) of the synthetic glucocorticoid Dex. Genes were ranked by differential expression and grouped by functional categories. An example of such an analysis is shown for the subset of the 12 most regulated genes (Table I). The gene up-regulated to the greatest extent by Dex was the  $\delta$  sleep-inducing peptide (88% identity to murine glucocorticoid-induced leucine zipper (GILZ)); GILZ has been reported to inhibit T cell apoptosis induced by stimulation via the TCR (18). Among the top five genes induced by Dex was IL-7R $\alpha$ . This was of particular interest because, while a prominent effect of glucocorticoids is to induce T cell apoptosis, IL-7R $\alpha$  is a receptor that delivers antiapoptotic (prosurvival) signals to T cells.

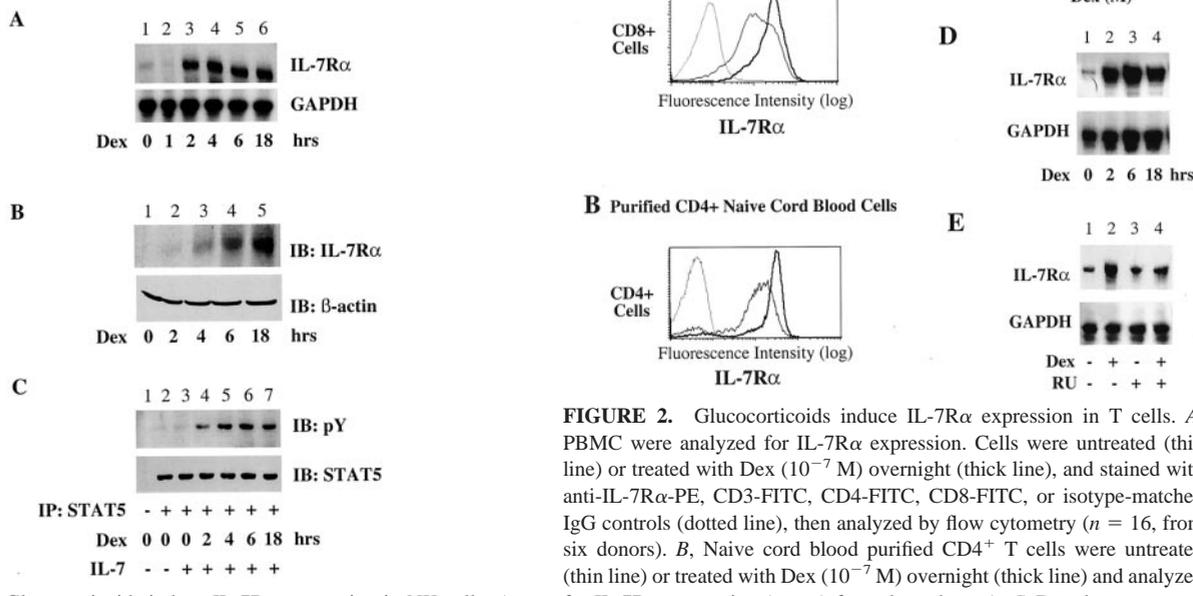
### Glucocorticoids induce IL-7R $\alpha$ expression

To confirm the induction of IL-7R $\alpha$  expression by glucocorticoids, the effect of Dex on an IL-7R $\alpha$ -negative NK cell line (NK 3.3) was examined (Fig. 1). Dex induced IL-7R $\alpha$  mRNA expression in these cells within 2 h of treatment (Fig. 1A). A similar induction of protein was observed by immunoblotting after 4 h of Dex treatment (Fig. 1B). IL-7 exerts its effects by inducing tyrosine phosphorylation of the transcription factor Stat5 (19). To assess whether the up-regulated IL-7R $\alpha$  was functional, we determined whether Dex treatment of NK cells permitted STAT5 phosphorylation by IL-7. Consistent with the lack of detectable IL-7R $\alpha$  expression, IL-7 alone had no effect on Stat5 activation in untreated

NK3.3 cells (Fig. 1C). However, preincubation with Dex permitted IL-7R $\alpha$  signaling and Stat5 phosphorylation. Thus, Dex induces the expression of functional IL-7R $\alpha$ .

