

Dysregulation of T-Cell Development in Adrenal Glucocorticoid-Deprived Rats

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A number of different experimental approaches have been used to elucidate the impact of basal levels of adrenal gland-derived glucocorticoids (GCs) on T cell development, and thereby T cell-mediated immune responses. However, the relevance of the adrenal GCs to T cell development is still far from clear. This study was undertaken to explore the relevance of basal levels of GCs to T cell differentiation/maturation. Eight days post-adrenalectomy in adult male rats the thymocyte yield, apoptotic and proliferative rate and the relationship amongst major thymocyte subsets, as defined by TCR $\alpha\beta$ /CD4/CD8 expression, were examined using flow cytometry. Adrenal GC deprivation decreased thymocyte apoptosis and altered the kinetics of T cell differentiation/maturation. In the adrenalectomized rats there was increased thymic hypercellularity and an over-representation of the CD4+CD8+ double positive (DP) TCR $\alpha\beta^{\text{low}}$ cells entering selection, as well as increased numbers of their DP TCR $\alpha\beta^-$ immediate precursors. These changes were accompanied with under-representation of the postselected DP TCR $\alpha\beta^{\text{high}}$ and the most mature CD4-CD8+ and, particularly, CD4+CD8-single positive (SP) TCR $\alpha\beta^{\text{high}}$ cells. This data suggests that withdrawal of adrenal GCs produces alterations in the thymocyte selection processes, possibly affecting the diversity of functional T cell repertoire and generation of potentially self-reactive cells as indicated by the reduced proportion and number of CD4-CD8- double negative TCR $\alpha\beta^{\text{high}}$ cells. In addition, it indicates that GCs influence the post-selection maturation of thymocytes and plays a regulatory role in controlling the ratio of mature CD4+CD8-/CD4-CD8+ SP TCR $\alpha\beta^{\text{high}}$ cells. *Exp Biol Med* 234:1067–1074, 2009

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Introduction

Considerable evidence has established the occurrence and relevance of the reciprocal regulation of the immune and endocrine systems to sustain a state of homeostasis (1, 2). A pivotal role in this regulatory communication has been ascribed to glucocorticoids (GCs). They belong to a class of steroid hormones that are synthesized by the adrenal gland and released in response to inflammatory and stress stimuli (3). Their secretion is under the control of hypothalamo-pituitary-adrenal (H-P-A) axis feedback mechanisms. Once in the circulation, GCs exert pleiotropic effects ranging from the regulation of energy metabolism and the control of cognitive functions, to the modulation of the immune system (3). These hormones can diffuse easily across the plasma membrane, and once in the cytosol they can bind to their respective cytoplasmic receptors. Ligand-bound receptors can dimerize and translocate to the nucleus of the cell to promote or prevent transcription of target genes (4, 5).

It has been well established that synthetic GCs at pharmacological doses exhibit immunosuppressive action. Accordingly, they are used in therapy of various immunological disorders (6–8). Impairment of GC action, due to changes at the level of organs of the H-P-A axis, or at the level of the GC receptor (GR), has been associated with dysregulation of the immune response (8–10). In contrast, it is believed that at physiological concentrations the endogenous GCs have modulatory rather than outright suppressive effects on the immune system (8), thus implicating them in the regulation of both innate and adaptive immunity.

T lymphocytes orchestrate adaptive immune responses that clear pathogens from infected hosts. The modulatory influence of GCs on adaptive immunity has been partly related to their thymic actions. Thymus is the primary lymphoid organ providing immunocompetent T lymphocytes

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generated from bone marrow-derived CD4-CD8- double-negative (DN) T-cell receptor (TCR)⁺ progenitors through a series of differentiation and selection steps (11). Briefly, these cells rearrange TCR β gene locus, and the cells which successfully rearrange this gene locus express a pre-TCR complex consisting of CD3, and the nonpolymorphic pre- α and β chains (11). Subsequent to pre-TCR surface expression, thymocytes divide rapidly, acquire CD4 and CD8 molecules, and undergo TCR α gene rearrangement. Those cells that generate a functional α chain, replace the pre-TCR with mature TCR $\alpha\beta$, thereby becoming CD4+CD8+ double positive (DP) TCR $\alpha\beta^{\text{low}}$ cells (12). These cells interact with major histocompatibility complex (MHC) molecules with bound self-peptides on the thymic non-lymphoid cells. The cells that do not recognize the MHC molecules that are present in the thymus undergo a default death pathway, i.e. "death by neglect". The destiny of cells that express a TCR $\alpha\beta$ able to interact with the MHC/self-peptide complexes depends on the nature of TCR $\alpha\beta$ signal. Thymocytes receiving a strong TCR $\alpha\beta$ signal undergo activation-induced apoptosis, a major mechanism for promoting self-tolerance (negative selection). Those receiving a TCR $\alpha\beta$ signal of intermediate strength continue to differentiate (positive selection) into CD4+CD8- or CD8+CD4- TCR $\alpha\beta^{\text{high}}$ single-positive (SP) cells that complete their maturation in the thymic medulla and migrate into the periphery, forming the peripheral T cell repertoire (13, 14).

