Annu. Rev. Immunol. 2000. 18:309–345

GLUCOCORTICOIDS IN T CELL DEVELOPMENT AND FUNCTION*

Jonathan D. Ashwell, Frank W. M. Lu, and Melanie S. Vacchio

Laboratory of Immune Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; e-mail: jda@Box-j.nih.gov

Key Words thymocyte selection, T cell activation, immunosuppression, apoptosis

■ **Abstract** Glucocorticoids are small lipophilic compounds that mediate their many biological effects by binding an intracellular receptor (GR) that, in turn, translocates to the nucleus and directly or indirectly regulates gene transcription. Perhaps the most recognized biologic effect of glucocorticoids on peripheral T cells is immunosuppression, which is due to inhibition of expression of a wide variety of activation-induced gene products. Glucocorticoids have also been implicated in Th lineage development (favoring the generation of Th2 cells) and, by virtue of their down-regulation of *fasL* expression, the inhibition of activation-induced T cell apoptosis. Glucocorticoids are also potent inducers of apoptosis, and even glucocorticoid concentrations achieved during a stress response can cause the death of $CD4^+CD8^+$ thymocytes. Perhaps surprisingly, thymic epithelial cells produce glucocorticoids, and based upon in vitro and in vivo studies of T cell development it has been proposed that these locally produced glucocorticoids participate in antigen-specific thymocyte development by inhibiting activation-induced gene transcription and thus increasing the TCR signaling thresholds required to promote positive and negative selection. It is anticipated that studies in animals with tissue-specific GR-deficiency will further elucide how glucocorticoids affect T cell development and function.

INTRODUCTION

The development and function of cells that comprise the immune system are subject to regulation by many intrinsic and extrinsic factors. Immunologists are extremely familiar with many of the ligands and receptors that induce cells to become activated, to migrate, adhere, and express effector functions. Cytokines, typically glycoproteins synthesized and/or secreted de novo in response to an immune or inflammatory stimulus, are the paradigm for soluble molecules that

can act at a distance and modulate the nature and intensity of the immune response. Another class of molecules that can affect immune cells belongs to the neuroendocrine system. Despite a vast literature on the effects of these soluble products on lymphocytes, the characterization of the molecular mechanisms by which they work, and an appreciation of their fundamental importance in the immune response, lags far behind that for cytokines. Nevertheless, countless observations in humans and animal models and in vitro strongly suggest that neuroendocrine influences can and do participate in shaping the immune response. Perhaps the best-studied mediators in this category are steroids, small lipophilic molecules that participate in an enormous number of normal and pathologic processes. Steroids bind intracellular DNA-binding factors that, in turn, regulate gene transcription in virtually all cell types. This review concentrates on one particular type of steroid: glucocorticoids. The case that this hormone has substantial and important physiologic and pharmacologic effects on the immune response is solid, and accumulating evidence has suggested an unexpected role for glucocorticoids in regulating thymocyte development and selection.

OVERVIEW OF GLUCOCORTICOIDS AND GLUCOCORTICOID RECEPTORS

Steroid Hormones

The term steroids refers to a group of small lipophilic compounds derived from a common precursor, cholesterol. The four major types of steroids: progestins, androgens, estrogens, and corticoids, differ in the number of carbon atoms they contain, the receptors they bind, and the biological activities they possess. One can further divide the corticoids into two groups: mineralocorticoids, which regulate ion transport and thus fluid and electrolyte balance, and glucocorticoids, which have many activities, including resistance to stress, regulation of intermediary metabolism, and immunosuppressive and anti-inflammatory effects. The conversion of cholesterol to the various steroids is performed by an array of dehydrogenases and cytochrome P450 enzymes, membrane-bound and heme-containing monooxygenases that catalyze dehydroxylation-oxidation reactions (Figure 1). The first and rate-limiting step in steroid biosynthesis is the cleavage of the side chain of cholesterol by P450scc to generate the first steroid, pregnenolone. P450scc expression seems to be limited to steroidogenic tissues such as the adrenals, placenta, gonads, brain, and thymus (1–5). Pregnenolone is hydroxylated at position 17 by P450c17, resulting in two possible parallel pathways of corticoid synthesis. Although rodents express P450c17 in the gonads, in adult animals it is not detectable in the adrenal cortex (6), so the major circulating glucocorticoid in mice (corticosterone) differs slightly from the preponderant circulating glucocorticoid in most species, including human (cortisol). Progesterone (or its 17-OH form) is hydroxylated in the endoplasmic reticulum by P450c21 to

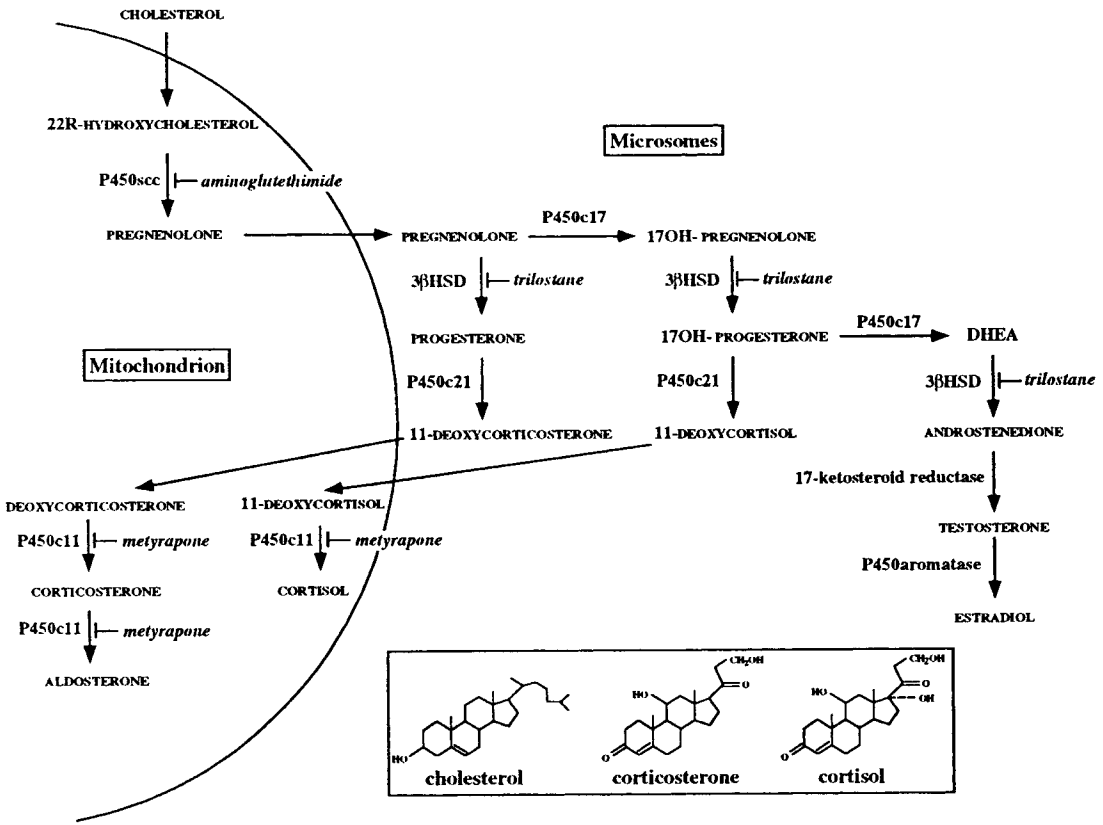

Figure 1 Simplified scheme detailing some of the major steps in steroid biosynthesis. The major enzymes shown are P450scc (CYP11A, ''scc'' denotes ''side chain cleavage''), P450c11 (CYP11B1, or 11β-hydroxylase), P450c17 (CYP17, or 17 α-hydroxylase/lyase), P450c21 (CYP21, 21-hydroxylase), P450aromatase (CYP19, or aromatase). The structures of cholesterol, corticosterone, and cortisol are shown.

yield 11-deoxycorticosterone (or 11-deoxycortisol), which has little glucocorticoid activity, and is then converted in mitochondria to the active glucocorticoid corticosterone (or cortisol) by P450c11. A small amount of corticosterone undergoes a series of intermediate steps that result in conversion to the mineralocorticoid aldosterone.

Because steroids are lipophilic they are transported in the blood in a reversible complex with protein. The major high-capacity low-affinity carrier is albumin; the major low-capacity high-affinity protein is transcortin, also known as corticosteroid-binding globulin (CBG). Only a small fraction (on the order of 1–10%) of glucocorticoids is free and available to mediate biological functions, so the effective glucocorticoid concentration in plasma is considerably lower than the total that is typically measured. The synthetic glucocorticoid dexamethasone (Dex) is particularly potent in vivo because of its high affinity for the GR and relatively low level of binding to plasma proteins (7, 8). Secretion of glucocorticoids by the adrenals is under control of the hypothalamo-pituitary axis. Adrenocorticotrophic hormone (ACTH) produced by the anterior pituitary causes an immediate increase in the secretion of glucocorticoids as well as an increase in the production of steroid biosynthetic enzymes (9). Glucocorticoid levels are maintained, in part, by a feedback loop with the hypothalamus and anterior pituitary in which low systemic glucocorticoid levels increase, and high levels suppress, ACTH secretion (8). Under normal conditions, secretion of ACTH occurs with a circadian pattern, in humans peaking prior to waking and reaching a nadir in the evening. Perhaps of special note to immunologists, in nocturnal animals such as rodents this pattern is reversed, with the peak in ACTH secretion occurring in the late afternoon (8). The resulting changes in glucocorticoid secretion typically yield fluctuations in plasma concentration, with a range of threefold to as much as tenfold over the course of the day (10). The major stimulus to increased ACTH, and thus glucocorticoid, secretion is ‘‘stress,’’ a term that covers a wide range of physiologic (e.g. exercise, emotional disturbance, etc) and pathologic (e.g. trauma, hemorrhage, fever, etc) situations (11).

Glucocorticoid Receptors

Nonprotein-bound corticosteroids passively diffuse across the plasma membrane into the cell where they encounter the glucocorticoid receptor (GR). The GR is a member of a large superfamily that includes receptors for other steroid hormones, thyroid hormone, vitamin D_3, retinoic acid, and a number of orphan receptors, including Nur77. Receptors of this superfamily have many conserved structural elements, including a COOH-terminal ligand-binding domain (which also contains residues required for dimerization and hormone-dependent gene transactivation), a nearby hinge region containing nuclear localization signals, a central zinc-finger-containing DNA-binding domain, and an NH_2-terminal variable region important for ligand-independent gene transactivation (12). Although many of these receptors are nuclear-resident, the GR exists in the cytosol in a

complex with heat shock proteins such as hsp90, hsp70, and the immunophilins hsp56 and CyP-40 (13), and it translocates to the nucleus when it is occupied by ligand (14). Once in the nucleus, the GR binds as a homodimer to specific DNA sequences (*g*lucocorticoid *r*esponsive *e*lements, or GREs), where it acts to enhance or inhibit transcription of corresponding genes. The classic consensus GRE consists of two conserved 6-nucleotide halves separated by three nonconserved bases, and it typically acts as an enhancer element (15). However, ''negative'' GREs that suppress gene transcription have also been described (15, 16), and some GREs contain sites for other transcription factors embedded in the GR-binding region, yielding a ''composite'' response element that may have enhancing or repressing activity (17). Thus, the GR is a ligand-regulated transcription factor. The members of this receptor superfamily interact with a cohort of molecules to mediate their function as transcriptional regulators, and the GR is no exception. For example, GRs are thought to stabilize the formation of a preinitiation complex that contains components of the basal transcriptional machinery (18). Furthermore, the ligand-binding domain contains a region (AF-2) that binds a number of proteins important for GR (and other members of this receptor superfamily) function. One group of co-activators includes steroid receptor coactivator-1 (SRC-1), activator of thyroid hormone and retinoid receptors (ACTR), and transcriptional intermediary factor 2 (TIF2)/glucocorticoid receptor interacting protein 1 (GRIP1) gene products (18). A second co-activator group includes proteins such as CREB binding protein (CBP) and its homolog p300, and p300/CBP-associated factor (P/CAF). One of the more exciting developments in this area in recent years is the realization that SRC-1, ACTR, p300/CBP, and P/CAF all have intrinsic histone acetytransferase (HAT) activity (19–23). Acetylation of core histones alters nucleosomal packing to allow increased access of transacting factors and components of the basal transcriptional machinery to the local DNA (24). Thus, chromatin remodeling in response to recruitment of HATs by the liganded GR is at least one mechanism by which glucocorticoids enhance gene transcription.

The GR is constitutively phosphorylated on serines and threonines. All of the potential phosphorylation sites (eight in the mouse) are N-terminal of the DNA-binding domain (25, 26). Many of these sites are ''proline-directed'' consensus sequences, favored by cyclin-dependent kinases (CDKs) and MAP kinases, and in fact in vitro cyclin/CDK complexes and ERK2 have been found to phosphorylate different serine/threonine residues in the GR (27). Baseline GR phosphorylation is cell-cycle-dependent, being highest in G_2/M and lowest in S phase. GR phosphorylation is enhanced after ligation with hormone in as short a time as 5–10 min, the degree of increase correlating inversely with the basal phosphorylation level (i.e. greatest in S phase) (reviewed in 26, 28). The biologic consequences of GR phosphorylation are controversial. Initial studies with GRs with mutated phosphorylation sites found little effect on nuclear localization or transactivation (29–31). A more recent study, however, reported that while transiently expressed GR phosphorylation-defective mutants transactivated a reporter driven by the

MMTV promoter, they were less good (by two- to fourfold) at transactivating a simple GRE-containing reporter (32). Moreover, receptors containing multiple mutated phosphorylation sites were, unlike wild-type GRs, incapable of down-regulating transcription of their own gene, and the half-life of GR protein was substantially increased. Thus, although at this time it seems that the phosphorylation status of the GR does not have an obvious major impact on function, this posttranslational modification may have subtle but perhaps biologically significant effects on receptor expression and transactivation of some target genes.