### Glucocorticoids up-regulate IL-7R $\alpha$ expression on T cells

The physiological functions of IL-7 for T cells include promotion of survival and proliferation (20). To determine whether glucocorticoid up-regulation of IL-7R $\alpha$  expression is pertinent to T cells, we analyzed the effect of Dex on peripheral blood T lymphocytes. Dex induced an increase of ~3-fold in cell surface IL-7R $\alpha$  expression (Fig. 2A, upper panel, thick line). Furthermore, both CD4 and CD8 subpopulations up-regulate IL-7R $\alpha$  (Fig. 2A), supporting the idea that glucocorticoids up-regulate IL-7R $\alpha$  on all T cells but not on monocytes or B cells (data not shown). Dex also up-regulated IL-7R $\alpha$  expression on naive CD4<sup>+</sup> human cord blood cells, indicating that the state of activation or differentiation of the T cells was not important for this effect (Fig. 2B). The Dex dose response was determined by FACS analysis, and as little as  $10^{-10}$  M Dex induced IL-7R $\alpha$ , with its expression being enhanced by increased Dex doses (Fig. 2C). These results were also confirmed at the mRNA level using RNase protection assays (data not



**FIGURE 1.** Glucocorticoids induce IL-7R $\alpha$  expression in NK cells. *A*, Time response of Dex. NK 3.3 cells were untreated (*lane 1*) or stimulated with Dex ( $10^{-7}$  M) at the indicated times (*lanes 2–6*). The cells were lysed, RNA was extracted, and IL-7R $\alpha$  expression was evaluated by RNase protection assay. *B*, NK 3.3 cells were treated as described in *A*, and then subjected to immunoblotting with anti-IL-7R $\alpha$  or anti- $\beta$ -actin Abs. *C*, IL-7R $\alpha$  signaling. NK 3.3 cells were untreated (*lanes 1–3*) or pretreated with Dex ( $10^{-7}$  M) for 2–18 h as indicated (*lanes 4–7*), left unstimulated (*lanes 1* and *2*), or stimulated with IL-7 (20 ng/ml; *lanes 3–7*). The cells were lysed and immunoprecipitated with nonimmune rabbit polyclonal Ig (*lane 1*) or anti-Stat5a (*lanes 2–7*) and subjected to immunoblotting with anti-phosphotyrosine and anti-Stat5a as indicated. One representative experiment of at least three independent experiments is shown.

