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Thymus-Derived Glucocorticoids Set the Thresholds for Thymocyte Selection by Inhibiting TCR-Mediated Thymocyte Activation

Melanie S. Vacchio,* Jan Y. M. Lee,* and Jonathan D. Ashwell†

Selection processes in the thymus eliminate nonfunctional or harmful T cells and allow the survival of those T cells with the potential to recognize Ag in association with self-MHC-encoded molecules (Ag/MHC). We have previously demonstrated that thymus-derived glucocorticoids antagonize TCR-mediated deletion, suggesting a role for endogenous thymic glucocorticoids in promoting survival of thymocytes following TCR engagement. Consistent with this hypothesis, we now show that inhibition of thymus glucocorticoid biosynthesis causes an increase in thymocyte apoptosis and a decrease in recovery that are directly proportional to the number of MHC-encoded molecules present and, therefore, the number of ligands available for TCR recognition. Expression of CD5 on CD4+CD8+ thymocytes, an indicator of TCR-mediated activation, increased in a TCR- and MHC-dependent manner when corticosteroid production or responsiveness was decreased. These results indicate that thymus-derived glucocorticoids determine where the window of thymocyte selection occurs in the TCR avidity spectrum by dampening the biological consequences of TCR occupancy and reveal that glucocorticoids mask the high percentage of self-Ag/MHC-reactive thymocytes that exist in the preselection repertoire. The Journal of Immunology, 1999, 163: 1327–1333.

Selection processes in the thymus eliminate nonfunctional or harmful T cells and allow the survival of those T cells with the potential to recognize Ag in association with self-MHC-encoded molecules (Ag/MHC).2 Thymocytes that express TCRs with high avidity for Ag/MHC undergo apoptosis (negative selection), while thymocytes that have not successfully rearranged their TCR genes or that express a receptor with subthreshold avidity for self Ag/MHC enter a default apoptosis pathway (death by neglect) (1, 2). Thymocytes bearing TCRs with moderate avidity for self Ag/MHC are rescued from the default death pathway and differentiate into mature T cells (positive selection). The molecular mechanism underlying the rescue of cells with moderate avidity and the death of cells with high avidity for self Ag/MHC is not known.

We have previously shown that the thymic epithelium produces steroids and have identified a role for endogenously produced glucocorticoids in thymocyte development. Analysis of transgenic mice that express antisense transcripts to the 3′-untranslated region of the glucocorticoid receptor (GR) in thymocytes, and which therefore have reduced GR levels and are hyporesponsive to glucocorticoids, revealed that glucocorticoids are critical at least two points during thymocyte development: progression from the CD4−CD8− to the CD4+CD8+ stage of development and maintenance of viability at the CD4+CD8+ stage (3). Moreover, addition of metyrapone, a selective inhibitor of corticosteroid synthesis (4), to thymic organ culture of TCR αβ transgenic mice resulted in the specific apoptotic death of cells that would otherwise undergo positive selection (5, 6). These findings indicate that under normal conditions endogenous glucocorticoids prevent thymocyte apoptosis when the TCR binds self Ag/MHC with sufficient avidity to result in positive selection. Importantly, inhibition of glucocorticoid synthesis did not cause a decrease in cell recovery from thymi expressing nonselecting MHC-encoded molecules, demonstrating that TCR occupancy is necessary for cell death under these conditions.

In this report we have assessed the role of glucocorticoids in thymocyte development in nontransgenic mice. Unexpectedly, inhibition of glucocorticoid biosynthesis caused an increase in thymocyte apoptosis, a decrease in cell recovery, and an increase in cell surface markers of activation that was αβ TCR-dependent and directly proportional to the number of MHC-encoded molecules present. Moreover, in the absence of TCR ligands, inhibition of corticosteroid production actually enhanced thymocyte survival. These results demonstrate that the fraction of thymocytes that recognize self Ag/MHC with biologically significant avidity is large and form the basis of a model in which glucocorticoids inhibit activation (and therefore both positive and negative selection) of thymocytes in vivo.

