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Reversible Disruption of Thymic Function by Steroid Treatment[1]

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The effect of steroid treatment on the thymic output of T cells was examined in an avian model. Recent thymic emigrants in chickens transiently express the chicken T cell Ag 1 thymocyte marker, and thymic function can be monitored indirectly by measuring the levels of TCR gene rearrangement excision circles in peripheral T cells. Both parameters were used to show that intensive steroid treatment induces thymic involution and a profound reduction in the supply of naive T cells to the periphery. Conversely, resident T cells in the peripheral lymphocyte pool were relatively spared. Thymopoiesis immediately recovered following cessation of steroid treatment, concurrent with restoration of the thymic output of newly formed T cells. Repopulation of the peripheral T cell pool recapitulated the ontogenetic pattern of $\gamma\delta$ T cell replenishment before $\alpha\beta$ T cell reseeding, thereby indicating the complete recovery of thymic function after a course of steroid treatment. ***The Journal of Immunology,*** **2002, 168: 6500–6505.**

Because of their immunosuppressive and anti-inflammatory effects, glucocorticoid hormones are widely used in the treatment of autoimmune diseases, allergic and inflammatory disorders, allograft rejection, and lymphoid malignancies (1, 2). Both positive and negative effects of steroids on lymphoid cells are mediated through the glucocorticoid receptor (3, 4), a transcription factor belonging to the superfamily of steroid/thyroid hormone receptors (5, 6). Following steroid binding, the receptor/steroid ligand complex is translocated into the nucleus, where it either interacts directly with glucocorticoid receptor elements in the promoter regions of positively regulated genes or associates with other transcription factors to indirectly regulate transcription of genes that may play important roles in immune responses (3, 6, 7).

The apoptotic effect of glucocorticoids is particularly notable for the immature thymocyte subpopulation denoted double-positive cells because they express both the CD4 and CD8 coreceptors (8). Accordingly, treatment with dexamethasone, a potent glucocorticoid agonist, may result in thymic involution (9). Although mature thymocytes and T cells in the periphery appear relatively resistant to the apoptotic effects of glucocorticoid hormones (10), in vivo appraisal of the peripheral T cell pool is complicated because of its mixed composition by recent thymic emigrants (RTE)[3] and the more abundant established T cell residents which may have engaged a variety of immune stimuli.

Glucocorticoids have very similar apoptotic effects on thymic cells in avian and mammalian species, indicating the conservation of this steroid effect (10, 11). An avian model was used therefore in the present studies of steroid-induced thymic dysfunction to take advantage of the fact that the chicken T cell thymocyte Ag 1 (chT1) can be used as a direct marker for RTE in the peripheral T cell pool. Measurement of the chT1$^+$ T cell levels can thus be used to accurately monitor thymic function in these gallinaceous birds (12). A comparable RTE marker in primates is presently unavailable, although the levels of TCR V(D)J gene rearrangement excision circles (TREC) in the peripheral T cell population provide a useful surrogate marker for estimating the numbers of RTE in both humans and birds (12–17). These extrachromosomal DNA circles are stable, nonreplicating structures that are concentrated in the naive RTE population and diluted in the proliferating pool of mature T cells. TREC measurements thus have been used as surrogate markers to assess the functional status of the thymus in acquired and congenital immunodeficiency diseases (14, 17–19). In the present studies, we evaluated the effects of an intensive course of dexamethasone treatment on thymic function by direct assessment of the thymus and by monitoring the RTE subpopulation in the peripheral T cell pool through serial determination of chT1$^+$ T cells and TREC levels. In parallel studies of thymectomized animals, we also evaluated the in vivo effects of glucocorticoids on the peripheral pool of mature T cells.

Materials and Methods

Animals, dexamethasone treatment, and thymectomy

Fertilized eggs of the SC strain (Hy-Line International, Dallas City, IA) were incubated at 41°C and intermittently rotated in a humidified incubator. Chicks were maintained under conventional vivarium conditions. Intramuscular injections of dexamethasone (Elkins-Sinn, Cherry Hill, NJ) were initiated at 4 wk of age. Pilot studies were conducted to determine a dexamethasone dosage (5 mg/kg per day) that induced thymic involution and growth retardation, but which did not affect survival and general health status of the treated animals. Thymectomy or sham thymectomy surgical procedures were performed on 4-wk-old chicks anesthetized by i.m. injection of Nembutal (Abbott Laboratories, North Chicago, IL). The thymic lobes were removed through a dorsal incision in the neck as previously described (20).