For over a century it has been known that adrenal insufficiency in humans leads to thymic hypertrophy (15). Moreover, it has been shown that: i) adrenalectomy in experimental animals results in thymic hypertrophy that cannot be reversed by adrenalin, produced by the adrenal medulla (16–19), and ii) adrenalectomy prevents stress and drug-induced involution of the thymus (19). Although the ability of GCs to induce apoptosis in thymocytes is widely recognized (20–23), their role in T cell maturation is still controversial (24–28). It has been suggested that GCs represent a key factor in determining positive vs. negative selection, although their precise role in this regulation is disputed (24, 25, 29, 30). However, data showing no differences between GR-deficient (–/–) mice, in which glucocorticoid signalling via GR is specifically abolished, GR+/+ mice, and wild-type littermates argue against a central role for GCs in determining the outcome of positive and negative selection (26). Furthermore, it has been shown that both thymic epithelial cells and DP thymocytes express genes encoding enzymes required for de novo GC synthesis (31–34). Moreover, in the thymus, the presence of mediators of the H-P-A axis, such as CRH and ACTH, has been demonstrated (35–37).

Although various functions have been attributed to thymus-derived GCs, ranging from regulation of T cell development and selection (28, 38) to the control of thymic involution (39), the degree of their synthesis in relation to levels of circulating GCs and their relevance for the control of thymopoiesis are still unclear.

A number of different experimental approaches have been used to address the impact of basal levels of adrenal gland-derived GCs on thymic homeostasis and T cell development. However, their impact on these processes is still not fully understood. With this in mind, the present study was designed to examine T cell maturation in adrenal GC-deprived rats by analysing cellularity and relationship among the thymocyte subsets at different stages of differentiation/maturation, as defined by TCR $\alpha\beta$ /CD4/CD8 expression.

Materials and Methods

Animals. In the present study, 10-week-old male DA (Dark Agouti) rats, bred in the animal housing facility at the Immunology Research Centre "Branislav Janković" (Belgrade, Serbia), were used. The animals were kept under standard laboratory conditions, in a tightly controlled environment (temperature 20–22°C, relative humidity 40–60%, 12 h light/dark cycle) in macrolone cages (3–4 rats/cage) with free access to food pellets and tap water. All procedures involving animals and their care were approved by our Institutional Animal Care and Use Committee and followed principles described in the European Community's Council Directive of 24 November 1986 (86/609/EEC).

Surgery. Bilateral adrenalectomy was performed under sodium pentobarbitone (40 mg/kg; Sagatal, Rhône Mérieux, Ltd., Harlow, UK) anaesthesia via single longitudinal lateral incisions. Control rats were sham-adrenalectomized (Adx) using the same surgical procedure, but without removal of the adrenals. Both Adx and sham-Adx animals were provided with 0.9% saline in drinking water. Completeness of adrenalectomy was confirmed by histological examination of removed tissue, by checking for residual adrenal tissue at post-mortem, and by measuring the blood corticosterone concentration.

Chemicals and Antibodies. Sodium azide, propidium iodide (PI), and RNase I-A were purchased from Sigma (Deisenhofen, Germany). Merocyanine 540 (MC540) was supplied by Sigma-Aldrich Chemie (Taufkirchen, Germany). Foetal calf serum (FCS) was acquired from Gibco (Grand Island, NY, USA). Fluorescein-isothiocyanate (FITC)-conjugated anti-CD8 (clone MRC OX-8), phycoerythrin (PE)-conjugated anti-CD4 (clone W3/25) and peridinin chlorophyll protein (PerCP)-conjugated anti-TCR $\alpha\beta$ (clone R73) monoclonal antibodies (mAbs) from Serotec (Oxford, UK) were used for immunophenotyping.