Humans have two GR isoforms: α (the classic GR) and β (33). These receptors share the first 727 amino acids (encoded by the first 8 exons of the GR gene and containing the transactivating and DNA-binding domains), but due to alternate mRNA splicing the receptors contain carboxy-terminal residues encoded by either exon 9α or 9β, respectively. Thus, in GRβ the last 50 amino acids of GRα are replaced with a unique 15 amino acid sequence that renders the molecule incapable of binding glucocorticoids and therefore transcriptionally inactive. Unlike GRα, GRβ constitutively resides in the nucleus (34). Indeed, GRβ can bind a GRE consensus sequence, and overexpression of GRβ can inhibit gene transactivation mediated by GRα in a dominant negative fashion. Screens for mRNA expression found that GRα and GRβ are widely expressed in the same tissues (33, 34), leading to the speculation that these GR isoforms might interact, perhaps via heterodimerization, to regulate the transcriptional effects of glucocorticoids. This possibility was supported by the finding that peripheral blood mononuclear cells from patients with glucocorticoid-resistant asthma had decreased binding of the GR to DNA, accompanied by elevated numbers of cells in which GRβ was detected by immunohistochemistry (35). Binding analyses indicated that the decrease in GR-DNA binding was due to lowered GR affinity for the GRE. Moreover, overexpression of GRβ in HepG2 cells (hepatocytes) had the same effect on binding of the endogenous GRα to DNA. GR DNA binding activity increased in peripheral blood mononuclear cells cultured in medium but not those cultured in IL-2 and IL-4, and culture of cells from normal donors with IL-2 and IL-4 resulted in increased GRβ expression. Together, these data suggest that GRβ expression can be regulated by cytokines and that there may in fact be situations in vivo in which the interplay between GRα and GRβ has significant biological consequences.

Glucocorticoid Signaling

Although the activity of the GR is often thought of simply in terms of direct gene transactivation, considerable cross-talk also occurs between the GR and other transcription factors that can modify each of their biologic activities. The first such interaction described was between the GR and AP-1 (17, 36–38). Typically, these factors antagonize each other's transcription enhancing activity, although AP-1 consisting of c-Jun dimers can also enhance GRE-mediated transactivation (reviewed in 39, 40). Most, although not all, studies that examined the issue

detected a direct physical interaction between the GR and AP-1, suggesting at least one direct molecular basis for cross-talk, although simple sequestration of these factors into inactive complexes does not seem to be likely to explain the phenomenon. Other nuclear factors that have been found to bind the GR and modify its activity are NF-κB (41–44), the cAMP response element binding protein CREB (45), and the signaling and transcription factors STAT3 and STAT5 (46, 47). Another potential mechanism is competition for co-activators (''squelching''). Both AP-1 and the GR are co-activated by CBP/p300, and in fact overexpression of CBP or p300 reverses the antagonism between AP-1 and the GR (46). Similarly, overexpression of CPB or SRC-1 reverses the transcriptional antagonism between the GR and NF-κB (47). These results support the notion that in some circumstances cross-talk between the GR and other transcription factors is due to competition for limiting co-activators of transcription. In the case of NF-κB, yet another mechanism for cross-talk with the GR has been proposed: Glucocorticoids increase the transcription and synthesis of IκB and thus may inhibit NF-κB by promoting its retention in the cytosol (48, 49). Although the possibility that glucocorticoids inhibit NF-κB by upregulating IκB is attractive, its biological relevance is uncertain at best, as there is a steadily increasing number of examples in which inhibition of NF-κB occurs in the absence of IκB upregulation (50–53). Regardless of the particular mechanism involved, the extensive degree of cross-talk between the GR and other transcription factors provides a rich framework for mutual regulation (positive or negative) between glucocorticoids and other signaling pathways.

GLUCOCORTICOIDS AND APOPTOSIS

At least since the end of the nineteenth century it has been known that adrenal insufficiency in humans (54) and adrenalectomy of animals result in thymic hypertrophy that cannot be reversed by the adrenal medullary product epinephrine (55–58), and that stress and drug-induced involution of the thymus is prevented by adrenalectomy (58). These observations were followed by the findings that administration of ACTH to mice caused a marked reduction in thymus and lymph node mass (59), and that a purified corticosteroid caused the regression of a lymphosarcoma (60)[1]. It is now appreciated that lymphoid cells, especially

[1]In an oddly touching footnote to this article, the editor explains that although the manuscript was received in June, 1942, it was withheld from publication, at the authors' request, until April, 1944. Among the reasons the authors gave for this voluntary delay are two that would now be considered remarkable: 1) ''Publication of the results would necessarily create hopes for a prompt enlargement of the scope of the investigation to certain forms of malignancy in clinical medicine for which answers could not be given'', and 2) ''The amount of 11-dehydro-17-hydroxycorticosterone available was so limited that extension of the investigation even to larger experimental animals was impossible. Also confirmation of the work by other laboratories could not be undertaken until a satisfactory source of the material became available.''

$CD4^+CD8^+$ (double positive, or DP) thymocytes, are among the few cell types that undergo apoptosis in response to corticosteroids [among the rare reports of other cells that have been observed to undergo glucocorticoid-induced apoptosis are osteoclasts and osteocytes (61, 62), dendritic cells (63), and some neuronal subsets (61)].

Despite the enormous strides made in our understanding of regulated cell death, the mechanism(s) by which glucocorticoids cause apoptosis is still largely unknown. What is known is that glucocorticoid-induced thymocyte apoptosis is mediated via the ''mitochondrial'' pathway: inhibitable by Bcl-2 and Bcl-x_L and requiring Apaf-1 and caspase-9 (64–69). Since the GR is a transcriptional regulator, the simplest model is that glucocorticoids induce the expression of one or more gene products that directly or indirectly cause cell death. Data with thymocytes support this, since glucocorticoid-induced apoptosis requires ATP (70) and is prevented by inhibitors of protein synthesis (71, 72), although reportedly the latter is not true of splenic T cells (73). An alternative possibility is that the lethal activity of glucocorticoids could be indirect, due to interference with other transcription factors required for cell survival (transcriptional repression). Such a possibility was suggested by the finding that expression of a transcriptionally inactive form of the GR that can still interact with transcription factors such as AP-1 was capable of signaling for apoptosis when expressed in GR-negative Jurkat T cells (74). This observation was countered, however, by a perhaps more physiological study using mice in which the wild-type GR was replaced with a point mutant that cannot dimerize and, therefore, cannot directly transactivate gene transcription, although it can interact with other transcription factors (75). Although grossly normal, thymocytes from these animals were refractory to corticosteroid-induced apoptosis. Therefore, while receptor cross-talk may account for some biological responses to corticosteroids, at this time it seems likely that glucocorticoid-induced thymocyte apoptosis requires GR-mediated gene transactivation.

Many attempts have been made to isolate steroid-induced genes that mediate cell death (76–80). Unfortunately, to date there are no convincing data that any of the candidates play such a role. However, a growing number of gene products have been implicated in blocking glucocorticoid-induced apoptosis. Among these are the now classic inhibitors of mitochondrial-dependent cell death such as Bcl-2 and Bcl-x_L (64, 66, 81) as well as IAPs (*i*nhibitors of apoptosis), which are thought to work at least in part by directly binding and inhibiting some caspases, including caspase-3, -7, and -9 (82–85). In addition to these, a number of gene products are not ''pure'' apoptosis inhibitors but nonetheless prevent glucocorticoid-induced apoptosis. One example is Notch, a transmembrane receptor that has been implicated in regulating the $CD4^+$ vs. $CD8^+$ and perhaps the TCR $\alpha\beta$ vs. $\gamma\delta$ differentiation decisions in the thymus (86, 87), and which has a developmental pattern of expression reminiscent of Bcl-2: highly expressed in early thymocyte progenitors ($CD4^-CD8^-$), absent in DP thymocytes, and expressed at intermediate levels in single positive thymocytes (88). Overexpression of a

constitutively active intracellular portion of Notch in DP thymocytes rendered them relatively resistant to, and in T cell hybridomas completely prevented, glucocorticoid-induced apoptosis (89). Another example is RAP46 (the murine homolog is BAG-1), which binds the GR and inhibits its function (90). Inhibitors of the proteasome, a complex intracytoplasmic protease complex that degrades ubiquitinated substrates, prevent thymocyte apoptosis induced by p53-dependent and -independent stimuli such as corticosteroids (91). Also, as discussed in detail below, activation via the TCR (or other transmembrane molecules that can transduce activating signals) potently inhibits glucocorticoid-induced cell death (5, 92, 93). Reportedly the interaction of B7 with CD28 and/or CTLA-4 can rescue thymocytes even in the absence of TCR occupancy (94). Although these disparate molecules and signaling pathways do not at this time move us appreciably closer to the goal of defining the molecular mechanisms by which glucocorticoids kill, they do emphasize the multiple levels at which this process can be controlled.

$CD4^+CD8^+$ thymocytes are exquisitely sensitive to glucocorticoid-mediated cell death, and even the physiologic concentrations achieved during a stress response can be sufficient to cause their apoptosis (95–97). Resting peripheral T cells, however, are comparatively resistant to glucocorticoid-induced death (72). The reason for the difference appears to be the expression of Bcl-2, which is present in $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes and peripheral T cells but not in $CD4^+CD8^+$ thymocytes (98). Although normally relatively resistant to glucocorticoid-induced apoptosis, TCR^{hi} thymocytes and mature T cells derived from Bcl-2-deficient ES cells are just as sensitive as $CD4^+CD8^+$ thymocytes (99). It is noteworthy in this regard that, after initially developing normally, the lymphoid organs of Bcl-2-deficient animals undergo massive apoptotic involution at approximately 4 weeks of age (99, 100), the same time at which circulating corticosteroids achieve adult levels (101, 102). It should be possible to determine if there is a cause-and-effect relationship between these two events by characterizing lymphoid development in adrenalectomized Bcl-2-deficient mice. Activation of peripheral T cells (which does not cause a decrease in Bcl-2) makes them more sensitive to glucocorticoids (103, 104), perhaps at least in part due to upregulation of GR levels (105). Glucocorticoid-induced apoptosis, unlike that induced by irradiation or genotoxic reagents, does not require p53 (106, 107).

GLUCOCORTICOIDS AND PERIPHERAL T CELLS

Immunosuppression

Immunosuppression is arguably the most widely appreciated effect of exogenously administered corticosteroids. Cortisone was first administered to patients suffering from rheumatoid arthritis in 1948, and soon thereafter glucocorticoids became a staple in the treatment of a myriad of autoimmune and inflammatory conditions (108, 109). Early evidence that glucocorticoids interfere with the

immune response was provided by the observation that cortisone or ACTH prolongs the survival of allogeneic skin grafts (transplantation immunity) (110, 111). Once it became possible to study lymphocytes in vitro, it was found that corticosteroids inhibit proliferative responses to a variety of mitogenic stimuli, largely by inhibiting the secretion of T-cell growth factor (i.e., the growth-promoting activity of T cell–derived lymphokines) (112).

Corticosteroids are now known to inhibit the production of a large number of cytokines, including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, GM-CSF, TNFα, and γ-interferon (113–116). In those instances in which the mechanism has been studied, inhibition was found to be largely due to interference with gene expression. In the case of IL-2, for example, it was initially reported that glucocorticoids inhibited a reporter construct containing the entire IL-2 enhancer but not a reporter construct containing a triplicated NF-AT binding site; this effect was mapped to two proximal regions in the IL-2 promoter that bind AP-1-like proteins (117). At the same time an AP-1 site in the IL-2 enhancer was reported to confer sensitivity to glucocorticoid inhibition, but only when present in conjunction with an NF-AT binding site (118). Another study found that Dex prevented binding of AP-1 and NF-AT to their corresponding binding sites in the IL-2 gene promoter and blocked activation of an AP-1 but not an NF-AT reporter (119). A direct effect of glucocorticoids on the generation of these and other important transcription factors seems unlikely. Initial studies using electrophoretic DNA mobility shift assays (gel shifts) to quantitate transcription factors reported that the levels of activation-induced transcription factors that bind the IL-2 enhancer, including NF-AT, AP-1, AP-3, Oct-1, and NF-κB, were not decreased by glucocorticoids (117, 120). The consensus from these studies seems to be that glucocorticoids generally do not prevent activation-induced generation of transcription factors; even in cases in which AP-1 binding to DNA was inhibited, there was no decrease in the actual amount of c-Fos and c-Jun protein in the nucleus (121). Rather, glucocorticoids appear to interfere with the binding and/or function of critical transcription factors (notably AP-1 in the case of the IL-2 gene), probably by direct protein-protein interactions between these factors and the liganded GR. The differences between reports on the effect (or lack thereof) of glucocorticoids on gel shift assays may be accounted for at least in part by the methods used to prepare nuclear extracts, some of which do not recover the GR (117). Direct inhibition of gene transcription by the GR is another possible mechanism of interference, and in fact a functional ''negative'' GRE has been identified in the promoter region of the IL-1β gene (16).