Immunofluorescence analysis and cell sorting

Peripheral blood leukocytes, thymocytes, and splenic leukocytes were prepared as single-cell suspensions (12). The anti-chT1, CD3, CD4, CD8, TCR$\gamma\delta$, and TCR$\alpha\beta$ (Vβ1 plus Vβ2) mAbs (21, 22) were conjugated with either FITC or PE by Southern Biotechnology Associates (Birmingham,

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[3] Abbreviations used in this paper: RTE, recent thymic emigrant; chT1, chicken T cell thymocyte Ag 1; TREC, TCR gene rearrangement excision circle.

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AL). Cells were stained with the mAbs and analyzed with a FACScan instrument using the CellQuest software package (BD Biosciences, MountainView, CA).

$\gamma\delta$ and Vβ1$^+$ $\alpha\beta$ T cells were purified by a FACS (FACStar; BD Biosciences) after staining blood lymphocytes with the PE-conjugated anti-TCR$\gamma\delta$ and FITC-conjugated anti-TCRVβ1 mAbs. Purity of the sorted cells in each experiment was >99%.

PCR determination of TCR gene rearrangement excision circles

Genomic DNA was isolated from FACS-sorted TCRVβ1$^+$ $\alpha\beta$ and TCR$\gamma\delta^+$ T cells in blood samples of dexamethasone-treated and age-matched control as described elsewhere (12). Equal amounts of DNA from each sample were serially diluted and used as templates for the PCR amplification of β-actin, Vγ1-Jγ1, and Vβ1-Dβ TREC. The primers, PCR conditions, and the detection method for PCR products were the same as previously described (12). PCR products separated on agarose gels were transferred to Genescreen Plus nylon membranes (MEN Life science Products, Boston, MA) and hybridized with [γ-^{32}P]ATP-labeled internal probes. After autoradiography, the signal intensities of TREC PCR products of serial dilutions of each sample were plotted. The midpoint was chosen and normalized against that of β-actin to compare the relative TREC levels. TREC index indicates the signal intensity ratio of Vγ1-Jγ1 or Vβ1-Dβ TREC in the dexamethasone-treated and nontreated control chickens.

Results

Differential steroid sensitivity of thymocyte subpopulations

To evaluate the thymic effects of intensive glucocorticoid treatment, 4-wk-old chicks were treated with 5 mg/kg dexamethasone daily for 1 wk and on alternate days thereafter. Pilot experiments indicated that while this steroid dosage induced thymic involution and growth retardation, the treated birds appeared to be otherwise healthy. The thymic mass was rapidly and persistently reduced by the treatment, whereas thymic weight in the young controls increased over the ensuing 6 wk (Fig. 1*a*). Thymocyte numbers were decreased by ~100-fold after 1 wk of dexamethasone therapy and remained depressed throughout the treatment period (Fig. 1*b*). The CD4$^+$CD8$^+$TCR^{low} subpopulation of thymocytes was most dramatically affected (Fig. 1*c*), with a 99.9% decline in cell numbers. The CD4$^-$CD8$^-$ double-negative subpopulation and the CD4 and CD8 single-positive subpopulations of $\alpha\beta$ T cells were also significantly reduced (24-, 8-, and 44-fold, respectively) within the first week of steroid treatment. In parallel, the numbers of $\gamma\delta^+$ thymocytes were decreased by ~100-fold, indicating that this thymocyte sublineage is also steroid sensitive. Among the residual thymocytes, the mature $\alpha\beta$ TCR^{high} T cells were relatively enriched in treated animals. The differential effect of steroid treatment on the mature CD4$^+$ and CD8$^+$ subpopulations was further manifested by an alteration of the CD4:CD8 ratio from 0.75:1 in controls to 4.4:1 in treated animals (Fig. 1*c*). These observations suggested a possible sparing effect of the steroid treatment on mature CD4$^+$ T cells.