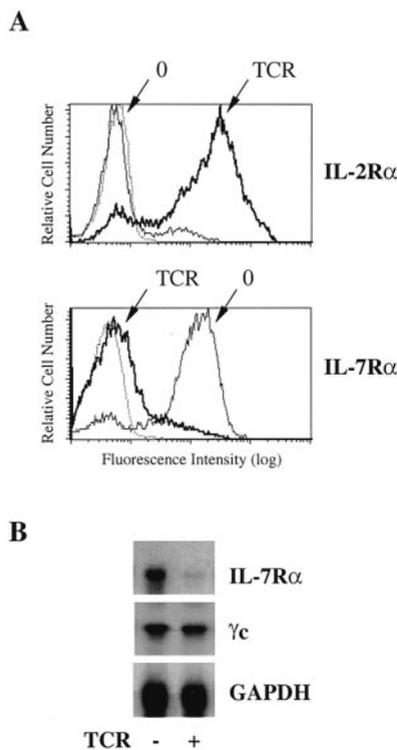
**FIGURE 2.** Glucocorticoids induce IL-7R $\alpha$  expression in T cells. *A*, PBMC were analyzed for IL-7R $\alpha$  expression. Cells were untreated (thin line) or treated with Dex ( $10^{-7}$  M) overnight (thick line), and stained with anti-IL-7R $\alpha$ -PE, CD3-FITC, CD4-FITC, CD8-FITC, or isotype-matched IgG controls (dotted line), then analyzed by flow cytometry ( $n = 16$ , from six donors). *B*, Naive cord blood purified CD4<sup>+</sup> T cells were untreated (thin line) or treated with Dex ( $10^{-7}$  M) overnight (thick line) and analyzed for IL-7R $\alpha$  expression ( $n = 6$ , from three donors). *C*, Dex dose response. PBMC were activated with PHA (2  $\mu$ g/ml) for 72 h and cultured for an additional day in the presence of IL-2 (40 IU/ml). Cells were rested and stimulated with the indicated concentration of Dex. CD3<sup>+</sup> cells (>95%) were gated and analyzed for IL-7R $\alpha$  expression. Values are the mean  $\pm$  SEM of three independent experiments. *D*, Dex time response. Peripheral T cells were untreated (*lane 1*) or were treated with Dex ( $10^{-7}$  M) for 2–18 h as indicated (*lanes 2–4*). This experiment was repeated three times on separate donors. *E*, Dex stimulation is reversed by RU-496. Peripheral T cells were treated for 18 h with Dex ( $10^{-7}$  M; *lanes 2* and *4*) and with RU-496 ( $10^{-7}$  M; *lanes 3* and *4*). *D* and *E*, The cells were lysed, RNA was extracted, and IL-7R $\alpha$  expression was evaluated by RNase protection assay using IL-7R $\alpha$ - and GAPDH-specific probes.

shown). Furthermore, Dex caused up-regulation of IL-7R $\alpha$  mRNA in peripheral T cells as early as 2 h after initiation of treatment (Fig. 2D). The glucocorticoid antagonist RU-486 (Fig. 2E), but not the protein synthesis inhibitor cycloheximide (data not shown), inhibited the Dex-induced increase in IL-7R $\alpha$  mRNA, consistent with a direct effect of the liganded GR on IL-7R $\alpha$  transcription. The enhancement of IL-7R $\alpha$  expression occurred in a dose-dependent manner. Thus, glucocorticoids up-regulate IL-7R $\alpha$  mRNA and protein in T cells.

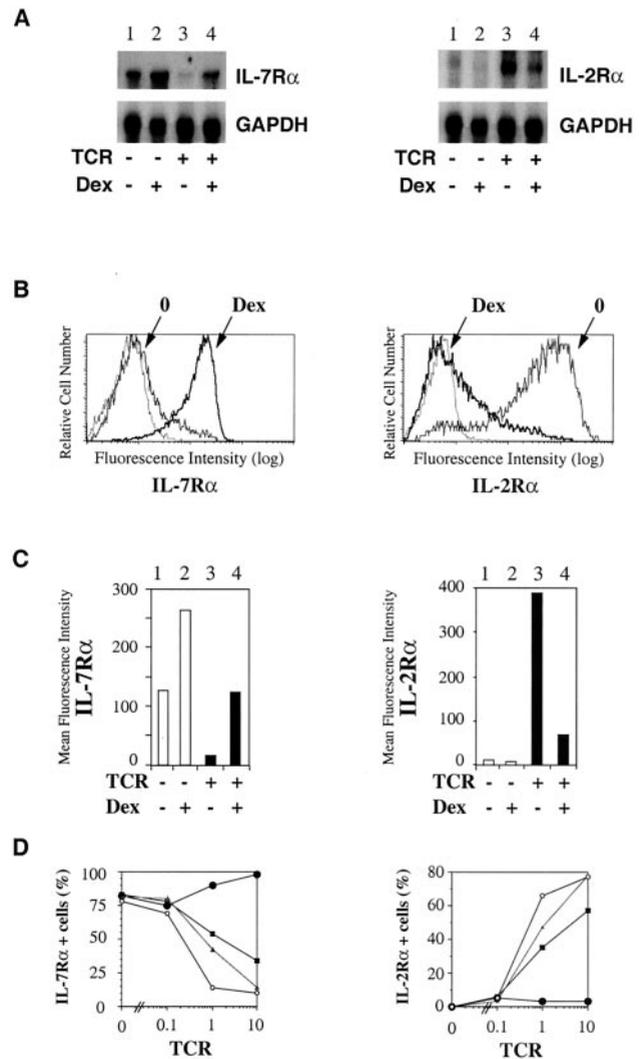
*Antagonism between TCR- and glucocorticoid-mediated activation on IL-7R $\alpha$  expression*