Materials and Methods

Mice and reagents

C57BL/6 (B6), C57BL/10 (B10), B10.BR, and B10.D2 mice were obtained from the Developmental Therapeutics Program, National Cancer Institute (Frederick, MD), and were used to generate timed pregnant animals. Males were removed from females after 16 h, and the day of separation was counted as day 0 of gestation. Mice in which β2m expression has been eliminated by homologous recombination (β2m−/−) were bred in our facility and had been backcrossed to C57BL/6 for at least six generations (7). C57BL/6 TCRαΔ50 transgenic mice (B6.TCRαΔ50; 8) were obtained from The Jackson Laboratory (Bar Harbor, ME). Fetal thymic organ cultures (FTOC) were performed in serum-free Nutrizoma-SP medium (Boehringer Mannheim, Indianapolis, IN) supplemented with 20 mM HEPES, 100 μM nonessential amino acids, 1 mM sodium pyruvate, and 50 μM 2-ME. Metyrapone was purchased from ICN Biochemicals (Costa Mesa, CA). The

*Experimental Immunology Branch and †Laboratory of Immune Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

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1 Address correspondence and reprint requests to Dr. Jonathan Ashwell, Room 1B-40, Building 10, National Institutes of Health, Bethesda, MD 20892. E-mail address: jda@bxv.jnh.gov

2 Abbreviations used in this paper: Ag/MHC, Ag in association with self-MHC-encoded molecules; GR, glucocorticoid receptor; FTOC, fetal thymic organ culture; DP, double positive.
Abs Y3P (anti-I-\(A^b\)) (9) and MKD6 (anti-I-\(A^b\)) (10) were purified by affinity chromatography. Abs used for flow cytometry, anti-CD4, anti-CD8, anti-TCR, anti-MHC, anti-CD5, and anti-CD69, were purchased from PharMingen (San Diego, CA).

**Fetal thymic organ cultures**

FTOC were performed as described (5). Fetal day 17 thymi were separated into lobes and cultured with 200–225 \(\mu\)g/ml of freshly diluted metyrapone or ethanol as a control on a Millipore filter (Bedford, MA) floating on a Gelfoam sponge in complete serum-free Nutridoma-SP medium (Boehringer Mannheim). Metyrapone, a selective inhibitor of the enzyme \(P_{450c11}\), blocks the conversion of biologically inactive deoxycorticosterone to the active form, corticosterone (4). In some experiments 10\(^{-9}\) M corticosterone, diluted in ethanol, was added. Cultures were conducted in either 1.5 ml of medium/well in 12-well plates or 3 ml of medium in six-well plates. Abs, 30 \(\mu\)g of Y3P (anti-I-\(A^b\)) or MKD6 (anti-I-\(A^b\)), or PBS as a control were added to cultures by dropping directly onto the thymi. Lobes were harvested after either 24 h (for TUNEL assay) or 3 days of culture, and single cell suspensions were prepared for counting and analyzed for CD4, CD8, TCR, CD5, CD69, and MHC expression by flow cytometry.

**TUNEL assay**

A modified form of the TUNEL assay (11) was used to detect fragmented DNA in apoptotic thymocytes (3). Briefly, thymocytes were incubated with anti-CD4-Red 670- and anti-CD8-PE-labeled Abs before formaldehyde fixation and permeabilization with 0.1% Triton X-100/0.1% sodium citrate, then subjected to a modified in situ nick translation using fluorescein-dUTP (Boehringer Mannheim). Labeled cells were visualized by flow cytometry with a FACSscan. To determine the amount of apoptosis in the double-positive (DP) population, thymocytes were gated for expression of both CD4 and CD8 and analyzed for dUTP-FITC incorporation. The percent specific apoptosis was calculated by the formula: Percent specific apoptosis = \(\frac{\text{[experimental apoptosis - spontaneous apoptosis]}}{100 - \text{spontaneous apoptosis}}\) \(\times 100\).