Thymic Single Cell Suspensions. For single-cell suspension of thymocytes, each thymus was dissociated by grinding on a sterile 60- μ m sieve screen submerged in ice-cold phosphate-buffered saline (PBS) pH 7.3 supplemented with 2% FCS and 0.01% NaN₃ (FACS buffer). The obtained cells were washed in FACS buffer and enumerated using improved Neubauer haemocytometer.

Flow Cytometric Analysis (FCA). Surface

Marker Expression. As previously described in detail (40), thymocytes were stained by incubating with saturating concentrations of fluorochrome-conjugated (anti-CD4, anti-CD8, anti-TCR $\alpha\beta$) mAbs. Data were collected and analyzed using a FACScan flow cytometer (Becton Dickinson) and CellQuest software (Becton Dickinson). Irrelevant isotype-matched controls were used to discriminate background staining for each fluorochrome type. Selective gating, based on forward vs. side light scatter, was used to eliminate dead cells and debris from the analyses.

Cell Cycle. The frequency of thymocytes in different phases of the cell cycle was estimated using PI incorporation, as previously described (41). Thymocytes were fixed on ice after a dropwise addition of ice-cold absolute ethanol. Next, the cells were incubated in DNase-depleted RNase I-A solution. Finally, PI solution was added, and the samples were incubated for at least 10 min, at room temperature, in dark. At the end of incubation the samples were filtered through a fine nylon mesh, and a FACScan flow cytometer and the CellQuest software (Becton Dickinson) were used for data acquisition and analysis. The data were expressed as proliferative index, i.e. the percentage of cells in the S and G2/M phases of the cell cycle.

Apoptosis. Apoptotic thymocytes were detected using MC540 lyophilic dye that labels a decreased packing order of phospholipids in the outer layer of the apoptotic cell plasma membrane. Freshly isolated thymocytes were labelled with MC540 according to the procedure described by Mower *et al.* (42). Briefly, stock solutions of MC540 (1 mg/ml) were prepared in double-distilled H₂O, filtered through a 0.22 μ m filter and stored not longer than one month. Thymocyte suspensions were adjusted to 10^6 cells in 500 μ l PBS with 5% FCS and 0.01% NaN₃, and 4 μ l of MC540 stock solution was added to each sample prior to the analysis on FACScan flow cytometer (Becton Dickinson).

Circulating Corticosterone Levels. To avoid changes in the corticosterone levels due to diurnal variations, the animals were sacrificed between 0900 h and 1000 h. For corticosterone level determination, blood was taken by cardiac puncture, and the hormone level in plasma was determined using an ImmuChem™ Double Antibody Corticosterone (¹²⁵I) RIA Kit for Rats and Mice (ICN Biomedicals Inc., Costa Mesa, CA, USA) according to the manufacturer's instructions.

Statistical Analysis. Data are expressed as mean $\pm$ standard error of mean (SEM). The differences between group means were determined by Mann-Whitney *U* test, using the statistical package SPSS for Windows version 10.0. Significance level was set at 0.05.

Results

Serum Corticosterone Level. Efficacy of adrenalectomy was confirmed by demonstrating a significantly diminished ($P < 0.01$) blood corticosterone level in Adx

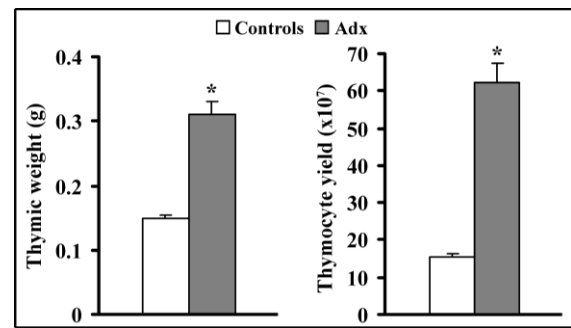


Figure 1. Adrenalectomy increased both thymic weight and cellularity in adult male DA rats. Histograms represent (left) thymic weight and (right) thymocyte yield in adrenalectomized (Adx) and sham-Adx rats (Controls). The values represent means $\pm$ SEM. $n = 7$ (Adx); $n = 6$ (Controls). * $P < 0.05$.

rats (9.73 ± 0.99 ng/ml) compared with controls (345.33 ± 46.42 ng/ml).