IL-7 has been shown to mediate homeostatic expansion of naive and memory T cells after viral infection (21). Therefore, we determined how IL-7R $\alpha$  expression was regulated by TCR stimulation. Purified human CD4<sup>+</sup> cord blood cells expressing high levels of IL-7R $\alpha$  were stimulated with anti-CD3/CD28 Abs. As expected, with simultaneous anti-CD3/CD28 stimulation there was a large increase in IL-2R $\alpha$  expression (Fig. 3A, upper panel). The same treatment caused a dramatic decrease in the cell surface level of IL-7R $\alpha$  (Fig. 3A, lower panel). We determined whether the down-regulation of IL-7R $\alpha$  expression occurred at the transcriptional level by quantitating the effect of TCR signaling on IL-7R $\alpha$  mRNA. Human peripheral T cells stimulated for 18 h with anti-CD3/CD28 Abs showed a decrease in expression of IL-7R $\alpha$

mRNA (Fig. 3B). This effect was specific because levels of GAPDH and the common  $\gamma_c$  cytokine subunit ( $\gamma_c$ ; the other chain of the IL-7R) did not change. Thus, TCR-mediated activation down-regulates IL-7R $\alpha$  on mature T cells.



**FIGURE 3.** TCR activation down-regulates IL-7R $\alpha$  expression. *A*, Naive cord blood-purified CD4<sup>+</sup> T cells were analyzed for IL-2R $\alpha$  (upper panel) and IL-7R $\alpha$  (lower panel) expression. Cells were untreated (0, thin line) or were treated with anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (1  $\mu$ g/ml) Abs for 2 days (TCR, thick line). Cells were then incubated with anti-IL-7R $\alpha$ -PE and anti-IL-2R $\alpha$ -FITC mAbs or isotype-matched IgG-controls (dotted line) and then analyzed by flow cytometry ( $n = 16$ , from six donors). *B*, Peripheral T cells were untreated or were stimulated with anti-CD3 and anti-CD28 Abs (5  $\mu$ g/ml) for 18 h. Cells were lysed, RNA was extracted, and IL-7R $\alpha$  and  $\gamma_c$  expression was evaluated by RNase protection assay using IL-7R $\alpha$ -,  $\gamma_c$ -, and GAPDH-specific probes. This experiment was performed three times on separate donors.