The literature is replete with reports of the effects of glucocorticoids on a host of transcription-dependent and -independent events that might be expected to affect the immune response. Glucocorticoids decrease the stability and half-life of mRNA encoding IL-1, IL-2, IL-6, IL-8, TNFα, and GM-CSF (122). Paradoxically, glucocorticoids also upregulate the expression of receptors for some of these same cytokines, including IL-1, IL-6, and GM-CSF, as well as IFN-γ

(reviewed in 113). The effect on IL-1 receptors is not straightforward: Dex augments mRNA levels in human peripheral mononuclear cells for both type I and type II IL-1 receptors and causes the shedding of the latter as a soluble protein (123). The IL-1 type II receptor binds IL-1 but does not signal, and thus it is thought to act as an inactive ''decoy'' for IL-1. In the same vein, glucocorticoids were found to increase expression of intracellular IL-1 receptor antagonist (IL-1ra) (124). Therefore, upregulation of IL-1 signaling antagonists may be yet another mechanism by which glucocorticoids mediate their anti-inflammatory effects. The effect of corticosteroids on IL-2 receptors is also not clear-cut. In fact, for T cells glucocorticoids have been reported to (*a*) increase IL-2Rα mRNA and protein, perhaps in synergy with IL-2 (125–127), and (*b*) decrease IL-2Rα and IL-2Rβ mRNA and protein levels (128, 129). In those cases in which IL-2Rα was decreased, it was likely a secondary effect due to inhibition of IL-2 production, since exogenous IL-2 prevented the downregulation. Whatever their effect on IL-2R expression, corticosteroids appear to directly decrease IL-2-dependent, but not IL-4- or IL-9-dependent, T cell proliferation, perhaps by inhibiting proximal signaling via the IL-2R (130, 131). Among the other reported effects of glucocorticoids are downregulation of cell surface adhesion molecules like ICAM-1 and E-selection (43, 132), inhibition of CD40 ligand upregulation on activated $CD4^+$ T cells (133), interference with transcription of the CTL serine protease granzyme B (134), and even the direct inhibition of early TCR signaling events (135). While the literature is confusing and inconclusive in many respects, what these studies make clear is that the immunosuppressive activity of glucocorticoids is a reflection of its activity on multiple molecular targets. While the inhibition of cytokine production is widely accepted to be biologically significant, the degree to which the other reported events (inhibition/induction of receptors and receptor antagonists, inhibition of receptor signaling, downregulation of adhesion molecules, etc) contribute to immunosuppression in vivo is not well established.

Th Lineage Commitment

Mature helper T cells can be divided into subsets that differ in the spectrum of cytokines they secrete (136). Th1 cells produce IL-2, IFN-γ, and TNFβ and contribute largely to T cell–mediated responses such as delayed-type hypersensitivity. Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 and participate in humoral and allergic responses. The means by which helper T cells are induced to differentiate down either of these pathways has been an area of intense interest, and clearly the cytokines the subsets secrete, and perhaps the nature of the antigen-presenting cell they interact with during differentiation (137), have an enormous influence. There is also a body of data indicating that glucocorticoids participate in guiding the differentiation of helper T cells. For example, in early studies mice were implanted with sustained-release pellets containing Dex and then immunized

with OVA (138). Upon antigen restimulation in vitro, OVA-specific T cells from animals that had received Dex produced substantially lower amounts of IL-2 but higher amounts of IL-4. A similar skewing of lymphokine production away from IL-2 and toward IL-4 was observed when T cells from OVA-primed mice were rechallenged in vitro with OVA in the presence of low concentrations of glucocorticoids. It was deduced that the effect of Dex was directly on the T cells, because glucocorticoid treatment of a cloned T cell line and a T cell hybridoma also favored the production of IL-4. Consistent with this, Dex either had no effect (139) or it enhanced anti-TCR-induced proliferation of the murine D10 Th2 cell line, presumably by increasing its secretion of IL-4 (140). Other studies have shown that the cytokine profile of $CD4^+$ rat T cells that had initially been activated in the presence of Dex was skewed toward Th2 cytokines when subsequently activated in the absence of glucocorticoids (141). Interestingly, it has also been possible to generate antigen-specific Th2 cells from unprimed mice by repeated simulation in vitro with antigen in the presence of IL-2 and anti-IL-10 antibodies in serum-free medium, but only in the presence of glucocorticoids (142).

In humans, acute glucocorticoid treatment of patients with nonsteroid-dependent asthma results in depressed levels of IgG and IgA and increased levels of IgE (143). This activity may or may not reflect a direct effect on the Th subsets, however, because glucocorticoids can synergize with IL-4 to increase IgE synthesis by enhancing isotype switching, even in the absence of T cells (144–146). In fact, and in contrast to the observations with murine cells mentioned above, many reports indicate that glucocorticoids inhibit the production of Th2 cytokines by human T cells, leading to the suggestion that murine and human T cells may be fundamentally different in their susceptibility to glucocorticoids (116, 147, 148). This seems unlikely, however, and although glucocorticoids do inhibit both Th1 and Th2 cytokines by activated human T cells, the effect on Th1 cytokines may be more pronounced (149). Furthermore, IL-12 production (which favors Th1 development) by LPS-stimulated PBL was inhibited by glucocorticoids, while IL-10 production (favoring Th2 development) was relatively resistant (150). Therefore, if Th differentiation is regulated by the balance between Th1 and Th2 cytokines rather than the absolute amount produced, glucocorticoids might be expected to support the generation of Th2 cells in humans. Furthermore, although activation of rat T cells in the presence of glucocorticoids resulted in the generation of Th2 cells, addition of Dex during the subsequent activation of these Th2 cells inhibited production of IL-4, just as it inhibits IL-4 production by human T cells (151). Collectively, the data are consistent with the hypothesis that while glucocorticoids inhibit the acute production of both Th1 and Th2 cytokines, their presence during initial activation may nevertheless promote the differentiation to the Th2 phenotype. The finding that Dex inhibits IL-4 production but that exogenous IL-4 potentiates the ability of Dex to enhance the generation of Th2 cells (141, 151) suggests that IL-4 and glucocorticoids may promote differentiation of Th2 cells by different mechanisms.

Antagonism of Activation-Induced Apoptosis

Activation-induced T cell apoptosis was first described with T cell hybridomas (152–154) and subsequently with pre-activated mature T cells and T cell clones (155–157). In the course of studies aimed at comparing activation- and glucocorticoid-induced apoptosis of T cell hybridomas, it was unexpectedly found that these lethal stimuli, when administered simultaneously, no longer caused cell death (92). This phenomenon was termed ''mutual antagonism,'' and was presumed to result from transcriptional interference between the GR and activation-induced transcription factors such as AP-1, although the relevant genes were not known (39). It is now clear that activation-induced apoptosis in these cells is caused by the upregulation of Fas ligand (FasL) expression and its subsequent interaction with Fas (158–161). Further studies found that glucocorticoids prevent activation-induced FasL upregulation but do not prevent signaling via Fas itself, demonstrating that interference with FasL expression by the GR is responsible for one arm of the mutual antagonism (161). One candidate for mediating this effect is the product of a newly described glucocorticoid-induced gene, GILZ (*g*lucocorticoid-*i*nduced *l*eucine *z*ipper) (162). Stable expression of GILZ in T cell hybridomas was found to block activation-induced FasL upregulation and subsequent apoptosis. Our increasing appreciation that many instances of apoptosis rely on the expression of FasL has fueled interest in understanding the molecular regulation of this molecule. At least three transcription factors have been directly implicated in its upregulation by activation: NF-AT (163, 164), Egr-2 and Egr-3 (165, 166), and NF-κB (167, 168). Of these, only the Egr family members are synthesized de novo, and the finding that inhibition of protein synthesis prevents activation-induced upregulation of *fasL* mRNA is consistent with the notion that these are critical mediators of activation-induced FasL upregulation (166). Furthermore, Egr-2 and -3 are themselves upregulated by NF-AT (165, 169, 170), and thus the contribution of NF-AT to FasL expression may be accounted for, at least in part, by indirect effects via the Egr transcription factors. In preliminary experiments, we have found that glucocorticoids inhibit *fasL* mRNA upregulation when cDNA encoding Egr-3 is introduced into HeLa cells (P Mittelstadt and JD Ashwell, unpublished data). It appears, then, that the interplay between the GR, perhaps its induced genes, and other transcriptional regulators may affect *fasL* transcription in an interesting but complex fashion.

Another molecule that may participate in the antagonism of activation-induced apoptosis is GITR (*g*lucocorticoid-*i*nduced *T*NFR family *r*elated), a transmembrane molecule homologous to the TNF/NFGR family members 4–1BB, CD27, and OX-40 that is expressed in thymocytes and peripheral T cells (171–173). GITR was initially identified as a murine glucocorticoid-induced gene that was also upregulated relatively late after activation, and its overexpression in T cell hybridomas inhibited CD3-mediated but not anti-Fas- or dexamethasone-induced apoptosis (171). However, although co-expression of the human homolog (hGITR or AITR) and its ligand, hGITRL/TL6, in Jurkat T cells also prevented anti-CD3-

induced cell death (presumably by interacting with TRAF2 to upregulate NF-κB activity), hGITR/AITR is upregulated by activation but not by glucocorticoids (172, 173). Thus, the possibility that GITR family members are glucocorticoid-induced genes that prevent activation-mediated apoptosis is uncertain. Finally, Nur77 is a nuclear orphan receptor whose induction is required for activation-induced death of T cell hybridomas and whose overexpression in thymocytes has been implicated in FasL upregulation, although as yet no Nur77 regulatory elements have been identified in the *fasL* promoter (174–176). Glucocorticoids inhibit the Nur77-dependent transcription of reporter constructs driven by either of the two known Nur77 DNA-binding response elements, one of which (NurRE) binds Nur77 homodimers and exhibits increased activity in T cells upon activation (177). Moreover, titration of Nur77 and the GR in transient transfection studies revealed that they each antagonize the transcriptional activity of each other (178). These results raise the possibility that antagonism between the GR and Nur77 may account, at least in part, for the observed antagonism between TCR- and glucocorticoid signaling in the induction of apoptosis.

Inhibition of activation-induced T cell death is not confined to transformed T cell hybridomas. Encephalitogenic myelin basic protein–specific T cell lines undergo apoptosis when restimulated with antigen and IL-2 in vitro; cell death is prevented by simultaneous exposure to glucocorticoids (179). Glucocorticoids also prevent activation-induced death of human $CD4^+$ T cells acutely infected with HIV and then restimulated with anti-TCR antibodies, and prevent the accelerated apoptosis of cultured $CD4^+$ and $CD8^+$ T cells from people infected with HIV (180). Since the susceptibility of T cells from HIV^+ individuals to spontaneous and activation-induced apoptosis is largely conferred by increased Fas expression and sensitivity to FasL-mediated apoptosis (181, 182), it is likely that the protective effect of glucocorticoids is due to their suppression of FasL expression.

GLUCOCORTICOIDS IN THYMOCYTE DEVELOPMENT

Thymocytes undergo an ordered series of phenotypic transitions as they differentiate from early precursors to mature T cells. Immature $CD4^-CD8^-$ (double negative) thymocytes that successfully rearrange the TCR β gene locus express a pre-TCR that consists of CD3-γ,-δ, and -ε, a ζ homodimer, and TCR β heterodimerized with the nonpolymorphic pre-TCR α chain (183). Subsequent to pre-TCR expression, thymocytes begin to divide rapidly, acquire CD4 and CD8, and undergo TCR α gene rearrangement. For those cells that generate a functional α chain, the pre-TCR is replaced with low levels of the mature αβ TCR (184). Due to the mostly random nature of the process by which TCR α and β chains are generated, each thymocyte will produce a unique αβ TCR, and from this point forward thymocyte development is tightly linked to the specificity of the TCR for self-peptides bound to MHC-encoded molecules. The prevailing view at this

time is that $TCR^{lo}CD4^+CD8^+$ thymocytes having TCRs with subthreshold avidity for self-antigen/MHC survive for approximately 3.5 days (185) and then undergo a default death pathway that has been called ''death by neglect.'' Thymocytes bearing TCRs that recognize self-antigen/MHC with high avidity undergo activation-induced apoptosis (negative selection), a major mechanism for promoting self-tolerance. The molecular mechanisms underlying this form of apoptosis are poorly defined, but antigen-specific negative selection appears to be largely independent of the Fas and TNF receptor signaling pathways (186–188). Finally, thymocytes bearing TCRs with intermediate avidity for self antigen/MHC are rescued from the default death pathway, differentiate into $TCR^{hi}CD4^+CD8^-$ or $TCR^{hi}CD4^-CD8^+$ cells, and migrate to the periphery (positive selection). The outcome of these selection processes largely determines the mature T cell antigen-specific repertoire.

The molecular means by which signaling via the TCR causes both rescue from default cell death and induction of cell death is an area of great interest. Presumably, low levels of TCR occupancy result in a change in expression or function of molecules involved in enhancing survival, while higher levels of occupancy alter the levels or activities of molecules that regulate apoptosis. Although the signaling pathways that lead to positive selection (which involves calcineurin and the Ras/MAP kinase signaling pathways) (189–191) and negative selection (which does not) may differ, the effector molecules regulated by these pathways have not been identified, making it difficult to determine how thymocytes make the critical decision of whether to live or die. However, considerable evidence suggests that glucocorticoids participate in this process and in doing so shape the peripheral T cell antigen-specific repertoire of adult animals. The initial observation that activation rescues T cell hybridomas from glucocorticoid-induced apoptosis (and vice versa) led to the speculation that similar interactions occurring in vivo could account for the relationship between TCR avidity for self-antigen/MHC and cell fate (92, 93, 192). In this ''mutual antagonism'' model, DP thymocytes with subthreshold avidity for self-antigen/MHC undergo death (by neglect) at least in part because of glucocorticoids. Encounter of self-antigen/MHC by thymocytes with intermediate avidity TCRs results in signaling that would otherwise lead to apoptosis, but due to antagonism by glucocorticoids the cells survive (positive selection). Finally, signals leading to apoptosis in thymocytes bearing TCRs with high avidity for self-antigen/MHC are too strong to be overcome by ambient corticosteroids, and these cells are deleted (negative selection).