Thymus Weight, Thymocyte Yield, Proliferative and Apoptotic Index. Adrenalectomy in adult male rats significantly increased both thymus weight and thymocyte number (Fig. 1).

In order to elucidate mechanisms which maintain thymic hypercellularity, in both Adx and Sham-Adx rats, we examined the main thymic homeostatic processes, viz. thymocyte apoptosis and proliferation. There was no difference in the frequency of cells in active phases of cell cycle (proliferative index) between freshly isolated thymocyte suspensions from Adx and control rats (Fig. 2). However, the frequency of apoptotic cells was significantly diminished ($P < 0.05$) in thymocyte suspensions from adrenal GC-deprived rats compared with controls (Fig. 2).

Expression of TCR $\alpha\beta$ /CD4/CD8 Molecules on Thymocytes. To get insight into the process of T cell differentiation/maturation in Adx rats, we quantified the expression of CD4, CD8 and TCR $\alpha\beta$, as previously described (39). According to density of surface TCR $\alpha\beta$ expression, three populations of thymocytes with distinct

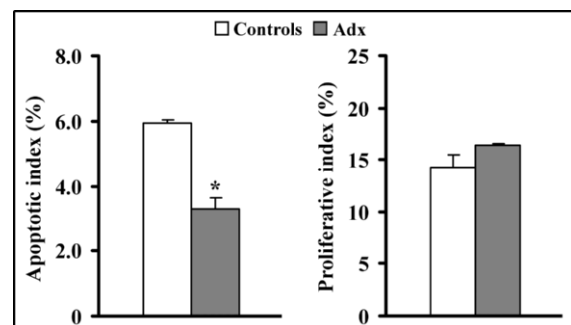


Figure 2. Adrenalectomy decreased the proportion of apoptotic thymocytes but did not affect percentage of cycling cells in adult male DA rats. Histograms represent percentage of (left) apoptotic thymocytes and (right) proliferating cells (cells in S/G2M phases of cell cycle) in adrenalectomized (Adx) and sham-Adx rats (Controls). The values represent means $\pm$ SEM. $n = 7$ (Adx); $n = 6$ (Controls). * $P < 0.05$.

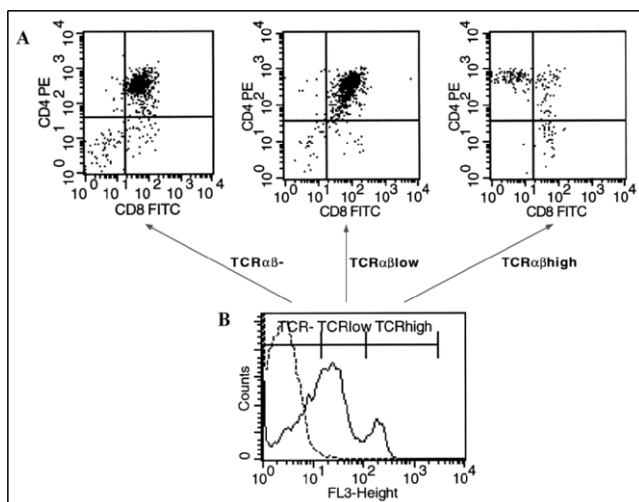


Figure 3. Three-color flow cytometric analysis of thymocytes from sham-Adx control rats stained with anti-CD8FITC, anti-CD4PE and anti-TCR $\alpha\beta$ PerCP mAbs. Thymocyte subsets at distinct stages of differentiation/maturation were delineated according to the characteristic pattern of CD4/CD8/TCR $\alpha\beta$ expression. The two-parameter dot plots (A) illustrate the expression of CD4 and CD8 molecules within TCR $\alpha\beta^-$, TCR $\alpha\beta^{\text{low}}$ and TCR $\alpha\beta^{\text{high}}$ thymocyte gates, which were set as shown in representative flow cytometric histograms of TCR $\alpha\beta$ expression (B). Dashed line in the histogram indicates irrelevant IgG isotype-matched control.

densities of these complexes—(a) high (TCR $\alpha\beta^{\text{high}}$), (b) low (TCR $\alpha\beta^{\text{low}}$), and (c) immeasurable (TCR $\alpha\beta^-$)—were distinguished (40). Plotting of the CD4+ vs. CD8+ expressing cells in each of these populations identified four subsets of thymocytes, allowing the estimation of their relative and absolute numbers (Fig. 3).