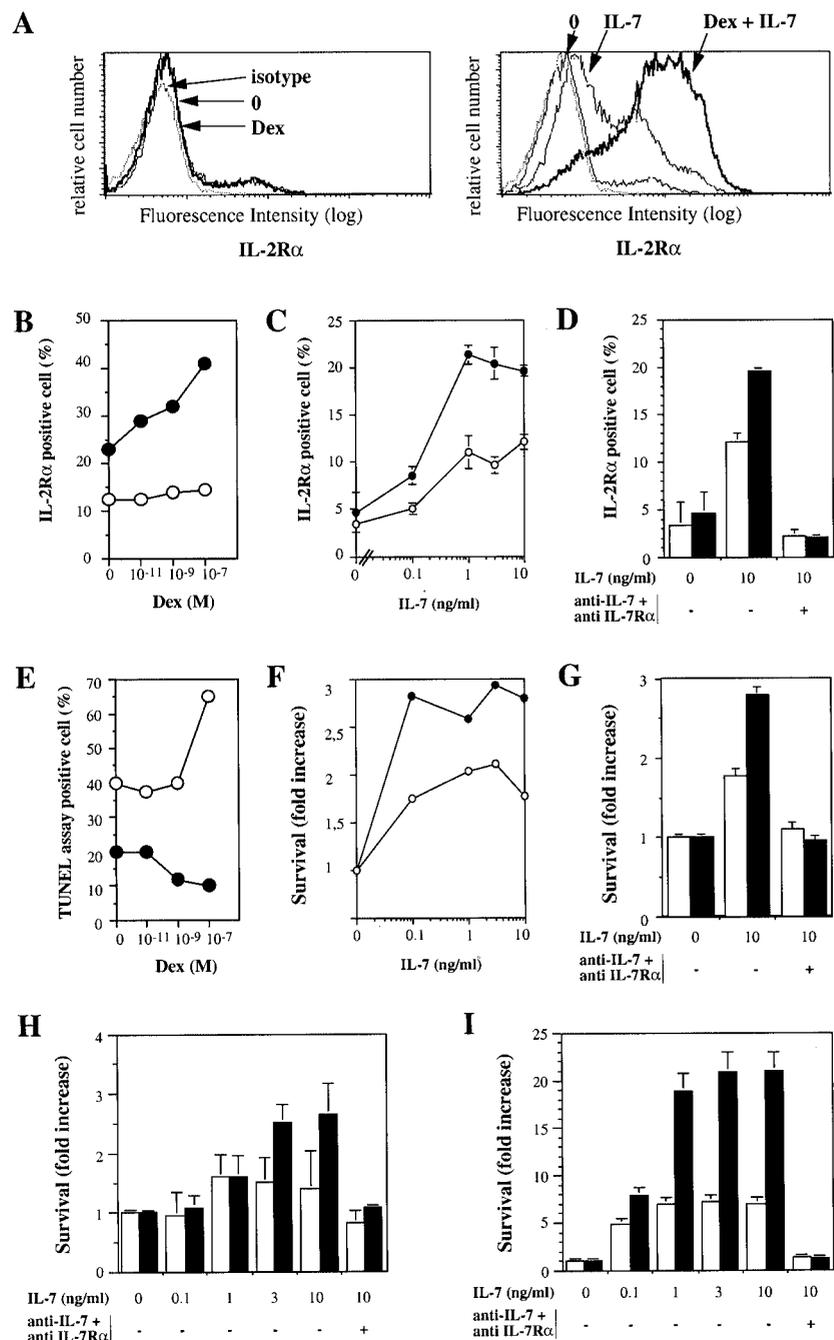
**FIGURE 4.** Antagonism between glucocorticoids and TCR activation on IL-2R $\alpha$  and IL-7R $\alpha$  expression. *A*, Peripheral T cells were not activated (lanes 1 and 2) or activated with anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (1  $\mu$ g/ml) (lanes 3 and 4) and untreated (lanes 1 and 3) or treated with Dex (10<sup>-7</sup> M; lanes 2 and 4) for 18 h. Cells were lysed, RNA was extracted, and IL-7R $\alpha$  and IL-2R $\alpha$  expression was evaluated by RNase protection assay. This experiment was performed three times on separate donors. *B*, Naive cord blood purified CD4<sup>+</sup> T cells were activated with anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (1  $\mu$ g/ml) and were untreated (thin line) or treated with Dex (10<sup>-7</sup> M) for 2 days (thick line). Cells were then incubated with anti-IL-7R $\alpha$ -PE and anti-IL-2R $\alpha$ -FITC mAbs or isotype-matched IgG-controls (dotted line) and then analyzed by flow cytometry. *C*, Naive cord blood purified CD4<sup>+</sup> T cells were not activated (lanes 1 and 2) or activated with anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (1  $\mu$ g/ml; lanes 3 and 4) and treated with Dex as described in *B*. Cells were analyzed for IL-7R $\alpha$  and IL-2R $\alpha$  expression, and mean fluorescence intensity is represented. This experiment was repeated six times using separate donors. *D*, The cells were activated with graded concentrations of anti-CD3 and anti-CD28 (0.1–10  $\mu$ g/ml) and were untreated (○) or were treated with graded concentrations of Dex (10<sup>-11</sup> M (▲), 10<sup>-9</sup> M (■), and 10<sup>-7</sup> M (●)) for 2 days, then analyzed for IL-7R $\alpha$  and IL-2R $\alpha$  expression.