There are a few inferences from this model that are not necessarily obvious. First, in this scenario TCR occupancy at any level does not in itself deliver a ''positive'' signal. That is, low-to-moderate levels of TCR-mediated activation do not initiate signaling pathways that intrinsically promote survival while higher levels of activation initiate apoptotic signals. Rather, biologically relevant levels of TCR signaling result in apoptosis. Intermediate levels of TCR signaling can be viewed as positive only in that they counter apoptotic signals delivered via the

GR (and vice versa)—in the absence of glucocorticoids, it would be expected that these same intermediate levels of TCR signals would result in activation-induced apoptosis. This is in many ways analogous to TCR-mediated activation of mature T cells. TCR occupancy alone causes anergy and apoptosis; only in the presence of costimulation, typically via CD28, does TCR occupancy result in proliferation and survival (193). Second, in addition to apoptotic signals, intermediate levels of TCR occupancy must result in signals that lead to differentiation, as evidenced by increases in expression of molecules such as the TCR, CD5, and CD69, and extinguishing of expression of CD4 or CD8. Differentiation is of course thwarted in cells also induced to die but will proceed if death is prevented by glucocorticoids.

If glucocorticoids play a significant role in thymocyte differentiation, they must necessarily be present in late fetal and neonatal life, a time during which a tremendous amount of thymocyte development occurs. However, the availability of circulating glucocorticoids is not uniform throughout development. In utero the placenta forms a partial barrier to transfer of maternal glucocorticoids (194), and in humans placental enzymes convert biologically active cortisol to the less active cortisone (195). Moreover, the expression of some steroidogenic enzymes is developmentally regulated, and as a result the levels of circulating glucocorticoids are low in fetal and neonatal life, not reaching adult levels in rodents until approximately 4 weeks of age (101, 102). These considerations prompted us to ask if the thymus itself produces glucocorticoids, and if so whether it does so during neonatal life. It was found that thymic epithelium, but not thymocytes, macrophages, or dendritic cells, produces pregnenolone and deoxycorticosterone (see Figure 1) when cultured in vitro, and that steroid production increased approximately twofold in response to ACTH (5). Immunohistochemical studies revealed that P450scc and P450c11 (Figure 1) are expressed mainly in cortical epithelium and that there is a subpopulation of large and intensely staining cells in a subcapsular and cortical periphery similar to that of thymic nurse cells. Subsequent studies have confirmed that thymic epithelial cells express steroidogenic enzymes and produce all of the steroids from pregnolone to corticosterone (196, 197). The ontogeny of thymic steroid production also appears to differ from that of the adrenal; cultured thymic epithelium from fetal and neonatal mice produced approximately twice as much as pregnenolone epithelium from four-week-old animals (5). A thymic epithelial cell line has been also reported to produce glucocorticoids, as evidenced by its ability induce apoptosis of a $CD3^+CD4^+CD8^+$ radiation leukemia virus–transformed thymocyte clone (197). This activity was inhibited by drugs that block either steroid production (aminoglutethimide) or GR occupancy (RU-38486). As with T cell hybridomas and normal thymocytes, death of the thymocyte clone was antagonized by activation via the TCR. Interestingly, the epithelial cell line appeared to produce glucocorticoids only when in contact with the $CD3^+$ thymocyte cell line, an activity that was not induced by contact with a $CD3^-CD4^-CD8^-$ thymocyte clone. Irradiated normal thymus cells were also reported to produce progesterone (3βHSD activity) but not corticosterone

(P450c11 activity) (196). These data raise the intriguing possibility that thymocyte-epithelial cell interactions may be required for the latter to produce glucocorticoids.

In Vitro Models

The mutual antagonism model of thymocyte selection predicts that diminishing glucocorticoid levels or responsiveness should affect antigen-specific thymocyte selection by causing the activation-induced death of cells that would ordinarily be positively selected. That is, levels of TCR-mediated signaling that normally result in positive selection should now result in negative selection. This prediction has been tested in a number of different experimental model systems. Since the thymus produces its own steroids, it is possible to ask how thymocyte development proceeds in fetal thymic organ culture (FTOC) in which glucocorticoid production is prevented. Blockade of corticosteroid production with metyrapone, or responsiveness with RU-486, did in fact make thymocytes much more sensitive to apoptosis induced by anti-TCR antibodies or a low avidity ligand (5). Importantly, the effect of metyrapone was largely reversed by exogenous corticosterone, ruling out a pharmacologic effect or toxicity independent of the blockade of glucocorticoid production. The most direct test of the prediction was performed with mice expressing a transgenic αβ TCR specific for the male H-Y antigen presented by the H-2D^b class I molecule. Thymocytes from H-2^{b}male, but not female, mice bearing this TCR are negatively selected (198). Importantly, thymocytes from female mice undergo positive selection and mature into clonotype-bearing CD4$^-$CD8$^+$ cells when expressed in an H-2^b, but not an H-2^d, animal, indicating that this transgenic TCR recognizes some unknown antigen plus an H-2^b-encoded molecule with low avidity (199). To determine if inhibition of glucocorticoid production could ''turn positive into negative selection,'' FTOC was performed with thymuses from RAG-2-deficient (to prevent expression of endogenous TCRs) H-2^b female mice that expressed the anti-H-Y/D^b TCR (200). Inhibition of corticosteroid production with metyrapone resulted in a substantial increase in DP apoptosis at 24 hr and a marked decrease in thymocyte recovery at 3 days that was largely prevented by the addition of physiologic levels of corticosterone. Notably, metyrapone did not induce apoptosis or affect thymocyte recovery when TCR αβ transgenic littermates of the H-2^d haplotype were analyzed, demonstrating that TCR occupancy was required for thymocyte death to occur.

In Vivo Models

A number of different methods have been used to ask how glucocorticoids affect thymocyte development in vivo. One approach was to adrenalectomize pregnant rat dams, which resulted in early maturation of thymic stroma in the fetuses (201). Although this was interpreted as the direct result of glucocorticoid insufficiency, the possibility that this effect might be due to increased ACTH secretion by the

adrenalectomized dams and secondary upregulation of corticosteroid production by the thymic epithelium of the fetuses was not addressed. Another approach has been to generate transgenic mice that express antisense transcripts to the 3' untranslated region of the GR. One set of animals (termed TKO mice) was created in which expression of the antisense transcripts is driven by the *lck* proximal promoter—a T lineage–specific promoter that is most active in immature thymocytes and relatively inactive in peripheral T cells (202). As expected, the transgene was expressed only in the thymus, and DP thymocytes homozygous for the antisense transgene had approximately a twofold reduction in GR mRNA and protein, with a corresponding reduction in sensitivity to corticosteroid-induced upregulation of a GRE-luciferase reporter construct and apoptosis (203). Thymuses from mice homozygous for the antisense transgene were as much as 90% smaller than controls due to a decrease in the number of DP thymocytes and a secondary decrease in $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes; heterozygous mice had an intermediate phenotype. A substantial increase occurred in the amount of spontaneous apoptosis of TKO DP thymocytes, and analysis of thymocyte ontogeny revealed that differences in thymocyte cellularity between wild-type and TKO mice were first apparent between d15 and d16 of gestation. As $\alpha\beta$ TCRs are not yet expressed at this stage of fetal development, it was speculated that glucocorticoids may be required to antagonize apoptotic signals delivered via the pre-TCR as well as the mature $\alpha\beta$ TCR. Furthermore, the DP cells that did develop in TKO mice were deleted by anti-TCR antibodies at approximately 100-fold lower concentration than was required for wild-type cells, indicating that, like deprivation of glucocorticoids in FTOC, glucocorticoid hyporesponsiveness in vivo rendered DP thymocytes exquisitely sensitive to activation-induced apoptosis. A different antisense GR mouse, using a similar construct driven by a neurofilament promoter, has been analyzed as well (204, 205). Despite the presumably tissue-specific promoter, GR levels were found to be reduced two- to threefold in all tissues analyzed, including the anterior pituitary gland, hippocampus, liver, thymus, and spleen, with resultant increases in circulating ACTH and corticosterone, and hyporesponsiveness of the lymphoid cells to glucocorticoid-mediated inhibition of mitogenesis. Unlike the TKO mice, there was no reduction in $CD4^+CD8^+$ thymocytes, and if anything a small increase in total thymocyte number, with a failure of the thymus to regress after puberty. An increase in the ratio of $CD4^+$ to $CD8^+$ cells in the spleen was also noted. There are several possible resolutions for the substantial differences between the two GR antisense animal models. First, the antisense GR was restricted to thymocytes in the TKO mice but was expressed widely in the other strain, and it is possible if not likely that systemic consequences of glucocorticoid hyporesponsiveness can indirectly affect thymocyte and peripheral T cell development. Second, because of the tissue distribution of the antisense transgenes, circulating (and perhaps thymus-derived) corticosterone levels were elevated due to increased secretion of ACTH only in the second model, and it may be that this compensated for the decreased thymocyte sensitivity to glucocorticoids. A GR ''knock-in'' mouse has also been

reported in which the wild-type GR was replaced with a point mutant that cannot dimerize and, therefore, cannot directly regulate gene transcription. Although a detailed analysis of thymocyte phenotype was not performed, DP thymocytes in these mice were resistant to glucocorticoid-induced apoptosis, and the gross thymus phenotype (cellularity and CD4 and CD8 staining) was normal (75). Once again, there are multiple possible interpretations for this result, and although it argues that direct gene transactivation is necessary for glucocorticoids to induce thymocyte apoptosis, it does not address the possibility that corticosteroids regulate thymocyte development by more indirect mechanisms (e.g., by interfering with or enhancing the activities of other transcription factors). Careful analysis of thymocyte phenotype and function in these animals and, ultimately, in conditional GR knockout mice will be required to fully explore the effect of eliminating glucocorticoid responsiveness in immature thymocytes.

Proposed Mechanism for the Effect of Glucocorticoids on Antigen-Specific Thymocyte Development

Both in vitro and in vivo methods of suppressing the response of thymocytes to corticosteroids have been used to address the mechanistic basis for possible effects of glucocorticoids on thymocyte selection. Metyrapone was used in FTOC to prevent local glucocorticoid production by thymuses from normal C57BL/6 (H-2^b) mice (206). Just as with the female H-2^b mice bearing the anti-H-Y/D^b $\alpha\beta$ TCR transgenes, this caused a decrease in DP thymocyte recovery (in this case approximately 50%) that was reversed by the addition of physiologic levels of free corticosterone (1 nM). If this decrease was due to deletion of cells with low-to-moderate avidity TCRs for self-antigen/MHC, it is much greater than expected given that only 3–4% of thymocytes undergo positive selection (207). To determine if TCR occupancy is indeed required for thymocyte loss in the absence of glucocorticoids, the availability of TCR ligands was varied by comparing the effect of metyrapone on thymuses from MHC-congenic C57BL mice. MHC-congenic animals differ in the number of different MHC-encoded molecules they express and therefore in the quantity and variety of potential TCR ligands. The following haplotypes (and the number of different MHC-encoded molecules they express) were tested: H-2^d (five), H-2^k (four), H-2^b (three), and β_2-microglobulin-deficient H-2^b (β2M -/-; one). A strict correlation was found between the ''complexity'' of the MHC and the loss of thymocytes caused by metyrapone, with H-2^d mice (five MHC-encoded molecules) being the most affected and β2M -/- mice (one MHC-encoded molecule) the least. The role of apoptosis in the decrease in thymocyte recovery was confirmed with a modified TUNEL assay after 24 hr of FTOC: metyrapone caused an increase in apoptotic cells that was directly related to the number of MHC molecules expressed (H-2^d $>$ H-2^b $>$ β2M -/-). Strikingly, when antigen-presentation was prevented by blocking the sole MHC-encoded molecule in β2M -/- mice (I-A^b) with a monoclonal antibody, metyrapone actually caused an increase in cell recovery. Since no ligands are available

for TCR recognition under these conditions, all thymocyte death must be ''by neglect,'' and thus the enhanced thymocyte recovery caused by metyrapone provides direct evidence for the notion that glucocorticoids in fact participate in this default death pathway.

These results demonstrate that TCR occupancy is required for the loss of local glucocorticoid production to result in DP thymocyte death, and they furthermore indicate that a large fraction (>50%) of the preselection TCR repertoire must recognize self-antigen/MHC with biologically significant avidity, consistent with recent data from a number of experimental systems (208–210). Moreover, since these self-reactive cells are ''revealed'' by the removal of glucocorticoids, the data suggest that glucocorticoids in fact prevent thymocytes bearing TCRs with relatively low (but still biologically significant) avidity for self-antigen/MHC from entering the positive selection ''window.'' To test this hypothesis, the effect of metyrapone on the levels of CD5, a transmembrane molecule whose upregulation on DP thymocytes is an early and sensitive measure of TCR/ligand interactions (209, 211), was assessed in FTOC of MHC-congenic H-2^b/β2M -/- (one MHC-encoded molecule) and H-2^a mice (5 MHC-encoded molecules). H-2^a DP thymocytes exhibited approximately a 40% increase in CD5 levels when cultured with metyrapone. In contrast, metyrapone caused no appreciable change in CD5 expression on β2M -/- DP thymocytes. To address the possibility that CD4 and/or CD8 interactions with MHC might have a primary role in this effect, wild-type H-2^b mice were compared to H-2^b mice deficient for TCRα, whose thymocytes progress to the DP stage of development but fail to express a mature αβ TCR. Metyrapone upregulated CD5 expression on wild-type but not TCRα-deficient DP thymocytes. Similar results were observed in the TKO mouse model in which GR levels are decreased in thymocytes: DP thymocytes from wild-type H-2^k mice expressed lower levels of CD5 than DP thymocytes from MHC-matched TKO animals. These results indicate that there is a direct relationship between TCR occupancy, glucocorticoids, and cellular activation.