TCR $\alpha\beta^-$ Thymocytes. Adrenalectomy significantly increased ($P < 0.01$) the percentage of the least mature CD4+CD8+ DP cells, but decreased ($P < 0.01$) the proportion of the CD4+CD8- SP cells (Fig. 4A). However, in Adx rats the number of cells within all thymocyte subsets, except for CD4-CD8- DN subset, was significantly ($P < 0.01$) increased compared with controls (Fig. 4B). The cellularity of CD4-CD8- DN subset remained unaffected by the surgery (Fig. 4B).

TCR $\alpha\beta^{\text{low}}$ Thymocytes. Withdrawal of adrenal CGs affected only the percentage of CD4+8+ DP thymocytes. The percentage of these cells was significantly ($P < 0.01$) increased compared with that in controls (Fig. 4A). The number of cells was significantly increased across all TCR $\alpha\beta^{\text{low}}$ thymocyte subsets ($P < 0.01$) in Adx rats compared with controls (Fig. 4B).

TCR $\alpha\beta^{\text{high}}$ Thymocytes. The relative proportion of all TCR $\alpha\beta^{\text{high}}$ thymocyte subsets was significantly diminished in adrenal GC deprived rats (Fig. 4A). Adrenalectomy increased ($P < 0.01$) the number of cells within CD4+CD8+ DP and CD4-CD8+ SP subsets (Fig. 4B), while it did not affect cellularity of CD4+CD8- SP subset (Fig. 4B). Therefore, CD4+CD8-/CD4-CD8+ ratio was decreased (2.50 in Adx vs. 3.00 in controls). In addition, the

cellularity of CD4-CD8- DN subset was diminished ($P < 0.05$) (Fig. 4B). In mice, these cells comprise: i) cells bearing natural killer (NK) receptor and ii) cells which are cytolytic toward syngenic target cells (43). Given that in rats most of TCR $\alpha\beta^+$ cells bearing NK receptor express CD8 (44), it is not likely that NKT cells significantly contributed to alterations in CD4-CD8- DN subset.

Discussion

This study clearly demonstrates that withdrawal of adrenal GCs in adult male DA rats affects the frequency of apoptotic thymocytes and influences kinetics of T cell differentiation/maturation. This leads to: i) thymic hypercellularity and ii) over-representation of the DP TCR $\alpha\beta^{\text{low}}$ cells entering selection and their immediate DP TCR $\alpha\beta^-$ precursors. This is accompanied with underrepresentation of the positively selected DP TCR $\alpha\beta^{\text{high}}$ and most mature CD4-CD8+, and particularly CD4+CD8- SP TCR $\alpha\beta^{\text{high}}$ cells.

The increased thymic weight and the organ hypercellularity in Adx rats are consistent with data showing that adrenalectomy in mice, or injection of an antagonist of GR (RU486), increases both the thymic size and the thymocyte number (45–47). This is in keeping with the finding that adrenalectomy in transgenic rats overexpressing a mutant GR with increased ligand affinity, restores the dramatically reduced thymocyte numbers (48).

The present finding that adrenalectomy does not affect thymocyte cell cycling pattern does not favour contribution of an enhanced cell proliferation to the thymic hypercellularity. However, the reduced frequency of apoptotic cells in suspensions of thymocytes freshly isolated from Adx rats contributing to thymic hypercellularity, further supports the notion that GCs control thymic cellularity by inducing thymocyte apoptosis (19–22). It has been demonstrated that, despite relatively low expression of GRs, DP cells are the most sensitive of the thymic cells to GC-induced apoptosis (49). Therefore, it seems plausible that increased thymic cellularity in Adx rats is a product of diminished apoptosis in the DP thymocytes. The presence of increased relative and absolute numbers of DP TCR $\alpha\beta^{\text{low}}$ cells in Adx rats is consistent with this interpretation. This notion is further supported by the unaltered relative and absolute numbers of the least mature DN TCR $\alpha\beta^-$ cells in adrenal GC-deprived rats. In particular, the similar frequency of proliferating thymocytes in these and control rats, in the absence of any data suggesting that GCs can influence progenitor entry into the thymus, suggests that the least mature cell transition to the next identifiable DP TCR $\alpha\beta^-$ stage of maturation did not exceed thymocyte precursor ingress. Given that DP TCR $\alpha\beta^-$ thymocytes represent cells that express pre-TCR or TCR $\alpha\beta$ at undetectable levels (12, 50), our findings are in line with the notion that the effect(s) of GCs on T cell development are dependent on signalling via the TCR $\alpha\beta$ (51). In addition, these findings