**Results**

**Inhibition of corticosteroid production in FTOC decreases T cell recovery**

Blockade of glucocorticoid synthesis in FTOC of thymi from TCR \(\alpha\beta\) transgenic mice has been shown to result in the death of thymocytes that normally undergo positive selection (6). To assess the contribution of endogenously produced glucocorticoids to the survival of thymocytes with an unrestricted range of TCR Ag specificities, we performed FTOC with B10 fetal day 17 thymi, which consist largely of TCR \(\alpha\beta\) CD4\(^+\)CD8\(^+\) DP thymocytes, cultured in medium alone or with metyrapone to inhibit corticosterone production. After 3 days the thymi were harvested, counted, and evaluated for expression of CD4 and CD8. Culture in medium alone resulted in the recovery of 74.7 \(\pm\) 12 \(\times\) 10\(^4\) DP thymocytes/lobe, whereas 38.9 \(\pm\) 6.0 \(\times\) 10\(^4\) DP thymocytes/lobe were recovered from thymi cultured in the presence of metyrapone (a 52% decrease; Fig. 1). The decrease in DP thymocytes was reflected in decreased numbers of mature CD4\(^+\) thymocytes that developed in these cultures (14.9 \(\pm\) 2.8 \(\times\) 10\(^4\) vs 8.5 \(\pm\) 0.7 \(\times\) 10\(^4\)). The number of CD4\(^+\)CD8\(^+\) thymocytes was similar in the two groups (10.6 \(\pm\) 2.5 \(\times\) 10\(^4\) vs 8.5 \(\pm\) 1.5 \(\times\) 10\(^4\)). As we have previously noted, replacement of corticosterone with physiologic levels (10\(^{-9}\) M) of free corticosterone largely reversed the effect of metyrapone (63.4 \(\pm\) 9.7 \(\times\) 10\(^4\) DP thymocytes/lobe).

**Effect of inhibiting glucocorticoid production on thymocyte recovery depends on the number of MHC-encoded molecules expressed**

If the decrease in cell recovery caused by the inhibition of endogenous glucocorticoid production was in fact due to TCR occupancy unopposed by glucocorticoid signaling, one would expect that cell loss under these conditions would be reduced by decreasing the number of available ligands (and thus TCR occupancy). It is possible to vary the number of MHC-encoded molecules available for Ag presentation by using C57BL H-2 congenic strains of mice. The level of MHC expression reflects gene copy number, and it has been shown that both MHC class I- and class II-mediated positive and negative selection are sensitive to MHC gene dosage (12–15). MHC-congenic animals differ in the number of different MHC-encoded molecules they express and, therefore, in the quantity and array of potential TCR ligands. That is, as the number of MHC molecules increases, so will the concentration of a given Ag/MHC TCR ligand. Moreover, as the number of different MHC molecules increases, so will the number of unique Ag/MHC complexes. B10.D2 (H-2\(^d\)) mice express the full complement of MHC-encoded molecules, with three class I gene products (K, D, and L) and two MHC class II gene products (I-A and I-E) (16). H-2\(^b\) haplotype (B10.BR) mice do not express an L allele (17) and therefore have four distinct MHC molecules. B10 (H-2\(^b\)) mice also do not express an L allele and in addition have a deletion in the 5’ portion of the gene encoding the \(\alpha\)-chain of the I-E molecule, so that the only MHC class II molecule they express is encoded by I-A (18). Therefore, the number of different MHC-encoded molecules expressed by these otherwise genetically identical animals varies from three (B10) to five (B10.D2). FTOC of day 17 thymi from these mice was performed in the absence or the presence of metyrapone. To compare the results from multiple independent experiments, the data are expressed as thymocyte recovery in the presence of metyrapone compared with that observed with B10 thymi, which were included in each experiment. There was indeed a hierarchy of DP thymocyte loss when corticosterone biosynthesis was inhibited; the relative effect was B10.D2 (H-2\(^d\), L\(^{d^+}\), I-E\(^{b}\)) > B10.BR (H-2\(^b\), L\(^{d^+}\), I-E\(^{b}\)) > B10 (H-2\(^b\), L\(^{d^+}\), I-E\(^{b}\)) (Fig. 2). To further restrict the diversity of TCR ligands, thymi were tested from H-2\(^d\) mice in which the \(\beta_m\) locus has been disrupted by homologous recombination (\(\beta_m^{+/1}\)) and which therefore do not express MHC class I molecules (7, 19). For these thymi, which express only a single MHC-encoded molecule (I-A), the decrease in cell recovery was substantially less than that observed with B10 thymi. Therefore, cell loss due to inhibition of glucocorticoid synthesis varies directly with the number of MHC-encoded molecules expressed.
Apoptosis caused by inhibiting glucocorticoid production is MHC dependent