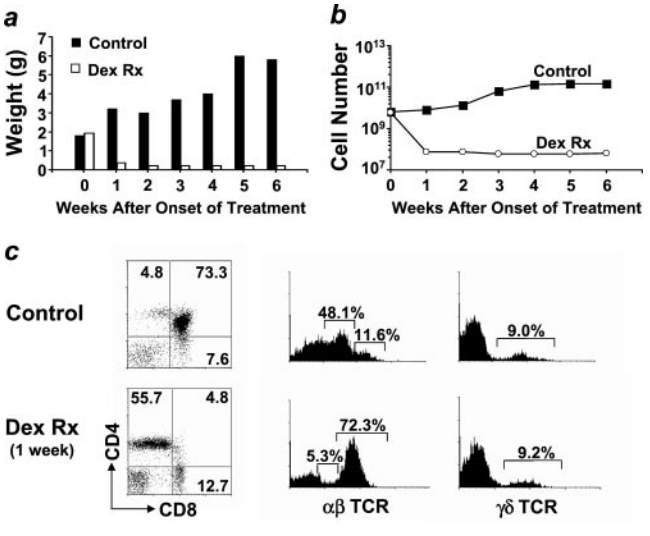

FIGURE 1. Effects of dexamethasone treatment on the thymus. Four-week-old chicks were injected with dexamethasone daily for 1 wk and on alternate days thereafter. *a* and *b*, Thymus weight (*a*) and thymocyte numbers (*b*) were monitored in age- and sex-matched controls and dexamethasone-treated (Dex Rx) chickens. *c*, Immunofluorescence analysis of thymocyte subpopulations in an untreated animal and one that received dexamethasone injections for 1 wk. The data are representative of three individual analyses.

The severe reduction in total thymic cellularity persisted throughout the duration of dexamethasone treatment and an increasing size of the thymocyte population in untreated juvenile birds exaggerated the gap between the two groups. After 4 wk of treatment, the numbers of CD4$^-$CD8$^-$, CD4$^+$CD8$^+$, CD4$^+$, CD8$^+$, and $\gamma\delta$ T cells in the treated animals were 1/1,000, 1/30,000, 1/400, 1/500, 1/1,200, respectively, of control values (Fig. 1*b* and data not shown). Intensive steroid treatment thus compromises all phases of thymocyte development, although the large CD4$^+$CD8$^+$ subpopulation of immature cortical thymocytes is the most profoundly depressed and mature CD4$^+$ T cells appear the least affected.

Steroid treatment impairs thymic output of naive T cells

The numbers of RTE marked by their transient expression of the chT1 thymocyte Ag have been shown to progressively decline to undetectable levels by 4 wk after thymectomy (12), and a similar decline in circulating chT1$^+$ T cells was observed in the dexamethasone-treated chicks (Fig. 2*a*). The chT1$^+$ RTE levels were reduced by ~50% by 10 days and >99% after 21 days of steroid treatment (Fig. 2*b*).

To evaluate TREC levels in the circulating T cells during the course of steroid treatment, TREC created by TCR Vγ1-Jγ1 rearrangement were examined in purified $\gamma\delta$ T cells, and Vβ1-Dβ TREC were assayed in the $\alpha\beta$ T cell subpopulation of Vβ1$^+$ cells. The levels of TREC representing both types of rearrangements were reduced by 50–60% after 3 wk of steroid treatment and were ~10% of the control values by 5–7 wk of therapy (Fig. 2*c*). Notably, however, low levels of the Vγ1-Jγ1 TREC and Vβ1-Dβ TREC were detectable well beyond the disappearance of chT1$^+$ T cells in the circulation (Fig. 2*b*) as further evidence that TREC levels are a less acute marker of thymic function.

Reversible steroid-induced impairment of thymic output

To evaluate whether the steroid treatment induced long-lasting effects on thymic function, we examined the recovery of thymocyte subpopulations after dexamethasone discontinuation. After 3 wk of therapy, the steroid injections were discontinued in one experimental subgroup. The cell numbers in each subset of chT1$^+$ thymocytes, including $\gamma\delta$, double-positive, and single-positive $\alpha\beta$ T cells, increased rapidly in the first 3 wk after discontinuation of the steroid treatment (Fig. 3 and data not shown). Interestingly, $\gamma\delta$ T cell recovery preceded the $\alpha\beta$ T cell recovery. Recovery of thymic function was also reflected by the increase in thymic weight to normal levels by 1 mo after treatment cessation (data not shown).