Based upon these data, we suggest that for immature thymocytes, just as for mature peripheral T cells, glucocorticoids are ''immunosuppressive.'' That is, by a variety of mostly transcriptional mechanisms, glucocorticoids blunt the biological consequences of TCR signaling. In the case of peripheral T cells, this means inhibition of effector function; for DP thymocytes the consequence is inhibition of differentiation (positive selection) or apoptosis (negative selection). As shown in Figure 2, the simplest conventional model of thymocyte selection holds that the vast majority of thymocytes die by neglect, with only a small number bearing TCRs with sufficient avidity for self-antigen/MHC to undergo positive or negative selection. The proposed model holds that, in fact, most thymocytes are not ''neglected,'' but rather there is a bias of preselection TCRs toward those with an avidity for self-antigen/MHC sufficient to signal. Just as for mature T cells, the biological consequences of TCR signaling in DP thymocytes will be inhibited by glucocorticoids. Reductions in glucocorticoid signaling will increase the thymocyte response at any given degree of TCR occupancy, the result being

that cells bearing TCRs with avidities in the ''normal'' positive selection window will undergo activation-induced apoptosis, and some cells bearing TCRs with avidities that are normally inadequate for selection will now fall in the positive selection window. This model, therefore, predicts that alterations in glucocorticoid effects will change the average TCR avidity for selecting self-antigen/MHC and thus the peripheral T cell antigen–specific repertoire.

The ability of glucocorticoids to regulate the T cell repertoire by setting the avidity window for selection is the most important implication of the mutual antagonism model. Two distinct experimental approaches employing the TKO GR antisense mice have been taken to test this hypothesis. The first was based upon the notion that the introduction of this transgene into an autoimmune mouse strain should decrease disease because of the decrease in the average TCR avidity for self-antigen/MHC. To do this, the GR antisense transgene was introduced into MRL-*lpr/lpr* mice (212), a model of spontaneous autoimmune disease that, due to a defect in the *fas* gene and the accompanying decrease in T cell activation–induced apoptosis, exhibits progressive lymphadenopathy because of the accumulation of T cells with the $TCR^+Thy\text{-}1^+CD4^-CD8^-B220^+$ cell surface phenotype. Although the number of thymocytes was decreased by the introduction of the antisense transgene, lymph node T cell numbers between the wild-type and lpr mice and the lpr.TKO were within 20% of each other by 7 weeks of age. Notably, although the proliferative response to mitogens, anti-TCR antibodies, and alloantigen was identical in these two animals, there was a marked and transgene-dose-dependent decrease in lymphadenopathy, anti-double stranded DNA antibodies, glomerulonephritis, and mortality in the TKO animals. Some TCR Vβs promote positive selection in mice of certain MHC haplotypes. This is not superantigen-mediated but is a consequence of recognition of unknown self-antigens presented by MHC-encoded molecules (213–215). As a result, overexpression of an affected V_β is seen in the $CD4^+$ or $CD8^+$ T cell subset, but not both. To determine if the beneficial effect of the transgene was indeed due to a change in the TCR repertoire, TCR Vβ use was quantitated. In each of the five cases examined, T cells bearing Vβs that are normally overexpressed in one of the T cell subsets in $H\text{-}2^k$ mice (the MRL-*lpr/lpr* haplotype) were reduced in fractional representation toward the levels found in non-$H\text{-}2^k$ mice. This result is analogous to the FTOC results obtained with metyrapone and the anti-$H\text{-}Y/D^b$ αβ TCR transgenic mice: A decrease in response to glucocorticoids leads to the deletion of cells that would otherwise be positively selected.

Another approach to address the nature of the TCR repertoire of the TKO mice has been to look for ''holes in the repertoire,'' that is, antigens to which these mice cannot respond because the T cells that can respond to them are not present or are present at low numbers. Reasoning that the most easily demonstrated examples of nonresponsiveness, if they in fact exist, would be with small peptide antigens that generate a homogenous response, $H\text{-}2^k$ mice that do or do not express the antisense GR transgene were immunized with the 81–104 C-terminal fragment of pigeon cytochrome *c* in complete Freund's adjuvant (F Lu and JD

Ashwell, manuscript in preparation). This peptide fragment is presented by I-E^k MHC-encoded molecules, and normally the large majority of responding T cells in these animals express TCRs containing Vα11 and Vβ3 (216). T cells were isolated from the draining lymph nodes of immunized mice and assayed for their response to restimulation. The proliferative response to anti-CD3 and to PPD (present in complete Freund's adjuvant) was virtually identical between the two groups. However, while the wild-type mice responded well to pigeon cytochrome *c* fragment 81–104, the TKO mice proliferated very poorly. Moreover, the fre-

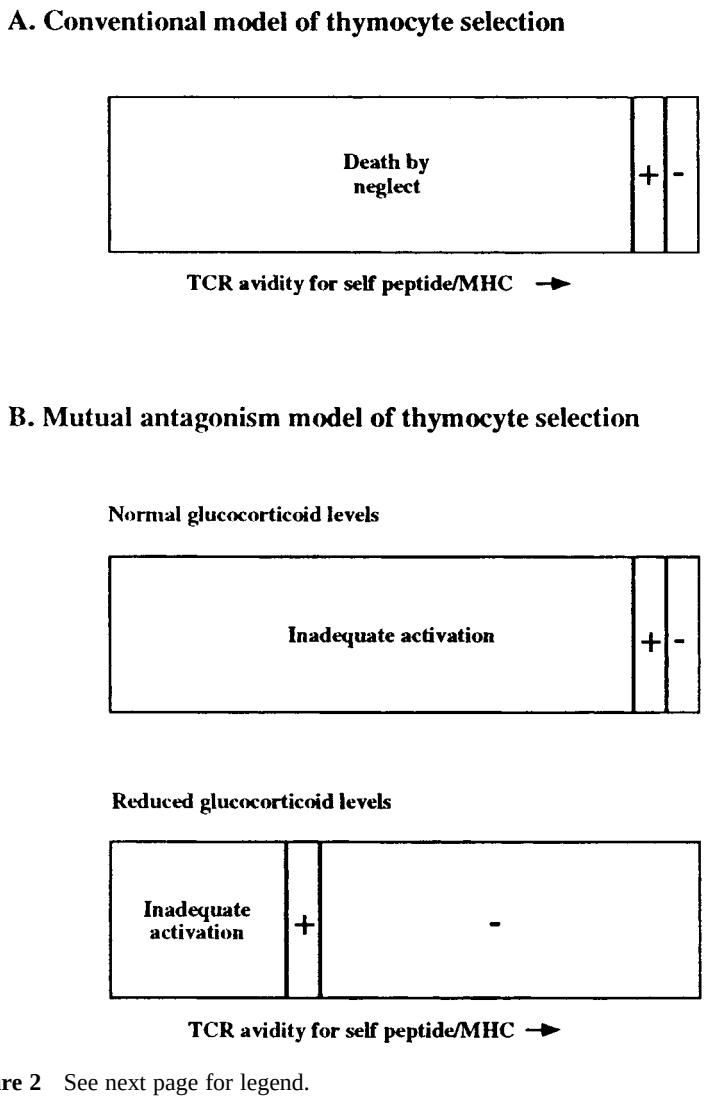

Figure 2 See next page for legend.

quency of Vα11 and Vβ3$^+$ T cells that responded to this antigen was markedly reduced in the TKO mice. It appears, therefore, that a responder animal has been converted to a nonresponder (or ''hyporesponder'') by a reduction in the expression of the GR during thymocyte development (recall that GR levels are normal in the peripheral T cells of TKO mice). Taken together, these data strongly support the prediction made by the mutual antagonism model that alterations in thymocyte glucocorticoid production or responsiveness will alter the antigen-specific T cell repertoire.

Implications

One of the more intriguing possible predictions of the mutual antagonism model is that increases in local glucocorticoid production and/or responsiveness might prevent thymocytes bearing TCRs with relatively high avidity for self-antigen/MHC from undergoing negative selection. These would represent untolerized cells that would provide a pool of potentially autoreactive T cells once in the periphery. At this time little evidence supports or militates against this possibility, but it is interesting to note that serum corticosterone levels are higher in autoimmune-prone mice than in normal animals (217) and that thymocytes from autoimmune obese chickens are resistant to glucocorticoid-induced apoptosis (218). It may be noteworthy in this regard that patients with the autoimmune disease multiple sclerosis also have significantly higher baseline plasma cortisol levels than do matched controls (219). Another interesting issue is the teleological reason for the involvement of corticosteroids in thymocyte selection. One possibility is that this evolved to deal with the problem posed by the elevation of systemic glucocorticoids that occurs during stress, including that incurred by acute infection. Given their potent immunosuppressive activity, it might be expected that

Figure 2 Proposed relationship between TCR avidity for self-antigen/MHC and thymocyte fate. (A) The conventional model in which the majority of thymocytes die by neglect (subthreshold biologically relevant avidity). Small numbers of cells with intermediate avidity are positively selected (+) and with high avidity are negatively (−) selected. (B) The mutual antagonism model. In a mouse with a full complement of MHC-encoded molecules, the large majority of thymocytes engage self-antigen/MHC with sufficient avidity to generate activating signals. In the presence of glucocorticoids (upper panel) most of these cells are inadequately activated to undergo positive selection and ultimately die in the thymus. As glucocorticoid levels are reduced (lower panel) their inhibition of activation is diminished, resulting in greater effective signal transduction at the same degree of receptor occupancy. Thus, occupancy of receptors that normally lead to only partial (inadequate) activation now lead to greater activation and either positive or negative selection. Note that this schematic does not address the question of whether fewer cells with very low avidity TCRs will die when glucocorticoid levels are reduced. Although there are data showing that in short-term FTOC thymocyte survival is enhanced by removing glucocorticoids, other factors that affect the viability of CD4$^+$CD8$^+$ thymocytes may keep this population relatively constant in vivo.

weakly or suboptimally activated T cells would be rendered useless under these conditions. The requirement that only thymocytes bearing TCRs with sufficient avidity to function in the presence of glucocorticoids (as evidenced by activation-induced positive selection) would help ensure that mature T cells would be capable of activation and expression of effector functions in the face of elevated glucocorticoid levels. It might be possible to explore such a possibility by challenging TKO mice with pathogens and assessing the robustness and efficacy of the T-dependent immune response.

CONCLUDING REMARKS

The anti-inflammatory and immunosuppressive effects of glucocorticoids are widely appreciated. What is less well known is that glucocorticoids influence T cells in a number of other, more subtle, ways. As detailed in this chapter, an extensive literature indicates that glucocorticoids are involved in Th lineage commitment, survival of activated T cells, and thymocyte development. Although much remains to be learned about molecular mechanisms, it is possible if not likely that all of these effects are mediated in large part by glucocorticoid-induced suppression of gene transcription. Fuller characterization and appreciation of the normal functions of glucocorticoids in vivo has been hampered by the multitude of effects glucocorticoids have on virtually all tissues, as well as the lack of a good GR-null model, since these animals die perinatally (220). It is to be expected, however, that the recent generation of viable mice with transcriptionally inactive GRs and, ultimately, mice with tissue-specific GR-deficiency will provide animal models that will make it possible to explore in more detail and with greater assurance the physiologic role for glucocorticoids in T cell development and the generation of the immune response.

ACKNOWLEDGMENTS

We are grateful to Barbara Osborne, Allan Weissman, and Georg Wick for critical review of this manuscript.

LITERATURE CITED

1. Mintami F, Shimizu T, Ueno R, Ishimura Y, Izumi S, Komatsu N, Watanabe K. 1982. Cytochrome P450–11β and P450scc in adrenal cortex: zonal distribution and intramitochondrial localization by the horseradish peroxidase-labeled antibody method. *J. Histochem. Cytochem.* 30:1066–74
2. Geuze HJ, Slot JW, Yanagibashi K, McCracken JA, Schwartz AL, Hall PF.

1987. Immunogold cytochemistry of cytochromes P-450 in porcine adrenal cortex. *Histochemistry* 86:551–57
3. Payne AH. 1990. Hormonal regulation of cytochrome P450 enzymes, cholesterol side-chain cleavage and 17α-hydroxylase/C17–20 lyase in Leydig cells. *Biol. Reprod.* 42:399–404
4. LeGoascogne C, Robel P, Gouezou M, Sananes N, Baulieu EE, Waterman M. 1987. Neurosteroids: cytochrome P-450scc in rat brain. *Science* 237:1212–15
5. Vacchio MS, Papadopoulos V, Ashwell JD. 1994. Steroid production in the thymus: implications for thymocyte selection. *J. Exp. Med.* 179:1835–46
6. Keeney DS, Jenkins CM, Waterman MR. 1995. Developmentally regulated expression of adrenal 17 alpha-hydroxylase cytochrome P450 in the mouse embryo. *Endocrinology* 136:4872–79
7. Meikle AW, Tyler FH. 1977. Potency and duration of action of glucocorticoids. Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *Am. J. Med.* 63:200–7
8. Vinson GP, Whitehouse B, Hinson J. 1992. *The Adrenal Cortex.* Englewood Cliffs, NJ: Prentice-Hall
9. Simpson ER, Waterman MR. 1988. Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. *Annu. Rev. Physiol.* 50:427–40
10. Vinson GP, Whitehouse BJ, Hinson JP. 1998. Structure and function of the adrenal cortex. In *Clinical Endocrinology,* ed. A Grossman, p. 395. London: Blackwell Sci.
11. Jones MT, Gillham B. 1988. Factors involved in the regulation of adrenocorticotropic hormone/β-lipotropic hormone. *Physiol. Rev.* 68:743–818
12. Beato M, Herrlich P, Schutz G. 1995. Steroid hormone receptors: many actors in search of a plot. *Cell* 83:851–57
13. Owens-Grillo JK, Hoffmann K, Hutchison KA, Yem AW, Deibel MR Jr, Handschumacher RE, Pratt WB. 1995. The cyclosporin A-binding immunophilin CyP-40 and the FK506-binding immunophilin hsp56 bind to a common site on hsp90 and exist in independent cytosolic heterocomplexes with the untransformed glucocorticoid receptor. *J. Biol. Chem.* 270:20479–84
14. Abraham N, Miceli MC, Parnes JR, Veillette A, Abbot S. 1991. Enhancement of T-cell responsiveness by the lymphocyte-specific tyrosine protein kinase p56*lck*. *Nature* 350:62–66
15. Beato M. 1991. Transcriptional control by nuclear receptors. *FASEB J.* 5:2044–51
16. Zhang G, Zhang L, Duff GW. 1997. A negative regulatory region containing a glucocorticosteroid response element (nGRE) in the human interleukin-1β gene. *DNA Cell Biol.* 16:145–52
17. Diamond MI, Miner JN, Yoshinaga SK, Yamamoto KR. 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* 249:1266–72
18. Shibata H, Spencer TE, Onate SA, Jenster G, Tsai SY, Tsai MJ, O'Malley BW. 1997. Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Prog. Horm. Res.* 52:141–64
19. Bannister AJ, Kouzarides T. 1996. The CBP co-activator is a histone acetyltransferase. *Nature* 384:641–43
20. Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM. 1997. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* 90:569–80
21. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* 87:953–59
22. Spencer TE, Jenster G, Burcin MM, Allis

CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ, O'Malley BW. 1997. Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* 389:194–98

23. Yang XJ, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y. 1996. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* 382:319–24
24. Wade PA, Pruss D, Wolffe AP. 1997. Histone acetylation: chromatin in action. *Trends Biochem. Sci.* 22:128–32
25. Bodwell JE, Orti E, Coull JM, Pappin DJ, Smith LI, Swift F. 1991. Identification of phosphorylated sites in the mouse glucocorticoid receptor. *J. Biol. Chem.* 266:7549–55
26. Bodwell JE, Webster JC, Jewell CM, Cidlowski JA, Hu JM, Munck A. 1998. Glucocorticoid receptor phosphorylation: overview, function and cell cycle-dependence. *J. Steroid Biochem. Mol. Biol.* 65:91–99
27. Krstic MD, Rogatsky I, Yamamoto KR, Garabedian MJ. 1997. Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol. Cell. Biol.* 17:3947–54
28. Weigel NL. 1996. Steroid hormone receptors and their regulation by phosphorylation. *Biochem. J.* 319:657–67
29. Mason SA, Housley PR. 1993. Site-directed mutagenesis of the phosphorylation sites in the mouse glucocorticoid receptor. *J. Biol. Chem.* 268:21501–4
30. Almlof T, Wright AP, Gustafsson JA. 1995. Role of acidic and phosphorylated residues in gene activation by the glucocorticoid receptor. *J. Biol. Chem.* 270:17535–40
31. Jewell CM, Webster JC, Burnstein KL, Sar M, Bodwell JE, Cidlowski JA. 1995. Immunocytochemical analysis of hormone mediated nuclear translocation of wild type and mutant glucocorticoid receptors. *J. Steroid Biochem. Mol. Biol.* 55:135–46
32. Webster JC, Jewell CM, Bodwell JE, Munck A, Sar M, Cidlowski JA. 1997. Mouse glucocorticoid receptor phosphorylation status influences multiple functions of the receptor protein. *J. Biol. Chem.* 272:9287–93
33. Bamberger CM, Bamberger AM, de Castro M, Chrousos GP. 1995. Glucocorticoid receptor β, a potential endogenous inhibitor of glucocorticoid action in humans. *J. Clin. Invest.* 95:2435–41
34. Oakley RH, Sar M, Cidlowski JA. 1996. The human glucocorticoid receptor β isoform. Expression, biochemical properties, and putative function. *J. Biol. Chem.* 271:9550–59
35. Leung DYM, Hamid Q, Vottero A, Szefler SJ, Surs W, Minshall E, Chrousos GP, Klemm DJ. 1997. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor β. *J. Exp. Med.* 186:1567–74
36. Schüle R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, Verma IM, Evans RM. 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 62:1217–26
37. Yang-Yen HF, Chambard JC, Sun YL, Smeal T, Schmidt TJ, Drouin J, Karin M. 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62:1205–15
38. Jonat C, Rahmsdorf HJ, Park K-K, Cato ACB, Gebel S, Ponta H, Herrlich P. 1990. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 62:1189–204
39. Zacharchuk CM, Ashwell JD. 1992. Fruitful outcomes of intracellular cross-talk. *Curr. Biol.* 2:246–48
40. Gottlicher M, Heck S, Herrlich P. 1998. Transcriptional cross-talk, the second

mode of steroid hormone receptor action. *J. Mol. Med.* 76:480–89
41. Stein B, Baldwin ASJ, Ballard DW, Greene WC, Angel P, Herrlich P. 1993. Cross-coupling of the NF-κB p65 and Fos/Jun transcription factors produces potentiated biological function. *EMBO J.* 12:3879–91
42. Ray A, Prefontaine KE. 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* 91:752–56
43. Caldenhoven E, Liden J, Wissink S, Van de Stolpe A, Raaijmakers J, Koenderman L, Okret S, Gustafsson JA, Van der Saag PT. 1995. Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol. Endocrinol.* 9:401–12
44. Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS Jr. 1995. Characterization of mechanisms involved in transrepression of NF-κB by activated glucocorticoid receptors. *Mol. Cell. Biol.* 15:943–53
45. Imai E, Miner JN, Mitchell JA, Yamamoto KR, Granner DK. 1993. Glucocorticoid receptor-cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids. *J. Biol. Chem.* 268:5353–56
46. Zhang Z, Jones S, Hagod JS, Fuentes NL, Fuller GM. 1997. STAT3 acts as a coactivator of glucocorticoid receptor singaling. *J. Biol. Chem.* 272:30607–10
47. Stocklin E, Wissler M, Gouilleux F, Groner B. 1996. Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* 383:726–28
48. Auphan N, Didonata JA, Rosette C, Hemberg A, Karin M. 1995. Immunosuppression by glucocorticoids: inhibition of NF-κB activity through induction of IκB synthesis. *Science* 270:286–90
49. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. 1995. Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. *Science* 270:283–86
50. Brostjan C, Anrather J, Csizmadia V, Stroka D, Soares M, Bach FH, Winkler H. 1996. Glucocorticoid-mediated repression of NFκB activity in endothelial cells does not involve induction of IκBα synthesis. *J. Biol. Chem.* 271: 19612–16
51. Heck S, Bender K, Kullmann M, Gottlicher M, Herrlich P, Cato AC. 1997. IκBα-independent downregulation of NF-κB activity by glucocorticoid receptor. *EMBO J.* 16:4698–707
52. De Bosscher K, Schmitz ML, Vanden Berghe W, Plaisance S, Fiers W, Haegeman G. 1997. Glucocorticoid-mediated repression of nuclear factor-κB-dependent transcription involves direct interference with transactivation. *Proc. Natl. Acad. Sci. USA* 94:13504–9
53. Wissink S, van Heerde EC, vand der Burg B, van der Saag PT. 1998. A dual mechanism mediates repression of NF-κB activity by glucocorticoids. *Mol. Endocrinol.* 12:355–63
54. Star P. 1895. An unusual case of Addison's disease; sudden death; remarks. *Lancet* 1:284
55. Boinet E. 1899. Recherches expérimetales sur les fonctions des capsules surrénales. *C.R. Seances Soc. Biol. Fil.* 51:671–74
56. Jaffe HL. 1924. The influence of the supradrenal gland on the thymus. I. Regeneration of the thymus following double supradrenalectomy in the rat. *J. Exp. Med.* 40:325–41
57. Jaffe HL. 1924. The influence of the suprarenal gland on the thymus. III. Stimulation of the growth of the thymus gland following double supradrenalectomy in young rats. *J. Exp. Med.* 40:753–59
58. Selye H. 1936. Thymus and adrenals in

the response of the organism to injuries and intoxication. *Br. J. Exp. Pathol.* 17:234–48
59. Dougherty TF, White A. 1943. Effect of pituitary adrenotropic hormone on lymphoid tissue. *Proc. Soc. Exp. Biol. Med.* 53:132–33
60. Heilman FR, Kendall EC. 1944. The influence of 11-dehydro-17-hydroxycorticosterone (compound E) on the growth of a malignant tumor in the mouse. *Endocrinology* 34:416–20
61. Mitchell IJ, Cooper AJ, Griffiths MR, Barber DJ. 1998. Phencyclidine and corticosteroids induce apoptosis of a subpopulation of striatal neurons: a neural substrate for psychosis? *Neuroscience* 84:489–501
62. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC. 1998. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *J. Clin. Invest.* 102:274–82
63. Brokaw JJ, White GW, Baluk P, Anderson GP, Umemoto EY, McDonald DM. 1998. Glucocorticoid-induced apoptosis of dendritic cells in the rat tracheal mucosa. *Am. J. Respir. Cell Mol. Biol.* 19:598–605
64. Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ. 1991. bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell* 67:879–88
65. Siegel RM, Katsumata M, Miyashita T, Louie DC, Greene MI, Reed JC. 1992. Inhibition of thymocyte apoptosis and negative antigenic selection in *bcl-2* transgenic mice. *Proc. Natl. Acad. Sci. USA* 89:7003–7
66. Grillot DA, Merino R, Nunez G. 1995. Bcl-XL displays restricted distribution during T cell development and inhibits multiple forms of apoptosis but not clonal deletion in transgenic mice. *J. Exp. Med.* 182:1973–83
67. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW. 1998. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* 94:739–50
68. Hakem R, Hakem A, Duncan G. S, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW. 1998. Differential requirement for caspase 9 in apoptotic pathways in vivo. *Cell* 94:339–52
69. Kuida K, Haydar TF, Kuan C. Y, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA. 1998. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* 94:325–37
70. Stefanelli C, Bonavita F, Stanic{\hacek} I, Farruggia G, Falcieri E, Robuffo I, Pignatti C, Muscari C, Rossoni C, Guarnieri C, Caldarera CM. 1997. ATP depletion inhibits glucocorticoid-induced thymocyte apoptosis. *Biochem. J.* 322:909–17
71. Thomas N, Edwards JL, Bell PA. 1983. Studies of the mechanism of glucocorticoid-induced pyknosis in isolated rat thymocytes. *J. Steroid Biochem.* 18:519–24
72. Cohen JJ, Duke RC. 1984. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J. Immunol.* 132:38–42
73. Perandones CE, Illera VA, Peckham D, Stunz LL, Ashman RF. 1993. Regulation of apoptosis in vitro in mature murine spleen T cells. *J. Immunol.* 151: 3521–29
74. Helmberg A, Auphan N, Caelles C, Karin M. 1995. Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. *EMBO J.* 14:452–60
75. Reichardt HM, Kaestner K. H, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schutz G. 1998. DNA binding of the glu-

cocorticoid receptor is not essential for survival. *Cell* 93:531–41
76. Harrigan MT, Baughman G, Campbell NF, Bourgeois S. 1989. Isolation and characterization of glucocorticoid- and cyclic AMP-induced genes in T lymphocytes. *Mol. Cell. Biol.* 9:3438–46
77. Baughman G, Harrigan MT, Campbell NF, Nurrish SJ, Bourgeois S. 1991. Genes newly identified as regulated by glucocorticoids in murine thymocytes. *Mol. Endocrinol.* 5:637–44
78. Baughman G, Lesley J, Trotter J, Hyman R, Bourgeois S. 1992. Tcl-30, a new T cell-specific gene expressed in immature glucocorticoid-sensitive thymocytes. *J. Immunol.* 149:1488–96
79. Owens GP, Hahn WE, Cohen JJ. 1991. Identification of mRNAs associated with programmed cell death in immature thymocytes. *Mol. Cell. Biol.* 11:4177–88
80. Goldstone SD, Lavin MF. 1991. Isolation of a cDNA clone, encoding a human β-galactoside binding protein, overexpressed during glucocorticoid-induced cell death. *Biochem. Biophys. Res. Commun.* 178:746–50
81. Memon SA, Petrak D, Moreno MB, Zacharchuk CM. 1995. A simple assay for examining the effect of transiently expressed genes on programmed cell death. *J. Immunol. Methods* 180:15–24
82. Deveraux QL, Takahashi R, Salvesen GS, Reed JC . 1997. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 388:300–4
83. Duckett CS, Li F, Wang Y, Tomaselli KJ, Thompson CB, Armstrong RC. 1998. Human IAP-like protein regulates programmed cell death downstream of Bcl-xL and cytochrome c. *Mol. Cell. Biol.* 18:608–15
84. Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC. 1998. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J.* 17:2215–23
85. Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. 1997. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J.* 16:6914–25
86. Robey E, Chang D, Itano A, Cado D, Alexander H, Lans D, Weinmaster G, Salmon P. 1996. An activated form of Notch influences the choice between CD4 and CD8 T cell lineages. *Cell* 87:483–92
87. Washburn T, Schweighoffer E, Gridley T, Chang D, Fowlkes BJ, Cado D, Robey E. 1997. Notch activity influences the αβ versus γδ T cell lineage decision. *Cell* 88:833–43
88. Hasserjian RP, Aster JC, Davi F, Weinberg DS, Sklar J. 1996. Modulated expression of notch1 during thymocyte development. *Blood* 88:970–76
89. Deftos ML, He YW, Ojala EW, Bevan MJ. 1998. Correlating notch signaling with thymocyte maturation. *Immunity* 9:777–86
90. Kullmann M, Schneikert J, Moll J, Heck S, Zeiner M, Gehring U, Cato AC. 1998. RAP46 is a negative regulator of glucocorticoid receptor action and hormone-induced apoptosis. *J. Biol. Chem.* 273:14620–25
91. Grimm LM, Goldberg AL, Poirier GG, Schwartz LM, Osborne BA. 1996. Proteasomes play an essential role in thymocyte apoptosis. *EMBO J.* 15:3835–44
92. Zacharchuk CM, Mercep M, Chakraborti P, Simons SS Jr, Ashwell JD. 1990. Programmed T lymphocyte death: cell activation- and steroid-induced pathways are mutually antagonistic. *J. Immunol.* 145:4037–45
93. Iwata M, Hanaoka S, Sato K. 1991. Rescue of thymocytes and T cell hybridomas from glucocorticoid-induced apoptosis by stimulation via the T cell receptor/CD3 complex: a possible in vitro model