To determine whether the decrease in cell recovery caused by inhibition of glucocorticoid synthesis was due to enhanced cell death, apoptosis of DP thymocytes in FTOC was determined. After 24 h of culture in the absence or the presence of metyrapone, thymi were harvested, and thymocyte apoptosis was determined by flow cytometry using a modified TUNEL assay to detect nicked or fragmented DNA (6). Culture in metyrapone reproducibly induced substantial apoptosis in B10.D2 thymi, while having only a small effect in $\beta_{2m^{-/-}}$ thymi (a representative experiment is shown in Fig. 3A). Specific apoptosis was determined by subtracting the percentage of TUNEL$^+$ thymocytes in the medium control from that observed in cultures with metyrapone. On the average, specific apoptosis of DP thymocytes induced by metyrapone in the $\beta_{2m^{-/-}}$ thymi was 2.2% ($\pm$0.6%), whereas in B10 thymi it was 5.4% ($\pm$1.2%). Metyrapone-induced specific apoptosis of DP cells was even greater in B10.D2 thymi (13.3 $\pm$ 2.8%). Therefore, just as for cell recovery, induction of cell death by inhibition of glucocorticoid production varied in direct proportion to the number of different MHC-encoded molecules. To eliminate any possibility of TCR occupancy by conventional Ag/MHC ligands, day 17 fetal thymi from H-2$^b$ B10 mice were cultured with or without metyrapone in medium alone or in medium containing mAbs to I-A$^b$ (expressed by B10 mice) or I-A$^d$ (an irrelevant Ab control; Fig. 4). Culture for 3 days in metyrapone resulted in recovery of 76% of the cells compared with culture in medium alone. Addition of anti-I-A$^d$ to metyrapone cultures had no effect (81% recovery). In contrast, when the only classic MHC-encoded molecule, I-A$^b$, was blocked with Ab, metyrapone caused no decrease in thymocyte recovery. In fact, there was a reproducible increase in thymocyte recovery (134% of medium alone). Therefore, in the absence of TCR ligands, prevention of glucocorticoid biosynthesis did not decrease and, in fact, enhanced, thymocyte survival.

FIGURE 2. Effect of MHC-encoded molecule diversity on DP thymocyte recovery in the absence of glucocorticoids. Fetal day 17 thymi from B10, B10.BR, B10.D2, and $\beta_{2m^{-/-}}$ mice that had been backcrossed to C57BL/10 for at least six generations were separated into lobes and cultured in pairs for 3 days with or without 225 $\mu$g/ml of metyrapone. The recovery of CD4$^+$CD8$^+$ thymocytes in the presence of metyrapone was calculated for $\beta_{2m^{-/-}}$ (n = 5–9/experiment), B10.BR (n = 2–9/experiment), and B10.D2 (n = 4–5/experiment) and then expressed relative to the recovery of B10 DP thymocytes (n = 4–9/experiment), given a value of 1, in that experiment. The mean recovery of B10 thymocytes from FTOC with metyrapone was 59.3% ($\pm$7.7%) that of recovery in medium alone.

FIGURE 3. Effect of MHC-encoded molecule diversity on DP thymocyte apoptosis in the absence of glucocorticoids. Fetal day 17 thymi from B10, B10.D2, and $\beta_{2m^{-/-}}$ mice were separated into lobes and cultured in pairs for 24 h with or without 225 $\mu$g/ml of metyrapone, then analyzed for TUNEL positivity and CD4/CD8 expression. A, Representative profiles from B10.D2 and $\beta_{2m^{-/-}}$ thymi cultured in either medium or metyrapone, gated on CD4 and CD8 expression, then analyzed for incorporation of dUTP-FITC in the TUNEL assay. B, Specific apoptosis of DP thymocytes ($\beta_{2m^{-/-}}, n = 20$; B10, $n = 19$; B10.D2, $n = 26$).