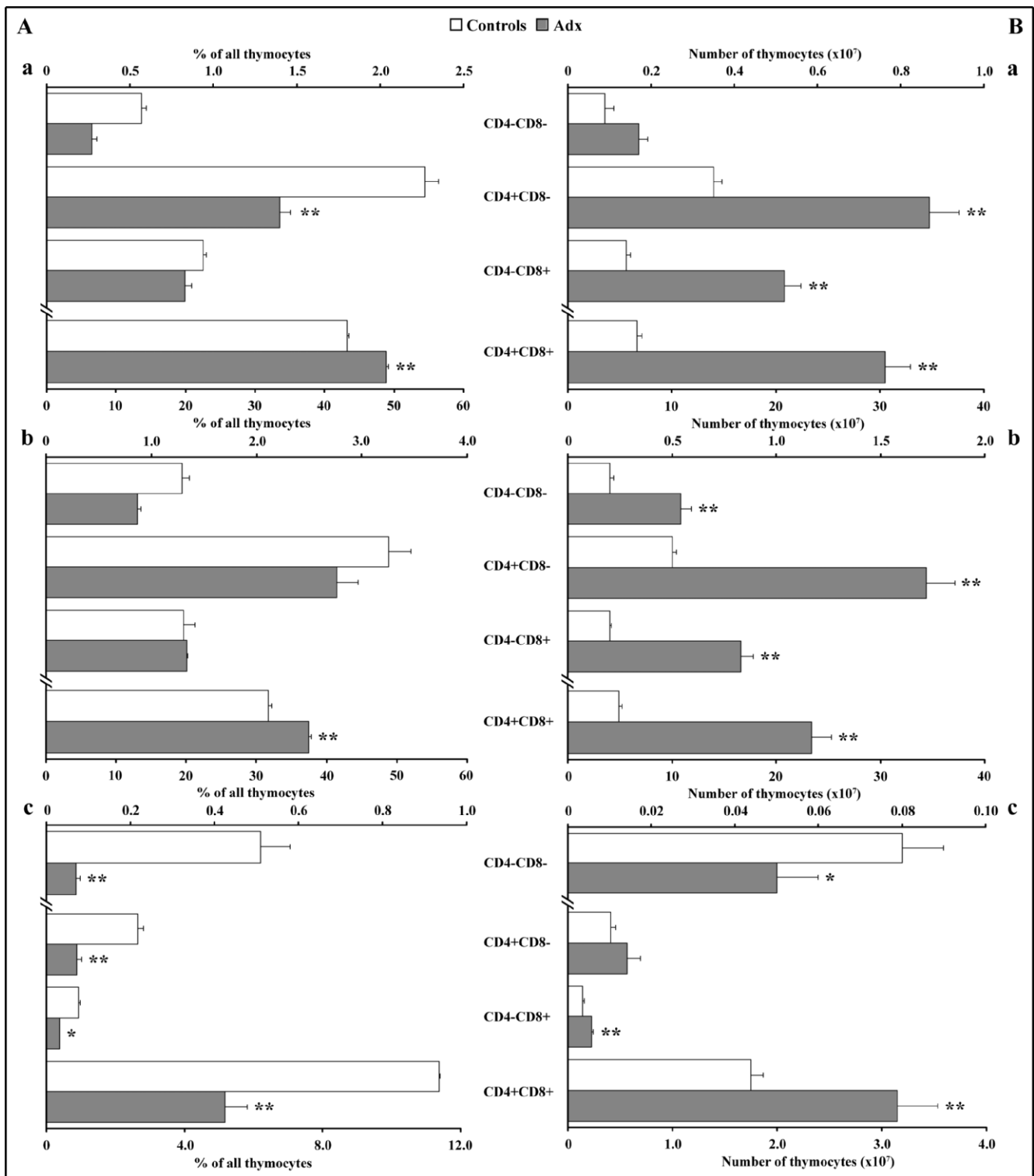


Figure 4. Adrenalectomy affected both the proportion (A) and the total numbers (B) of major thymocyte subsets delineated by expression of CD4/CD8/TCR $\alpha\beta$ in adult male DA rats. Flow cytometric analysis of (A) relative and (B) absolute numbers of thymocyte subsets delineated by CD4/CD8 expression within thymocyte populations gated according to surface density of TCR $\alpha\beta$: (a) undetectable (TCR $\alpha\beta^{-}$), (b) low (TCR $\alpha\beta^{\text{low}}$) and (c) high (TCR $\alpha\beta^{\text{high}}$) in adrenalectomized (Adx) and sham-Adx rats (Controls). The values represent means $\pm$ SEM. $n = 7$ (Adx); $n = 6$ (Controls). * $P < 0.05$; ** $P < 0.01$.