Given that glucocorticoids and TCR-mediated activation have opposite effects on IL-7R $\alpha$  expression, we hypothesized that a balance between TCR and GR signaling would determine IL-7R $\alpha$  expression levels and, hence, IL-7 responsiveness. RNase protection assays were conducted on RNA from T cells treated with anti-CD3/CD28 Abs, Dex, or both (Fig. 4A, left panel). While Dex alone enhanced (Fig. 4A, lane 2) and TCR-mediated activation alone inhibited (Fig. 4A, lane 3) IL-7R $\alpha$  expression, the two stimuli effectively antagonized each other (Fig. 4A, lane 4). By comparison, Dex had no effect on IL-2R $\alpha$  mRNA expression, whereas TCR-mediated stimulation resulted in increased IL-2R $\alpha$  expression, an effect that was inhibited by Dex (Fig. 4A, right panel). Analysis of cell surface expression of these receptors on CD3/CD28-activated CD4 $^+$  T cells from cord blood yielded similar results (Fig. 4, B and C). A concomitant dose response of glucocorticoid and TCR signaling resulted in various levels of IL-7R $\alpha$

expression and showed that the two signaling pathways had opposite effects on IL-7R $\alpha$  expression (Fig. 4D). Thus, a balance between GR and TCR signaling can regulate IL-7R $\alpha$  levels.

#### Glucocorticoid-induced IL-7R $\alpha$ enhances IL-7 responsiveness in T cells

To determine whether the glucocorticoid-induced increase in IL-7R $\alpha$  in mature T cells has functional relevance, the expression of IL-2R $\alpha$ , a cell surface molecule whose gene is up-regulated by IL-7 (22), was assessed in purified CD4 $^+$  cord blood T cells. Whereas Dex alone had little effect on IL-2R $\alpha$  expression (Fig. 5A, left panel), IL-7 induced a moderate increase (Fig. 5A, right panel). Dex greatly enhanced IL-7-induced IL-2R $\alpha$  expression (Fig. 5A, right panel). Indeed, escalating concentrations of Dex gradually enhanced IL-7-induced IL-2R $\alpha$  expression (Fig. 5B). Moreover, we confirmed that the up-regulation of IL-2R $\alpha$  was IL-7 dose



**FIGURE 5.** Glucocorticoids enhance IL-7-mediated action on IL-2R $\alpha$  expression and survival. *A*, Naive cord blood purified CD4 $^+$  T cells were untreated or treated with Dex ( $10^{-7}$  M), and untreated (left panel) or treated with IL-7 (10 ng/ml; right panel) for 6 days and analyzed for IL-2R $\alpha$  expression. The cells were stained with anti-IL-2R $\alpha$ -FITC and isotype-matched FITC-IgG as a control and analyzed by flow cytometry. *B*, Naive cord blood purified CD4 $^+$  T cells were treated for 4 days with increasing concentration of Dex ( $10^{-11}$ ,  $10^{-9}$ , and  $10^{-7}$  M), without ( $\circ$ ) or with IL-7 (10 ng/ml;  $\bullet$ ), and cells were analyzed for IL-2R $\alpha$  expression. One representative experiment of three is shown. *C*, Naive cord blood purified CD4 $^+$  T cells were treated for 1 day with increasing concentrations of IL-7, without ( $\circ$ ) or with Dex ( $10^{-7}$  M;  $\bullet$ ), and cells were analyzed for IL-2R $\alpha$  expression. *D*, Naive cord blood purified CD4 $^+$  T cells were treated for 1 day with IL-7 and with anti-IL-7 and anti-IL-7R $\alpha$  neutralizing Abs as indicated without ( $\square$ ) or with Dex ( $10^{-7}$  M;  $\blacksquare$ ), and cells were analyzed for IL-2R $\alpha$  expression. *E*, Cells were treated as described in *B* and analyzed for apoptosis by bromodeoxyuridine staining using a modified TUNEL assay. *F*, Cells were treated as described in *C* and analyzed for apoptosis using annexin V staining. *G-I*, Cells were treated as described in *D* and analyzed for apoptosis using annexin V staining after 1 (*G*), 3 (*H*), or 7 days (*I*). These experiments are representative of three donors, and the mean  $\pm$  SEM of three independent experiments is represented.