FIGURE 4. Blockade of glucocorticoid synthesis enhances thymocyte recovery in the absence of TCR ligands. Fetal day 17 thymi from H-2$^b$ $\beta_{2m^{-/-}}$ mice were cultured with 225 $\mu$g/ml metyrapone or an ethanol control for 3 days in medium alone (n = 30), 10 $\mu$g/ml Y3P (anti-I-A$^b$; n = 18), or 10 $\mu$g/ml of a control Ab, MKD6 (anti-I-A$^d$; n = 11). The recovery of CD4$^+$CD8$^+$ thymocytes was calculated for each treatment group, and the fractional change caused by culture with metyrapone is shown.
Removal of glucocorticoid effects reveals a large population of self-reactive thymocytes

Although the relationship between MHC expression and the effect of corticosteroid withdrawal on thymocyte survival indicated that glucocorticoids play an important role in the survival of cells recognizing self Ag/MHC, the actual number of cells affected by metyrapone was larger than would be predicted based upon the number of CD4<sup>+</sup>CD8<sup>−</sup> cells that normally undergo positive selection (estimated to be 3–4%) (20). Rather, the observation that TCR engagement by self Ag/MHC appears to result in substantial apoptotic death and decreased cell recovery when glucocorticoid production is inhibited suggests that a large number (>50%) of thymocytes express TCRs with biologically relevant avidity for self Ag/MHC. An independent means of testing this is to measure cell surface levels of CD5, a molecule whose expression is dependent upon TCR-mediated signaling and whose up-regulation on immature thymocytes is an early and sensitive marker of even low (non-selecting) avidity TCR/ligand interactions (21, 22). Two different methods were used to assess the effect of diminishing glucocorticoid signaling on DP thymocyte activation. First, thymi from β2m<sup>−/−</sup> (one MHC molecule) and B10.A mice (five MHC molecules) were compared (Fig. 5A). As previously noted, CD5 levels increase on DP, but not on CD4<sup>+</sup>CD8<sup>−</sup> thymocytes as a function of MHC expression, presumably reflecting low avidity TCR interactions (22). Fetal thymi from β2m<sup>−/−</sup> and B10.A mice were cultured with or without metyrapone, and CD5 expression was determined 18 h later (Fig. 5B). The low level of CD5 expression on β2m<sup>−/−</sup> DP thymocytes did not appreciably change in the presence of metyrapone, indicating that inhibiting glucocorticoid production has little effect on CD5 expression when there is minimal TCR occupancy. In contrast, CD5 levels on B10.A DP thymocytes increased; 40% in the presence of metyrapone (CD5 mean fluorescence intensities were 1037 ± 118 in medium (n = 12) and 1448 ± 105 with metyrapone (n = 6)). In other experiments, up-regulation of CD5 on DP thymocytes was found as early as 8 h after adding metyrapone, a time at which there was no detectable apoptosis, demonstrating that the increase in CD5 expression was not the result of selective survival of a thymocyte subpopulation (data not shown). To determine whether the increase in CD5 expression did indeed depend upon TCR occupancy, a comparison

![FIGURE 5. Inhibition of glucocorticoid synthesis results in enhanced CD5 expression on DP thymocytes. A, Flow cytometric analysis of CD5 expression on DP (gate R1) and CD4<sup>+</sup>CD8<sup>−</sup> (gate R2) thymocytes from 3- to 6-wk-old β2m<sup>−/−</sup> and B10.A mice. One representative experiment of five is shown. B, Fetal day 17 thymi from B10.A and β2m<sup>−/−</sup> mice were cultured for 24 h with or without 225 μg/ml of metyrapone, then analyzed for CD5, CD8, and TUNEL positivity. Representative profiles from B10.A and β2m<sup>−/−</sup> thymi cultured in either medium or metyrapone, gated on CD8<sup>+</sup> TUNEL<sup>−</sup> cells, were analyzed for CD5 expression. C, Fetal day 17 thymi from B6 (medium, n = 4; metyrapone, n = 5) or B6.TCRα<sup>−/−</sup> (medium, n = 3; metyrapone, n = 4) mice were cultured for 24 h with or without metyrapone and analyzed for CD5 expression as in B. Profiles representative of the average mean fluorescence intensity for each group are shown.](http://www.jimmunol.org/)