We also monitored the levels of chT1$^+$ RTE in the peripheral T cell pool after discontinuing the dexamethasone treatment. A gradual recovery of the thymic output resulted in restoration of the chT1$^+$ RTE levels to normal over the next 5 wk (Fig. 4, *a* and *b*), whereas in animals receiving continuous treatment the depletion of chT1$^+$ T cells persisted (Fig. 2*b*). During the first week after steroid withdrawal, very few chT1$^+$CD3$^+$ T cells could be detected in the circulation, but by the 10th day the chT1$^+$$\gamma\delta$ T cells began

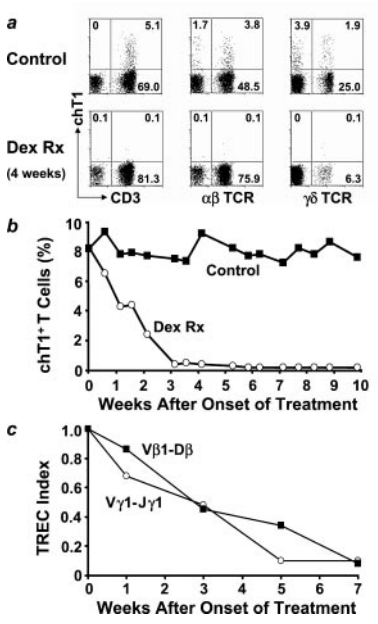

FIGURE 2. Dexamethasone effects on the level of RTE in the circulation. Four-week-old chicks were treated with dexamethasone and untreated chicks of the same age and sex served as controls. *a*, Immunofluorescence analysis of circulating $\alpha\beta$ and $\gamma\delta$ chT1$^+$ T cells of control subjects and chicks receiving dexamethasone treatment for 4 wk. The data are representative of three individual analyses. *b*, Time course analysis of the levels of circulating chT1$^+$ T cells during dexamethasone treatment. The frequency of circulating chT1$^+$ T cells was determined by immunofluorescence analysis, and the data points indicate the average value for three to six experimental animals. *c*, Time course analysis of dexamethasone effects on TREC levels in the circulating T cells. TREC index indicates the signal intensity ratio of the PCR products for Vβ1-Dβ or Vγ1-Jγ1 TREC of dexamethasone-treated vs control chickens (see *Materials and Methods*).

to reappear in the blood. This was followed by a re-emergence of the chT1$^+\alpha\beta$ T cells beginning on the 14th day of the recovery period (Fig. 4*a*). The frequency of chT1$^+$ $\gamma\delta$ and $\alpha\beta$ T cells then increased gradually to reach pretreatment levels 1 mo after steroid discontinuation (Fig. 4*a*). An increase in the Vγ1-Jγ1 and Vβ1-Dβ TREC levels from the peripheral T cell pool also reflected the recovery in thymic output of T cells. The Vγ1-Jγ1 TREC levels reached 60% of the normal control levels by 2 wk after dexamethasone discontinuation, whereas recovery of the Vβ1-Dβ TREC to this level required ~4 wk (Fig. 4*c*). At this time, the Vγ1-Jγ1 TREC levels were 20% higher than control levels (Fig. 4*c*), in agreement with the observation of a higher chT1$^+\gamma\delta$:chT1$^-\gamma\delta$ T cell ratio in the steroid withdrawal group (data not shown).

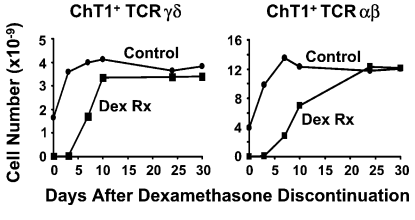

FIGURE 3. Recovery of thymopoiesis after discontinuation of dexamethasone treatment. Four-week-old chicks were treated with dexamethasone for 3 wk and the day of the final dexamethasone injection was designated day 0. The numbers of chT1$^+\alpha\beta$ and chT1$^+\gamma\delta$ thymocytes at each time point were calculated from determinations of the total thymocyte number and the frequency of each subpopulation.