dependent and was potentiated in the presence of glucocorticoids (Fig. 5C). Finally, we demonstrated that this enhanced IL-2R $\alpha$  expression was dependent upon IL-7 signaling, because it was completely inhibited by neutralizing Abs against the IL-7/IL-7R $\alpha$  complex (Fig. 5D). If the enhancement of IL-7 responsiveness by glucocorticoids has functional consequences, glucocorticoids might be expected to enhance the effect of IL-7 on T cell survival. As shown in Fig. 5E, IL-7 reduced by approximately half the spontaneous apoptosis of CD4<sup>+</sup> cord blood cells. Whereas a high concentration of Dex (10<sup>-7</sup> M) alone caused an increase in apoptosis, it inhibited apoptosis in the presence of IL-7. Using annexin V staining to measure apoptosis of purified CD4<sup>+</sup> T cells from cord blood, we analyzed the effect of IL-7 and neutralizing anti-IL-7/IL-7R $\alpha$  Abs on the antiapoptotic effects of glucocorticoids and examined this effect in a dose-dependent manner (Fig. 5, F and G). Indeed, escalation of IL-7 doses favors survival, and this effect was further enhanced in the presence of glucocorticoids (Fig. 5F), whereas neutralizing Abs against the IL-7/IL-7R $\alpha$  complex completely reversed the antiapoptotic effect of glucocorticoids and IL-7 (Fig. 5G). These results were confirmed with increasing doses of IL-7 in a time-dependent manner on days 3 (Fig. 5H) and 10 (Fig. 5I). Neutralizing Abs against the IL-7/IL-7R $\alpha$  complex completely reversed the antiapoptotic effect of glucocorticoids and IL-7 on days 3 (Fig. 5H) and 10 (Fig. 5I). Thus, the IL-7-mediated inhibition of apoptosis increased with increasing concentrations of glucocorticoids and increasing doses of IL-7 in the presence of glucocorticoids, consistent with an enhanced sensitivity of Dex-treated cells to IL-7. These actions were specifically mediated by the IL-7/IL-7R $\alpha$  complex, as demonstrated with the blocking experiments. Therefore, glucocorticoids can paradoxically promote T cell survival in the presence of IL-7.

## Discussion

IL-7 is a key cytokine for the development of T cells. Its importance is illustrated by the fact that mice deficient for IL-7 (16) or IL-7R $\alpha$  (17) or humans with loss-of-function mutations in IL-7R $\alpha$  (18) have a marked deficiency in T cell development. The receptor for IL-7 is composed of the IL-7-specific subunit (IL-7R $\alpha$ ) (23) and  $\gamma_c$ , the latter associating with the Janus kinase 3 (13, 20, 24, 25). Mutations in  $\gamma_c$  and Janus kinase 3 also result in a reduction in T cell number, and targeted mutations of other  $\gamma_c$ -using cytokines (IL-2, IL-4, and IL-15) clearly indicate that IL-7 has nonredundant functions in lymphopoiesis (26–30). Several important functions have been ascribed to IL-7 in the thymus, including promotion of survival and proliferation of immature thymocytes (31–33). IL-7 is also secreted by monocytes and dendritic and intestinal epithelial cells, participates in mature lymphoid survival and expansion (34–37), and inhibits T cell death in vitro (38, 39). Furthermore, IL-7 participates in regulation of the maintenance and the strength of the immune response and regulates the development and the cytolytic activity of intestinal  $\gamma\delta$  intraepithelial lymphocytes (36, 40, 41). These intraepithelial lymphocytes represent the frontline defense alimentary and bacterial gut lumen Ags and depend on IL-7R $\alpha$  signaling (42). Thus, IL-7R $\alpha$  signals IL-7-mediated critical specific actions on T cell development and function, yet the control of its expression remains obscure.