Cmetyrapone for 24 h had no effect on DP thymocyte CD5 expres-

sponded to at least one of the eight MHC haplotypes tested. Based

sponding to allogeneic MHC. More than 30% of the cells re-

II molecule, similar to the frequency of control hybridomas re-

discussion demonstrates that in the absence of thymus-
derived steroids participate in the death of thymocytes ex-

pressing receptors with very low avidity for self Ag/MHC.

was made between cells from B6 thymi and those from thymi from

B6 mice lacking TCRα (B6.TCRα−−/−). Thymocytes from these

animals express a pre-TCR, which allows them to progress to the

DP stage of development, but do not undergo selection because

they lack an αβ TCR (8). Treatment of TCRα−−/− thymi with

metyrapone for 24 h had no effect on DP thymocyte CD5 expres-

sion (Fig. 5C). In contrast, metyrapone reproducibly increased the

levels of CD5 on DP thymocytes from TCRα-expressing mice, dem-

onstrating that a TCR capable of recognizing Ag/MHC must be

present for a decrease in glucocorticoids to cause thymocyte

activation. For an independent approach to this issue, DP thymo-
cytes from transgenic mice expressing antisense GR transcri-

pts, and thus hyporesponsive to glucocorticoids (3), were analyzed for

CD5 expression (Fig. 6). As with inhibition of glucocorticoid syn-

thesis, expression of the GR antisense transgene resulted in up-

regulation of CD5 expression on CD4+CD8+ thymocytes. It is

interesting that the CD5 level on all DP thymocytes increased

when glucocorticoid synthesis or responsiveness was inhibited,
suggesting that TCRs on all thymocytes may have at least some
degree of biologically significant avidity for any MHC-encoded
molecule regardless of the specific peptide Ag it binds. Together,
these results demonstrate that reduction in endogenous glucocor-

ticoid production or responsiveness to glucocorticoids leads to an

increase in TCR-mediated activation of DP cells by endogenous

Ag/MHC.

Discussion

The data in this report demonstrate that in the absence of thymus-
derived glucocorticoids there is a direct relationship between de-

letion of DP thymocytes and the number of different MHC-en-
coded molecules expressed. A particularly interesting observation

is that under conditions in which thymocytes could not encounter

MHC/peptide ligands, inhibition of corticosteroid production ac-
tually increased thymocyte recovery. This suggests that locally

produced corticosterone, just as corticosterone produced by the

adrenals (23), causes immature thymocytes to die and implies that

thymus-derived steroids participate in the death of thymocytes ex-

pressing receptors with very low avidity for self Ag/MHC.

The magnitude of the effect of eliminating glucocorticoid production

on thymocyte survival in the presence of MHC-encoded molecules

raises another, quantitative, issue: how much of the preselection

TCR repertoire is capable of binding self Ag/MHC with biologi-

cally relevant avidity? It was long thought that only a small num-

ber of thymocytes express TCRs with biologically relevant avidity

for self Ag/MHC (that is, bear receptors capable of transducing

activating signals). Quantitative analyses of thymocyte turnover

suggest that only a small proportion of thymocytes (3–4%) survive

these selection processes and leave the thymus (19, 24). Further-

more, examination of tissue sections for apoptotic cells revealed no

obvious difference between thymi from mice that did and those

that did not express MHC-encoded molecules (25). These results

have been thought to indicate that only a small number of thymo-
cytes express TCRs that recognize self Ag/MHC with sufficient

avidity to trigger biological responses.