for positive selection of the T cell repertoire. *Eur. J. Immunol.* 21:643–48
94. Wagner DH Jr, Hagman J, Linsley PS, Hodsdon W, Freed JH, Newell MK. 1996. Rescue of thymocytes from glucocorticoid-induced cell death mediated by CD28/CTLA-4 costimulatory interactions with B7–1/B7–2. *J. Exp. Med.* 184:1631–38
95. Gruber J, Sgonc R, Hu YH, Beug H, Wick G. 1994. Thymocyte apoptosis induced by elevated endogenous corticosterone levels. *Eur. J. Immunol.* 24:1115–21
96. Ayala A, Herdon CD, Lehman DL, DeMaso CM, Ayala CA, Chaudry IH. 1995. The induction of accelerated thymic programmed cell death during polymicrobial sepsis: control by corticosteroids but not tumor necrosis factor. *Shock* 3:259–67
97. Tarcic N, Ovadia H, Weiss DW, Weidenfeld J. 1998. Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids. *J. Neuroimmunol.* 82:40–46
98. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. 1991. BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc. Natl. Acad. Sci. USA* 88:6961–65
99. Nakayama K, Nakayama K, Negishi I, Kuida K, Shinkai Y, Louie MC, Fields LE, Lucas PJ, Stewart V, Alt FW, Loh DY. 1993. Disappearance of the lymphoid system in Bcl-2 homozygous mutant chimeric mice. *Science* 261: 1584–88
100. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. 1993. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75:229–40
101. Henning SJ. 1978. Plasma concentrations of total and free corticosterone during development in the rat. *Am. J. Physiol.* 235:E451–56
102. Savu L, Zouaghi H, Nunez EA. 1985. Serum inflammatory responses of transcortin binding activities and of total and free corticosterone and progesterone levels in developing rats: a kinetic approach. *Int. J. Tissue React.* 7:443–48
103. Lanza L, Scudeletti M, Puppo F, Bosco O, Peirano L, Filaci G, Fecarotta E, Vidali G, Indiveri F. 1996. Prednisone increases apoptosis in in vitro activated human peripheral blood T lymphocytes. *Clin. Exp. Immunol.* 103:482–90
104. Horigome A, Hirano T, Oka K, Takeuchi H, Sakurai E, Kozaki K, Matsuno N, Nagao T, Kozaki M. 1997. Glucocorticoids and cyclosporine induce apoptosis in mitogen-activated human peripheral mononuclear cells. *Immunopharmacology* 37:87–94
105. Crabtree GR, Munck A, Smith KA. 1980. Glucocorticoids and lymphocytes. I. Increased glucocorticoid receptor levels in antigen-stimulated lymphocytes. *J. Immunol.* 124:2430–35
106. Clarke A. R, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH. 1993. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 362:849–52
107. Lowe SW, Schmitt E. M, Smith SW, Osborne BA, Jacks T. 1993. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 362:847–49
108. Hench PS, Kendall EC, Slocumb CH, Polley HF. 1950. Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions. *Arch. Int. Med.* 85:545–66
109. Plotz CM. 1975. Corticosteroids as immunosuppressive drugs: a brief perspective. *Transplant. Proc.* 7:5–6
110. Billingham RE, Krohn BA, Medawar PB. 1951. Effect of cortisone on survival of skin homografts in rabbits. *Br. Med. J.* 1:1157–63
111. Medawar PB, Sparrow EM. 1956. The

effects of adrenocortical hormones, adrenocorticotrophic hormone and pregnancy on skin transplantation immunity in mice. *J. Endocrinol.* 14:240–56
112. Crabtree GR, Gillis S, Smith KA, Munck A. 1980. Mechanisms of glucocorticoid-induced immunosuppression: inhibitory effects on expression of Fc receptors and production of T-cell growth factor. *J Steroid Biochem* 12:445–49
113. Almawi WY, Beyhum HN, Rahme AA, Rieder MJ. 1996. Regulation of cytokine and cytokine receptor expression by glucocorticoids. *J. Leukoc. Biol.* 60:563–72
114. Kunicka JE, Talle MA, Denhardt GH, Brown M, Prince LA, Goldstein G. 1993. Immunosuppression by glucocorticoids: inhibition of production of multiple lymphokines by in vivo administration of dexamethasone. *Cell. Immunol.* 149:39–49
115. Fushimi T, Shimura S, Suzuki S, Saitoh H, Okayama H, Shirato K. 1998. Suppression of gene expression and production of interleukin 13 by dexamethasone in human peripheral blood mononuclear cells. *Tohoku J. Exp. Med.* 185:157–60
116. Rolfe FG, Hughes JM, Armour CL, Sewell WA. 1992. Inhibition of interleukin-5 gene expression by dexamethasone. *Immunology* 77:494–99
117. Northrop JP, Crabtree GR, Mattila PS. 1992. Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. *J. Exp. Med.* 175:1235–45
118. Vacca A, Felli MP, Farina AR, Martinotti S, Maroder M, Screpanti I, Meco D, Petrangeli E, Frati L, Gulino A. 1992. Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. *J. Exp. Med.* 175:637–46
119. Paliogianni F, Raptis A, Ahuja SS, Najjar SM, Boumpas DT. 1993. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-ATJ. *Clin. Invest.* 91:1481–89
120. Granelli-Piperno A, Nolan P, Inaba K, Steinman RM. 1990. The effect of immunosuppressive agents on the induction of nuclear factors that bind to sites on the interleukin 2 promoter. *J. Exp. Med.* 172:1869–72
121. Adcock IM, Brown C. R, Gelder CM, Shirasaki H, Peters MJ, Barnes PJ. 1995. Effects of glucocorticoids on transcription factor activation in human peripheral blood mononuclear cells. *Am. J. Physiol.* 268:C331–38
122. Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF. 1992. Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts. *Blood* 79:45–51
123. Re F, Muzio M, De Rossi M, Polentarutti N, Giri J. G, Mantovani A, Colotta F. 1994. The type II ''receptor'' as a decoy target for interleukin 1 in polymorphonuclear leukocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *J. Exp. Med.* 179:739–43
124. Levine SJ, Benfield T, Shelhamer JH. 1996. Corticosteroids induce intracellular interleukin-1 receptor antagonist type I expression by a human airway epithelial cell line. *Am. J. Respir. Cell Mol. Biol.* 15:245–51
125. Lamas M, Sanz E, Martin-Parras L, Espel E, Sperisen P, Collins M, Silva AG. 1993. Glucocorticoid hormones upregulate interleukin 2 receptor a gene expression. *Cell. Immunol.* 151:437–50
126. Fernandez-Ruiz E, Rebollo A, Nieto MA, Sanz E, Somoza C, Ramirez F, Lopez-Rivas A, Silva A. 1989. IL-2 protects T cell hybrids from the cytolytic effect of glucocorticoids. Synergistic effect of IL-2 and dexamethasone in the induction of high-affinity IL-2 receptors. *J. Immunol.* 143:4146–51

127. Wiegers GJ, Labeur MS, Stec IE, Klinkert WE, Holsboer F, Reul JM. 1995. Glucocorticoids accelerate anti-T cell receptor-induced T cell growth. *J. Immunol.* 155:1893–902
128. Boumpas DT, Anastassiou ED, Older SA, Tsokos GC, Nelson DL, Balow JE. 1991. Dexamethasone inhibits human interleukin 2 but not interleukin 2 receptor gene expression in vitro at the level of nuclear transcription. *J. Clin. Invest.* 87:1739–47
129. Batuman OA, Ferrero AP, Diaz A, Berger B, Pomerantz RJ. 1994. Glucocorticoid-mediated inhibition of interleukin-2 receptor-α and -β subunit expression by human T cells. *Immunopharmacology* 27:43–55
130. Paliogianni F, Ahuja SS, Balow JP, Balow JE, Boumpas DT. 1993. Novel mechanism for inhibition of human T cells by glucocorticoids. Glucocorticoids inhibit signal transduction through IL-2 receptor. *J. Immunol.* 151:4081–89
131. Louahed J, Renauld JC, Demoulin JB, Baughman G, Bourgeois S, Sugamura K, Van Snick J. 1996. Differential activity of dexamethasone on IL-2-, IL-4-, or IL-9-induced proliferation of murine factor-dependent T cell lines. *J. Immunol.* 156:3704–10
132. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissmann G. 1992. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc. Natl. Acad. Sci. USA* 89:9991–95
133. Bischof F, Melms A. 1998. Glucocorticoids inhibit CD40 ligand expression of peripheral $CD4^+$ lymphocytes. *Cell. Immunol.* 187:38–44
134. Wargnier A, Lafaurie C, Legros-Maida S, Bourge JF, Sigaux F, Sasportes M, Paul P. 1998. Down-regulation of human granzyme B expression by glucocorticoids. Dexamethasone inhibits binding to the *ikaros* and *ap-1* regulatory elements of the *granzyme b* promoter. *J. Biol. Chem.* 273:35326–31
135. Baus E, Andris F, Dubois PM, Urbain J, Leo O. 1996. Dexamethasone inhibits the early steps of antigen receptor signaling in activated T lymphocytes. *J. Immunol.* 156:4555–61
136. Mosmann TR, Sad S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* 17:138–46
137. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 283:1183–86
138. Daynes RA, Araneo BA. 1989. Contrasting effects of glucocorticoids on the capacity of T cells to produce the growth factors interleukin 2 and interleukin 4. *Eur. J. Immunol.* 19:2319–25
139. Sierra-Honigmann MR, Murphy PA. 1992. T cell receptor-independent immunosuppression induced by dexamethasone in murine T helper cells. *J. Clin. Invest.* 89:556–60
140. Bertoglio JH, Leroux E. 1988. Differential effects of glucocorticoids on the proliferation of a murine helper and a cytolytic T cell clone in response to IL-2 and IL-4. *J. Immunol.* 141:1191–96
141. Ramirez F, Fowell DJ, Puklavec M, Simmonds S, Mason D. 1996. Glucocorticoids promote a Th2 cytokine response by $CD4^+$ T cells in vitro. *J. Immunol.* 156:2406–12
142. Dozmorov IM, Miller RA. 1998. Generation of antigen-specific Th2 cells from unprimed mice in vitro: effects of dexamethasone and anti-IL-10 antibody. *J. Immunol.* 160:2700–5
143. Posey WC, Nelson HS, Branch B, Pearlman DS. 1978. The effects of acute corticosteroid therapy for asthma on serum immunoglobulin levels. *J. Allergy Clin. Immunol.* 62:340–48

144. Jabara HH, Ahern DJ, Vercelli D, Geha RS. 1991. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. *J. Immunol.* 147:1557–60
145. Wu CY, Sarfati M, Heusser C, Fournier S, Rubio-Trujillo M, Peleman R, Delespesse G. 1991. Glucocorticoids increase the synthesis of immunoglobulin E by interleukin 4-stimulated human lymphocytes. *J. Clin. Invest.* 87:870–77
146. Bohle B, Willheim M, Baier K, Stadler B, Spitzauer S, Scheiner O, Kraft D, Ebner C. 1995. Hydrocortisone enhances total IgE levels—but not the synthesis of allergen-specific IgE—in a monocyte-dependent manner. *Clin. Exp. Immunol.* 101:474–79
147. Wu CY, Fargeas C, Nakajima T, Delespesse G. 1991. Glucocorticoids suppress the production of interleukin 4 by human lymphocytes. *Eur. J. Immunol.* 21:2645–47
148. Wang Y, Campbell HD, Young IG. 1993. Sex hormones and dexamethasone modulate interleukin-5 gene expression in T lymphocytes. *J. Steroid Biochem. Mol. Biol.* 44:203–10
149. Franchimont D, Louis E, Dewe W, Martens H, Vrindts-Gevaert Y, De Groote D, Belaiche J, Geenen V. 1998. Effects of dexamethasone on the profile of cytokine secretion in human whole blood cell cultures. *Regul. Pept.* 73:59–65
150. Visser J, van Boxel-Dezaire A, Methorst D, Brunt T, de Kloet ER, Nagelkerken L. 1998. Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. *Blood* 91:4255–64
151. Ramirez F. 1998. Glucocorticoids induce a Th2 response in vitro. *Dev. Immunol.* 6:233–43
152. Ashwell JD, Cunningham RE, Noguchi PD, Hernandez D. 1987. Cell growth cycle block of T cell hybridomas upon activation with antigen. *J. Exp. Med.* 165:173–94
153. Mercep M, Bluestone JA, Noguchi PD, Ashwell JD. 1988. Inhibition of transformed T cell growth in vitro by monoclonal antibodies directed against distinct activating molecules. *J. Immunol.* 140:324–35
154. Ucker DS, Ashwell JD, Nickas G. 1989. Activation driven T cell death. I. Requirements for de novo transcription and translation and association with genome fragmentation. *J. Immunol.* 143:3461–69
155. Russell JH, White CL, Loh DY, Meleedy-Rey P. 1991. Receptor-stimulated death pathway is opened by antigen in mature T cells. *Proc. Natl. Acad. Sci. USA* 88:2151–55
156. Ucker DS, Meyers J, Obermiller PS. 1992. Activation-driven T cell death. II. Quantitative differences alone distinguish stimuli triggering nontransformed T cell proliferation or death. *J. Immunol.* 149:1583–92
157. Wesselborg S, Janssen O, Kabelitz D. 1993. Induction of activation-driven death (apoptosis) in activated but not resting peripheral blood T cells. *J. Immunol.* 150:4338–45
158. Dhein J, Walczak H, Bäumler C, Debatin K.-M, Krammer PH. 1995. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* 373:438–41
159. Brunner T, Mogil RJ, LaFace D, Yoo NJ, Mahboubi A, Echeverri F, Martin SJ, Force WR, Lynch DH, Ware CF, Green DR. 1995. Cell-autonomous Fas CD95 / Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 373:441–44
160. Ju S-T, Panka DJ, Cui H, Ettinger R, El-Khatib M, Sherr DH, Stanger BZ, Marshak-Rothstein A. 1995. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 373:444–48
161. Yang Y, Mercep M, Ware CF, Ashwell JD. 1995. Fas and activation-induced Fas ligand mediate apoptosis of T cell hybridomas: inhibition of Fas ligand