Recognition of foreign Ags by mature T cells induces the expression of many cytokine receptors that drive the immune response toward either a Th1 cellular or Th2 humoral type (43, 44). Two types of signals are needed to generate immune responses. The first is the recognition of Ag/MHC by the TCR. The second signal, termed costimulation, involves costimulatory molecule interactions and the activation of cytokine receptors with their cognate cytokines (1). Mounting evidence suggests the critical role of

the ligand-receptor complex IL-7/IL-7R $\alpha$  in the course of the T cell immune response (32, 34). For example, peripheral T cells from IL-7R $\alpha$ <sup>-/-</sup> mice show impaired proliferation and increased apoptosis after TCR engagement. Therefore, IL-7R $\alpha$  expression appears to be important in the maintenance and strength of the immune response (34, 45, 46). In this regard it is noteworthy that TCR stimulation decreases IL-7R $\alpha$  expression. This down-regulation may represent a negative feedback loop to turn off lymphocyte activation and, hence, T cell-driven immune responses.

Adrenal-derived glucocorticoids suppress innate and specific immune responses through their negative actions on the expression of inflammatory mediators such as cytokines and cytokine receptors (5, 47, 48). Although widely used clinically, they clearly have protean effects and are far from optimal immunosuppressive agents. In this study we found that glucocorticoids increase IL-7R $\alpha$  expression in both naive and activated CD4<sup>+</sup> T cells. By doing so, glucocorticoids were able to enhance the expression of IL-7-inducible genes and IL-7-mediated rescue from glucocorticoid-induced apoptosis. The finding that glucocorticoids increase IL-7R $\alpha$  expression may provide a mechanism by which glucocorticoids can have a positive influence on the immune response. Based upon gene expression assessed with DNA microarrays, another top glucocorticoid-up-regulated gene is GILZ, with major antiapoptotic action. GILZ counteracts activation-induced T cell death and may contribute in part to the Dex-induced inhibition of TCR-activated apoptosis (19). Thus, glucocorticoids can have pro-survival activity during the T cell immune response by blocking TCR-induced clonal deletion and maintaining IL-7R $\alpha$  expression. During acute infections or flare-ups of inflammatory diseases, adrenal glucocorticoid secretion is crucial for the restraint and control of the adaptive immune response. In the neighborhood of IL-7-secreting epithelial or stromal cells, glucocorticoids may prevent T cell deletion by improving IL-7 signaling and help sustain the T cell immune response to overcome foreign Ags and pathogens.

New emigrants from generative lymphoid organs undergo positive or negative selection in the periphery (2, 40, 49). The homeostasis of naive mature and memory T lymphocytes is dependent on ligand-TCR interactions (4, 50–52). Also, T cell homeostasis is mediated by signals transmitted by growth factor receptors in secondary lymphoid and in nonlymphoid organs (3, 49, 53, 54). IL-7 potently enhances thymic-independent peripheral expansion and restores immunity in athymic T cell-depleted hosts (55). Furthermore, IL-7 is required for homeostatic expansion of naive CD8<sup>+</sup> and CD4<sup>+</sup> T cells in lymphopenic hosts and is partially required for the homeostatic proliferation of memory cells (22). These two studies point to IL-7 as an important cytokine in T cell homeostasis (22, 55). Therefore, improved IL-7R $\alpha$  signaling by glucocorticoids may contribute to IL-7-mediated expansion of memory T cells and maintenance of the peripheral T cell repertoire (56–58).

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