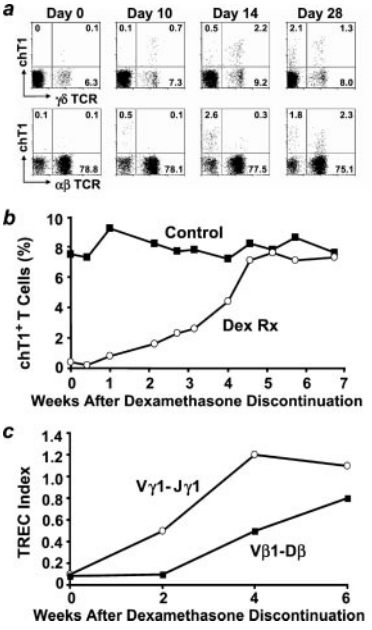

FIGURE 4. Recovery of thymic output of T cells after discontinuation of dexamethasone treatment. Four-week-old chicks were treated with dexamethasone for 3 wk and the day of the last dexamethasone injection was designated day 0. *a*, Sequential reappearance of chT1$^+\gamma\delta$ and chT1$^+\alpha\beta$ T cells in the circulation. The data are representative of three individual analyses. *b*, Time course analysis of the levels of circulating chT1$^+$ T cells. Data points indicate average frequency of chT1$^+$ T cells in three to six experimental animals. *c*, Time course analysis of TREC levels in circulating $\gamma\delta$ and $\alpha\beta$ T cells.

Relative steroid resistance of mature T cells in the peripheral pool

The intensive course of steroid treatment severely retarded growth of the young chicks. The body weights, spleen weights, and total splenocyte numbers were relatively static in the dexamethasone administration period, whereas these growth parameters increased progressively in untreated controls (Fig. 5, *a* and *b*, and data not shown). Relative to the thymocyte population, however, mature T cells in the periphery were much less affected by the intensive steroid treatment. Following the onset of steroid treatment, the numbers of splenic T lymphocytes increased only slightly in treated chickens, while they continued to increase over the next 2 wk in untreated controls undergoing normal growth (Fig. 5*b* and data not shown). After 4 wk of treatment, the numbers of CD4$^+$ T cells in blood samples had increased slightly, whereas CD8$^+$ and $\gamma\delta$ T cell levels were significantly decreased (Fig. 5*d*). Correspondingly the CD4:CD8 ratio in the peripheral T cell pool was modified from 2:1 to 7:1. In parallel with this subpopulation distribution shift in the periphery, a relatively severe depletion of CD8$^+$ T cells was also observed in the thymus of steroid-treated animals. Although not the primary focus of these studies, the steroid effects on B cells were noted to be especially severe. B cell numbers were reduced by >95% in the spleen and blood throughout the period of treatment. The normal T:B cell ratio of 5:1 in the peripheral blood of untreated birds was therefore increased to 40:1 in the steroid-treated group (data not shown).

Since the changes observed in the peripheral T cell pool during steroid treatment could either reflect a direct effect on the peripheral T cells or the indirect effect of diminished thymic output, we compared the effects of dexamethasone treatment on the peripheral T cell subpopulations in thymectomized vs nonthymectomized birds. The thymus was removed at 4 wk of age, and these subjects

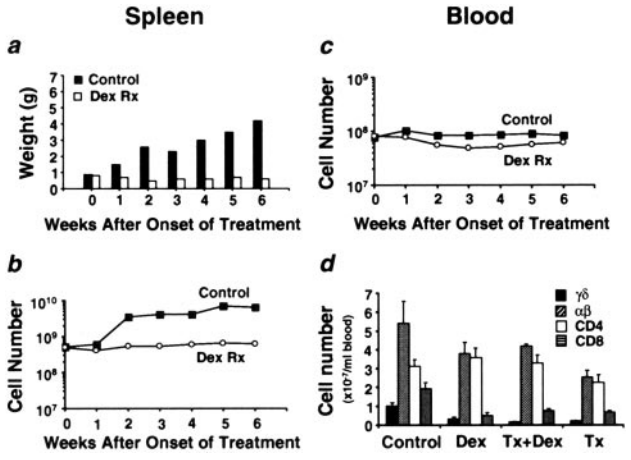

FIGURE 5. Evaluation of dexamethasone effects on peripheral lymphocytes. Four-week-old chicks were treated with dexamethasone, *a–c*, Spleen weights (*a*), splenic leukocyte numbers (*b*), and numbers of peripheral blood leukocytes (PBL, *c*) in the treated and control chicks were monitored weekly. PBL data points represent the average value for three to six chickens. *d*, Four-week-old chicks were untreated (Control), treated with dexamethasone for 3 wk (Dex), thymectomized (Tx), or thymectomized and dexamethasone-treated for 3 wk (Tx plus Dex). At 7 wk of age, the levels of circulating $\gamma\delta$, $\alpha\beta$, $CD4^+$, and $CD8^+$ T cells were calculated by determining the total lymphocyte numbers and frequencies of each T cell subpopulation. The data represent average values (bars) and 1 SE (I) for four chickens in each group. The modest increase observed in the $CD4^+$ subpopulation of dexamethasone-treated athymic chickens did not reach statistical significance ($p = 0.07$). In contrast, the decrease observed for the $CD8^+$ subpopulation in dexamethasone-treated euthymic chickens was highly significant ($p = 0.002$) as determined by a paired two-sample Student's *t* test.