expression by retinoic acid and glucocorticoids. *J. Exp. Med.* 181:1673–82
162. D'Adamio F, Zollo O, Moraca R, Ayroldi E, Bruscoli S, Bartoli A, Cannarile L, Migliorati G, Riccardi C. 1997. A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death. *Immunity* 7:803–12
163. Latinis KM, Norian LA, Eliason SL, Koretzky GA. 1997. Two NFAT transcription factor binding sites participate in the regulation of CD95 (Fas) ligand expression in activated human T cells. *J. Biol. Chem.* 272:31427–34
164. Holtz-Heppelmann CJ, Algeciras A, Badley AD, Paya CV. 1998. Transcriptional regulation of the human FasL promoter-enhancer region. *J. Biol. Chem.* 273:4416–23
165. Mittelstadt PR, Ashwell JD. 1999. Role of Egr-2 in up-regulation of Fas ligand in normal T cells and aberrant double-negative lpr and gld T cells. *J. Biol. Chem.* 274:3222–27
166. Mittelstadt PR, Ashwell JD. 1998. The cyclosporin A-sensitive transcription factor Egr-3 regulates Fas ligand expression. *Mol. Cell. Biol.* 18:3744–51
167. Matsui K, Fine A, Zhu B, Marshak-Rothstein A, Ju ST. 1998. Identification of two NF-κB sites in mouse CD95 ligand (Fas ligand) promoter: functional analysis in T cell hybridoma. *J. Immunol.* 161:3469–73
168. Kasibhatla S, Genestier L, Green DR. 1999. Regulation of fas-ligand expression during activation-induced cell death in T lymphocytes via NF-κB. *J. Biol. Chem.* 274:987–92
169. Mages HW, Baag R, Steiner B, Kroczek RA. 1998. Utilization of an NF-ATp binding promoter element for EGR3 expression in T cells but not fibroblasts provides a molecular model for the lymphoid cell-specific effect of cyclosporin A. *Mol. Cell. Biol.* 18:7157–65
170. Rengarajan J, Mittelstadt PR, Mages HW, Gerth A, Kroczek R, Ashwell JD, Glimcher LH. 1999. Sequential involvement of NFAT and Egr transcription factors in Th1-specific FasL regulation. Submitted.
171. Nocentini G, Giunchi L, Ronchetti S, Krausz LT, Bartoli A, Moraca R, Migliorati G, Riccardi C. 1997. A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc. Natl. Acad. Sci. USA* 94:6216–21
172. Gurney AL, Marsters SA, Huang RM, Pitti RM, Mark DT, Baldwin DT, Gray AM, Dowd AD, Brush AD, Heldens AD, Schow AD, Goddard AD, Wood WI, Baker KP, Godowski PJ, Ashkenazi A. 1999. Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR. *Curr. Biol.* 9:215–18
173. Kwon B, Yu KY, Ni J, Yu GL, Jang IK, Kim YJ, Xing L, Liu D, Wang SX, Kwon BS. 1999. Identification of a novel activation-inducible protein of the tumor necrosis factor receptor superfamily and its ligand. *J. Biol. Chem.* 274:6056–61
174. Liu ZG, Smith SW, McLaughlin KA, Schwartz LM, Osborne BA. 1994. Apoptotic signals delivered through the T-cell receptor of a T-cell hybrid require the immediate-early gene nur77. *Nature* 367:281–84
175. Woronicz JD, Calnan B, Ngo V, Winoto A. 1994. Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas. *Nature* 367:277–81
176. Weih F, Ryseck RP, Chen L, Bravo R. 1996. Apoptosis of *nur77*/N10-transgenic thymocytes involves the Fas/Fas ligand pathway. *Proc. Natl. Acad. Sci. USA* 93:5533–38
177. Philips A, Maira M, Mullick A, Chamberland M, Lesage S, Hugo P, Drouin J. 1997. Antagonism between Nur77 and glucocorticoid receptor for control of transcription. *Mol. Cell. Biol.* 17:5952–59

178. Philips A, Lesage S, Gingras R, Maira MH, Gauthier Y, Hugo P, Drouin J. 1997. Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells. *Mol. Cell. Biol.* 17:5946–51
179. Pelfrey CM, Tranquill LR, Boehme SA, McFarland HF, Lenardo MJ. 1995. Two mechanisms of antigen-specific apoptosis of myelin basic protein (MBP)-specific T lymphocytes derived from multiple sclerosis patients and normal individuals. *J. Immunol.* 1548:6191–202
180. Lu W, Salerno-Goncalves R, Yuan J, Sylvie D, Han DS, Andrieu JM. 1995. Glucocorticoids rescue $CD4^+$ T lymphocytes from activation-induced apoptosis triggered by HIV-1: implications for pathogenesis and therapy. *AIDS* 9:35–42
181. Bäumler CB, Böhler T, Herr I, Benner A, Krammer PH, Debatin K-M. 1996. Activation of the CD95 (APO-1/Fas) system in T cells from human immunodeficiency virus type-1-infected children. *Blood* 88:1741–46
182. Yang Y, Liu Z-H, Ware CF, Ashwell JD. 1997. A cysteine protease inhibitor prevents activation-induced T cell apoptosis and death of peripheral blood cells from HIV-infected individuals by inhibiting upregulation of Fas ligand. *Blood* 89:550–57
183. van Oers NS, von Boehmer H, Weiss A. 1995. The pre-T cell receptor (TCR) complex is functionally coupled to the TCR-ζ subunit. *J. Exp. Med.* 182:1585–90
184. Kisielow P, von Boehmer H. 1995. Development and selection of T cells: facts and puzzles. *Adv. Immunol.* 58:87–209
185. Huesmann M, Scott B, Kisielow P, von Boehmer H. 1991. Kinetics and efficacy of positive selection in the thymus of normal and T cell receptor transgenic mice. *Cell* 66:533–40
186. Singer GG, Abbas AK. 1994. The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity* 1:365–71
187. Page DM, Roberts EM, Peschon JJ, Hedrick SM. 1998. TNF receptor-deficient mice reveal striking differences between several models of thymocyte negative selection. *J. Immunol.* 160:120–33
188. Gravestein LA, Borst J. 1998. Tumor necrosis factor receptor family members in the immune system. *Semin. Immunol.* 10:423–34
189. Alberola-Ila J, Hogquist KA, Swan KA, Bevan MJ, Perlmutter RM. 1996. Positive and negative selection invoke distinct signaling pathways. *J. Exp. Med.* 184:9–18
190. O'Shea CC, Crompton T, Rosewell IR, Hayday AC, Owen MJ. 1996. Raf regulates positive selection. *Eur. J. Immunol.* 26:2350–55
191. Urdahl KB, Pardoll DM, Jenkins MK. 1994. Cyclosporin A inhibits positive selection and delays negative selection in $\alpha\beta$ TCR transgenic mice. *J. Immunol.* 152:2853–59
192. Zacharchuk CM, Mercep, M, Ashwell, JD. 1991. Thymocyte activation and death: a mechanism for molding the T cell repertoire. In *Antigen and Clone-Specific Immunoregulation*, ed. RL Edelson, p. 52. New York: Ann. NY Acad. Sci.
193. Noel PJ, Boise LH, Green JM, Thompson CB. 1996. CD28 costimulation prevents cell death during primary T cell activation. *J. Immunol.* 157:636–42
194. Ballard PL. 1979. Glucocorticoids and differentiation. In *Glucocorticoid Hormone Action*, ed. JD Baxter, GG Rousseau, p. 493. Berlin: Springer-Verlag
195. Murphy BEP, Diez d'Aux RC. 1972. Steroid levels in the human fetus: cortisol and cortisone. *J. Clin. Endocrinol. Metab.* 35:678–83
196. Lechner O, Wiegers GJ, Oliveira-dos-Santos AJ, Dietrich H, Recheis H, Waterman M, Boyd R, Wick G. 1999.

Glucocorticoid production in the murine thymus. *Eur. J. Immunol.* In press
197. Pazirandeh A, Xue Y, Rafter I, Sjovall J, Jondal M, Okret S. 1999. Paracrine glucocorticoid activity produced by mouse thymic epithelial cells. *FASEB J.* 13: 893–901
198. Kisielow P, Teh HS, Blüthmann H, von Boehmer H. 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature* 335:730–33
199. Teh HS, Kisielow P, Scott B, Kishi H, Uematsu Y, Blüthmann H, von Boehmer H. 1988. Thymic major histocompatibility complex antigens and the αβ T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature* 335:229–33
200. Vacchio MS, Ashwell JD. 1997. Thymus-derived glucocorticoids regulate antigen-specific positive selection. *J. Exp. Med.* 185:2033–38
201. Sacedon R, Varas A, Jimenez E, Munoz JJ, Vicente A, Zapata AG. 1999. Accelerated maturation of the thymic stroma in the progeny of adrenalectomized pregnant rats. *Neuroimmunomodulation* 6:23–30
202. Garvin AM, Abraham KM, Forbush KA, Farr AG, Davison BL, Perlmutter RM. 1990. Disruption of thymocyte development and lymphomagenesis induced by SV40 T-antigen. *Int. Immunol.* 2:173–80
203. King LB, Vacchio MS, Dixon K, Hunziker R, Margulies DH, Ashwell JD. 1995. A targeted glucocorticoid receptor antisense transgene increases thymocyte apoptosis and alters thymocyte development. *Immunity* 3:647–56
204. Pepin MC, Pothier F, Barden N. 1992. Impaired type II glucocorticoid-receptor function in mice bearing antisense RNA transgene. *Nature* 355:725–28
205. Morale MC, Batticane N, Gallo F, Barden N, Marchetti B. 1995. Disruption of hypothalamic-pituitary-adrenocortical system in transgenic mice expressing type II glucocorticoid receptor antisense ribonucleic acid permanently impairs T cell function: effects on T cell trafficking and T cell responsiveness during postnatal development. *Endocrinology* 136: 3949–60
206. Vacchio MS, Lee JYM, Ashwell JD. 1999. Thymus-derived glucocorticoids set the thresholds for thymocyte selection by inhibiting TCR-mediated thymocyte activation. *J. Immunol.* 163:1327–33
207. Shortman K, Egerton M, Spangrude GJ, Scollay R. 1990. The generation and fate of thymocytes. *Semin. Immunol.* 2:3–12
208. Zerrahn J, Held W, Raulet DH. 1997. The MHC reactivity of the T cell repertoire prior to positive and negative selection. *Cell* 88:627–36
209. Merkenschlager M, Graf D, Lovatt M, Bommhardt U, Zamoyska R, Fisher AG. 1997. How many thymocytes audition for selection? *J. Exp. Med.* 186:1149–58
210. Vidovic D, Boulanger N, Kuye O, Toral J, Ito K, Guenot J, Bluethmann H, Nagy ZA. 1997. The helper T-cell repertoire of mice expressing class II major histocompatibility complex β chains in the absence of a chains. *Immunogenetics* 45:325–35
211. Dutz JP, Ong CJ, Marth J, Teh HS. 1995. Distinct differentiative stages of $CD4^+CD8^+$ thymocyte development defined by the lack of coreceptor binding in positive selection. *J. Immunol.* 154: 2588–99
212. Tolosa E, King LB, Ashwell JD. 1998. Thymocyte glucocorticoid resistance alters positive selection and inhibits autoimmunity and lymphoproliferative disease in MRL-lpr/lpr mice. *Immunity* 8:67–76
213. Mountz JD, Zhou T, Eldridge J, Berry K, Blüthmann H. 1990. Transgenic rearranged T cell receptor gene inhibits lymphadenopathy and accumulation of $CD4^-CD8^-B220^+$ T cells in *lpr*/*lpr* mice. *J. Exp. Med.* 172:1805–17
214. Perkins DL, Listman JA, Marshak-Rothstein A, Kozlow W, Kelley VR, Finn PW,

Rimm IJ. 1996. Restriction of the TCR repertoire inhibits the development of memory T cells and prevents autoimmunity in lpr mice. *J. Immunol.* 156:4961–68

215. Peng SL, Fatenejad S, Craft J. 1996. Induction of nonpathologic, humoral autoimmunity in lupus-prone mice by a class II-restricted, transgenic αβ T cell. Separation of autoantigen-specific and -nonspecific help. *J. Immunol.* 157: 5225–30
216. Davis MM, McHeyzer-Williams M, Chien YH. 1995. T-cell receptor V-region usage and antigen specificity. The cytochrome c model system. *Ann. NY Acad. Sci.* 756:1–11
217. Lechner O, Hu Y, Jafarian-Tehrani M, Dietrich H, Schwarz S, Herold M, Haour F, Wick G. 1996. Disturbed immunoendocrine communication via the hypothalamo-pituitary-adrenal axis in murine lupus. *Brain Behav. Immun.* 10:337–50
218. Wick G, Sgonc R, Lechner O. 1998. Neuroendocrine-immune disturbances in animal models with spontaneous autoimmune diseases. *Ann. NY Acad. Sci.* 840:591–98
219. Michelson D, Stone L, Galliven E, Magiakou MA, Chrousos GP, Sternberg EM, Gold PW. 1994. Multiple sclerosis is associated with alterations in hypothalamic-pituitary-adrenal axis function. *J. Clin. Endocrinol. Metab.* 79:848–83
220. Cole TJ, Blendy JA, Monaghan AP, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K, Schütz G. 1995. Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. *Genes Dev.* 9:1608–21

Note Added in Proof

We referred to an article as “in preparation” on page 330. It has been accepted, and this is the current citation: Lu, F. W. M., Yasutomo, K., McHeyzer-Williams, L. J., McHeyzer-Williams, M. G., Goodman, G., Germain, R. N., and Ashwell, J. D. 2000. Thymocyte resistance to glucocorticoids leads to antigen-specific unresponsiveness due to “holes” in the T cell repertoire. *Immunity.* In press.