This issue has recently been revisited by a number of groups

using different experimental approaches, and the consensus is that

a sizable fraction of the preselection TCR repertoire is, in fact,
capable of recognizing Ag/MHC. For example, addition of a single

peptide Ag to cultured thymi from TAP1−/− mice (which cannot

themselves generate self peptides that bind to MHC class I mol-

ecules) caused the generation of a small, but measurable, popula-

tion of CD4+CD8− cells (26), as did the addition of β2m and a

single peptide to cultures of β2m−/− thymi (27). In both cases,
mixtures of peptides were more efficient than single peptides.

Another approach that analyzed the anti-MHC class II repertoire was
to generate transgenic mice that expressed only a single MHC

class II molecule, I-Αβ, covalently bound to an antigenic peptide

(28). This resulted in positive selection of a relatively large number

of CD4+ thymocytes. The peripheral CD4+ T cells in these ani-

mals had a diverse repertoire, as assessed by Vβ expression, and a

very high percentage of T cell hybridomas generated from these

cells responded to I-Αβ plus unknown self peptides or allogeneic

MHC-encoded molecules. The authors concluded that the TCR

repertoire is inherently biased toward MHC molecules, and that a

relatively large number of thymocytes do, in fact, recognize self

peptides/MHC with biologically significant avidity. They sugges-
ted that these cells are not apparent in normal animals after

positive selection on a low to moderate avidity peptide/MHC com-
bination because they are subsequently deleted on a cross-reactive,

but higher avidity, ligand. T cell reactivity has also been studied in

mice that lack the I-A α-chain and therefore express only an ab-

normal MHC class II molecule consisting of an I-Αβ homodimer

(29). The finding that T cells from these mice, which developed in

the absence of MHC class II αβ heterodimers, nonetheless had a

high frequency of cells that responded to normal allogeneic class

II molecules was taken to mean that the germline TCR repertoire

has been selected for MHC reactivity.

Several recent studies that attempted to directly analyze the pre-

selection TCR repertoire. In one case, CD4− T cell hybridomas

were made from anti-TCR-stimulated thymi from MHC class I/II-
negative mice (30). The frequency of MHC-reactive hybridomas

was high, with 4.4% of the cells responding to a given MHC class

II molecule, similar to the frequency of control hybridomas re-

sponding to allogeneic MHC. More than 30% of the cells re-

sponded to at least one of the eight MHC haplotypes tested. Based

upon these results and the assumption that anti-MHC class I cells

should arise with similar frequency, it was estimated that 20–30% of

thymocytes in an outbred mouse might be autoreactive. In an-

other study, the frequency of preselection thymocytes capable of

FIGURE 6. CD5 expression is increased on DP thymocytes that are

hyporesponsive to glucocorticoids. Representative profiles in which thymi

B6 mice with and without a GR antisense transgene are shown.

Cell Number

FIGURE 6. CD5 expression is increased on DP thymocytes that are

hyporesponsive to glucocorticoids. Representative profiles in which thymi

from 3- to 6-wk-old B6 and B6.TKO mice were analyzed for CD4, CD8,

and CD5 expression. CD5 profiles of DP thymocytes (R1 gate, upper pan-

els) are shown in the lower panel.
responding to MHC was determined using a short term thymic reaggregate culture system (22). It was found that 24 h after exposure to MHC+ stroma, 15% of thymocytes expressed CD69. Using CD5 as a measure of activation, it was reported that 11% of cells responded to I-A2, 10% responded to H-2Kb and Db, and 19% responded when both were present. Furthermore, while CD5 was not expressed on fresh DP thymocytes from mice lacking MHC molecules, over 20% of DP cells from mice expressing either MHC class I or I-A2 were positive for CD5, and it was mentioned that up to 50% were CD5+ in mice expressing the MHC class II I-E molecule.