were then treated or not treated with dexamethasone before comparative evaluation with nonthymectomized controls. Removal of the thymus alone resulted in an ~50% reduction in levels of circulating $\alpha\beta$ T cells and an ~80% reduction in $\gamma\delta$ T cells. Similar $CD8^+$ and $\gamma\delta$ T cell levels were found in thymectomized animals regardless of whether or not they were treated with steroids.

Discussion

Glucocorticoid hormones, when given in relatively high doses as in these experiments, can effectively extinguish thymopoiesis and thereby interrupt the supply of virgin $\alpha\beta$ and $\gamma\delta$ T cells to the periphery. Despite these severe deleterious effects, thymopoiesis was immediately restored to normal levels when steroid treatment was discontinued and this resulted in the resumption of T cell seeding to the periphery. The remarkable poststeroid restoration of thymopoiesis recapitulated the normal ontogenetic pattern with $\gamma\delta$ T cell production preceding $\alpha\beta$ T cell repopulation. This rapid and complete recovery of thymopoiesis suggests that intensive steroid therapy for limited periods of time has no lasting effect on the T cell compartment of the immune system.

The loss of RTE as a consequence of steroid-induced thymic involution could be directly monitored in these studies by determining the levels of T cells marked by chT1 cell surface expression in the chick model and indirectly monitored by measuring the levels of TCR excision circles. The RTE decline indicated by these parameters in the steroid-treated birds resembled that observed following surgical thymectomy (Fig. 2, *b* and *c*; Refs. 12 and 13), although the steroid-induced decline was less acute. This could reflect the transient supply of a limited number of relatively mature, steroid-resistant thymocytes from the preexisting thymic reservoir. In both steroid-treated and thymectomized birds, the decline in TREC levels trailed the decline in the RTE subpopulation marked by expression of the chT1 thymocyte Ag. Although chT1-marked T cells were rarely found after 3 wk of steroid treatment, TREC could be persistently detected among the peripheral $\alpha\beta$ and $\gamma\delta$ T cell subpopulations, albeit in reduced levels. In thymectomized birds, the $t_{1/2}$ of chT1 expression by RTE is 3.5 days vs a 2–3 wk $t_{1/2}$ for TREC levels in the peripheral T cell pool (12, 13). The difference in acuity for gauging thymic impairment by the two parameters reflects the stability of the TREC, the levels of which are reduced only through T cell division and cell death. TREC measurements thus provide a less precise means for monitoring thymic function than the assessment of RTE using an identifying cell surface molecule.

Mature $\alpha\beta$ T cells appear to be relatively resistant to steroid-induced apoptosis (23), except for those in splenic germinal centers, which exhibit thymocyte-like sensitivity (24). Our assessment of the steroid effects on peripheral T cells in thymectomized birds confirms their relative glucocorticoid resistance. The $CD4^+$ subpopulation of peripheral T cells was slightly increased by the intensive dexamethasone treatment. Although larger numbers of experimental animals are needed to evaluate the significance of this enhancement, $CD4^+$ T cells were also found to be preponderant among the residual thymocytes. These findings could reflect the ability of glucocorticoids to accelerate the TCR-mediated induction of T cell proliferation via the enhanced expression of the IL-2 receptor or other cytokine receptors (25, 26). The steroid-induced up-regulation of IL-7Rα and IL-7R expression observed in human $CD4^+$ T cells may also contribute to enhanced survival and function of helper T cells (27). The up-regulation of CD8$\alpha\alpha$ expression on the $CD4^+$ T cells (28), that was also seen in our experiments, is another indication of glucocorticoid enhancement of T cell activation.

Dexamethasone effects on the peripheral T cell pool have previously been examined in patients with systemic lupus erythematosus (29) and in cattle (30). Although the $CD4^+$, $CD8^+$, and $\alpha\beta$ T cell levels were not significantly influenced by the dexamethasone treatment, $\gamma\delta$ T cell levels in the circulation declined rapidly. These findings suggested that either $\gamma\delta$ T cells are unusually susceptible to glucocorticoid-induced apoptosis (29, 30) or that steroids induce their redistribution from the circulation into the lymphoid tissues (30). Our analysis of thymectomized and nonthymectomized animals indicates that the dexamethasone-induced decline in circulating $\gamma\delta$ T cells is primarily due to reduced thymic output. Lacking the remarkable capacity that $\alpha\beta$ T cells possess for peripheral expansion, $\gamma\delta$ T cell maintenance is especially dependent upon continuous thymic output (31, 32).

Mature and immature T cells possess equivalent numbers of glucocorticoid receptors, the function of which is essential in the steroid-induced apoptosis pathway (9, 33). The sensitivity of lymphocytes to glucocorticoid-dependent apoptosis nevertheless may vary depending on lymphocyte differentiation status and availability of supportive factors (34). In this regard, the variation in sensitivity to glucocorticoid-induced apoptosis observed as a function of T cell differentiation is associated with changes in the levels of Notch and Bcl-2 expression (35, 36). Bcl-2, an antiapoptotic factor, is preferentially expressed by $CD4^+$ and $CD8^+$ thymocytes and peripheral T cells and not by the $CD4^+CD8^+$ thymocytes (37). Correspondingly, Notch is highly expressed in the progenitor subpopulation of $CD4^-CD8^-$ thymocytes, absent in $CD4^+CD8^+$ double-positive thymocytes, and re-expressed at intermediate levels in single-positive thymocytes (38). This coordinate pattern of expression is due to the fact that activation of the Notch signaling pathway results in the up-regulation of Bcl-2 expression, thereby

conferring resistance to glucocorticoids in the $CD4^+$ and $CD8^+$ single-positive thymocytes (36).

When thymopoiesis was resumed after discontinuation of the dexamethasone treatment, exportation of T cells rapidly ensued. The $chT1^+$ $\gamma\delta$ and $\alpha\beta$ T cells began to reappear in the circulation by the 10th and 14th days, respectively. Complete recovery of thymic mass and levels of circulating $chT1^+$ RTE were achieved within 1 mo after the final dexamethasone injection. These findings indicate that the glucocorticoid-induced thymic involution has no lasting effects on either the lymphoid progenitor population or the inductive thymic microenvironment, both of which are crucial to normal thymic function and regeneration of the T cell system (39, 40). Interestingly, the regeneration of T cells during the recovery phase recapitulated the normal ontogenetic pattern of $\gamma\delta$ and $\alpha\beta$ T cell development. During ontogeny, $\gamma\delta$ T cells appear in the thymus 3 and 4 days before the $\alpha\beta$ T cells, and the two subpopulations later begin migrating to the periphery in the same order (41). The present analysis of a transient interruption of thymopoiesis indicates that intrathymic development and export of $\gamma\delta$ T cells from the thymus precedes that of $\alpha\beta$ T cells even in adolescent animals. Studies in chicks and mice indicate that the fledgling $\gamma\delta$ T cells have a relatively rapid turnover rate and shorter period of intrathymic residence than do $\alpha\beta$ T cells, possibly because the $\gamma\delta$ T cell subpopulation does not undergo positive selection in the thymus (42, 43).

A reliable marker of human RTE has not yet been identified, although TREC measurement can be used as a less direct estimate of thymic function in humans (14–17). The RTE subpopulation can be identified as $Thy1^+CD45RC^-RT6^-$ T cells in rats (44, 45), whereas a suitable method for monitoring TREC levels is unavailable in mice. This leaves the chicken as the sole current model in which both methods of RTE assessment can be used to monitor thymic function (12, 13). It is likely, however, that the profound interruption of thymic function observed during intensive glucocorticoid treatment in this avian model also applies to steroid treatment in humans, since steroid-treated infants undergo a dramatic reduction in thymic mass (46). Moreover, human thymocytes treated with dexamethasone rapidly undergo apoptosis (47). These observations predict that comparable effects of steroid treatment on thymic function will be observed in humans. In this context, it should be emphasized that our studies were conducted during the height of thymopoietic activity in the chick model, and recovery from a comparable degree of steroid-induced involution of the human thymus would likely be limited by reduction in thymic regenerative capacity with increasing age (40, 48).

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