The data in the present study indicate that glucocorticoids interfere with the activation of thymocytes with low, but biologically relevant, avidity for self Ag/MHC, as reflected in lowered levels of CD5. The up-regulation of CD5 by metyrapone did not occur in the absence of a mature TCR, indicating that signaling via CD4 or CD8 alone cannot account for this effect. However, the data do not rule out a supplementary role for signaling through these molecules, either independent of the TCR signal or, more likely, in their capacity as TCR coreceptors. Glucocorticoids are well known to potently antagonize activation of mature T cells and inhibit transcriptional activation of, among other gene products, cytokines such as IL-1, IL-2, IL-4, and IFN-γ (31). There is a variety of distinct mechanisms by which glucocorticoids may mediate these effects (reviewed in Ref. 32). The occupied GR may bind to a response element in the regulatory region of a target gene and repress transcription. Alternatively, the GR may interfere, by direct or indirect physical interactions, with the activity of other transcription factors such as AP-1. It has also been found that glucocorticoids up-regulate the expression of IkB, sequestering NF-κB in the cytosol and decreasing its transcriptional activity (33, 34), although direct interactions between the GR and NF-κB p65 may also contribute to this inhibition (35, 36). Yet another mechanism for inhibitory cross-talk between the GR and a host of other transcription factors is competition for coactivators of gene transcription such as p300/CBP (37). Finally, it has been reported that glucocorticoids may inhibit some proximal events in TCR signaling, such as phosphatidylinositol turnover and Ca2+ flux (38).

The conventional model of thymocyte selection holds that the large majority of thymocytes die in the thymus because their TCRs fail to bind self Ags/MHC with biologically significant avidity (2). Only a small fraction of thymocytes bind self ligands with sufficient avidity to either be rescued from this default “neglect” death pathway or be induced to undergo activation-induced apoptosis. As detailed above, it is becoming clear that a substantial fraction of thymocytes do, in fact, recognize self Ag/MHC with sufficient avidity to receive activation signals, although only a small number actually traverse the entire process to become mature SP thymocytes. To accommodate this and our results with glucocorticoids, we suggest the following relationship between TCR avidity for self Ag/MHC and cell fate. In this model, as with the conventional model, thymocytes bearing TCRs with high avidity are negatively selected, those with intermediate avidity are positively selected. There is a large additional population of cells, however, with TCRs that recognize self Ag/MHC with low, but biologically significant, avidity (sufficient to cause up-regulation of CD5, for example). The activation of all thymocytes in all TCR avidity groups is inhibited by glucocorticoids. In the case of cells with intermediate avidity, that recognize self Ag/MHC with low, but biologically significant, avidity (sufficient to cause up-regulation of CD5, for example), glucocorticoids levels decrease, the biological consequences of receptor occupancy are enhanced, as reflected by increased expression of CD5 and CD69, so that thymocytes signaled via lower avidity interactions in the absence of glucocorticoids respond like thymocytes signaled via higher avidity interactions in the presence of glucocorticoids. As a result, thymocytes that would be positively selected in the presence of glucocorticoids become negatively selected in their absence, and cells with low avidity that would normally be inadequately activated, and thus be lost, would progress to the SP stage of development. In vivo, the ultimate consequence of this would be the selection of T cells whose receptors would, on the average, have lower avidity for self Ag/MHC than the normally selected repertoire.

This model is consistent with a variety of observations. Inhibition of glucocorticoid production in FTOC makes DP thymocytes exquisitely sensitive to anti-TCR-mediated deletion (5). In a more physiological setting, lack of thymus-derived corticosteroids results in the TCR-mediated deletion of cells that are otherwise positively selected because they recognize self Ag/MHC with intermediate avidity (6). Finally, reduction in thymocyte GR levels, which renders them hyporesponsive to glucocorticoids, results in loss of MHC-dependent positive selection of T cells bearing particular Vβs and markedly reduces the autoimmunity and lymphoproliferative disease of MRL-lpr/lpr mice (39). Thus, the effect of glucocorticoids on thymocyte development can be viewed as analogous to their effect on peripheral T cells. They act as immunosuppressants by preventing expression of activation-induced genes. In the case of peripheral T cells, this activity results in inhibition of effector function. In the case of thymocytes, this activity determines what range of TCR avidities for self Ag/MHC will fall in the positive selection window (i.e., affects development). It is an interesting speculation that thymic production of corticosteroids may have evolved to ensure that T cells surviving selection have sufficient avidity for self MHC to respond to Ags even in the face of stress-induced glucocorticoid levels, such as occur during acute illness.

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