suggest that: i) adrenal-derived GCs are necessary to keep thymic cellularity within the normal range, and ii) that thymic-derived GCs cannot compensate for lack of adrenal production.

It has been strongly suggested that in the absence of GCs, thymocytes that do not recognize self-MHC, or bear TCR $\alpha\beta$ with extremely low avidity to MHC/self-peptides, i.e. useless thymocytes (12), are rescued, rather than undergo GC-mediated apoptosis (33, 51, 52). Therefore, our finding of the increased relative and absolute numbers of CD4+CD8+ DP TCR $\alpha\beta^{\text{low}}$ cells in Adx rats may indicate that in the absence of adrenal GCs their local thymic production (31–34) is not sufficient to provide efficient elimination of the cells that do not recognize self-MHC, or bear TCR $\alpha\beta$ with extremely low avidity to MHC/self-peptide (the majority of thymocytes). However, the presence of a greater number of DP TCR $\alpha\beta^{\text{low}}$ cells in Adx rats, without a proportional rise in the numbers of DP TCR $\alpha\beta^{\text{high}}$ cells, which are supposed to be cells that have just passed positive selection (53), and their SP (CD4+CD8- and CD4-CD8+) TCR $\alpha\beta^{\text{high}}$ descendants (leading to a reduction in their proportional representation), suggests decelerated transition of DP TCR $\alpha\beta^{\text{low}}$ cells to downstream maturational stages and/or increased negative selection. The latter option is fully consistent with the mutual antagonism theory stating that in the absence of GCs, not only thymocytes with high avidity for self-MHC (which are potentially harmful), but also the cells with low-to-moderate avidity for self-MHC, i.e. useful cells (13), are being forced into activation-mediated apoptosis (negative selection), rather than being rescued and positively selected as would occur in the presence of GCs (2, 13, 33, 52, 54). Therefore, it seems conceivable that, in the absence of adrenal GCs, locally produced GCs cannot maintain selection of useful cells at optimal level. In other words, the previous assumption suggests that in the absence of adrenal GCs, despite the presence of an increased number of positively selected cells, *de novo* generated repertoire of useful, i.e. functional T cells, may be skewed. Moreover, reduction in Adx rats of both relative and absolute numbers of DN TCR $\alpha\beta^{\text{high}}$ cells, which are supposed to be mature self-reactive cells arising normally in the thymus (42, 43), gives further strength to the thesis that adrenal GC ablation leads to an overexaggerated negative selection. This is fully consistent with data showing the presence of a higher percentage of these cells in rats with increased blood corticosterone levels due to chronic stress (55). Finally, this is consistent with the data showing a higher basal level of GCs in animal models of autoimmune disease and in patients with autoimmune multiple sclerosis (56, 57).

Although adrenalectomy reduced the relative numbers of both SP TCR $\alpha\beta^{\text{high}}$ thymocyte subsets, it caused a more pronounced effect on CD4+CD8- SP TCR $\alpha\beta^{\text{high}}$ subset, so that the number of CD4-CD8+ SP TCR $\alpha\beta^{\text{high}}$ cells remained unchanged, while that of the CD4+CD8- SP TCR $\alpha\beta^{\text{high}}$ cells was increased. Therefore, in Adx rats the ratio of

CD4+CD8-/CD4-CD8+ SP TCR $\alpha\beta^{\text{high}}$ thymocytes was reduced. It should be pointed out that using Lck(Pr)-sGR transgenic mice with increased GC sensitivity restricted to the T cell lineage, GCs have been demonstrated to regulate the CD4+/CD8+ T cell ratio in the periphery, but not the CD4+CD8-/CD4-CD8+ SP cell ratio in the thymus (39). This discrepancy between these and our findings may be reconciled by the fact that Pazirandeh and collaborators calculated the ratio between all CD4+CD8- and CD4-CD8+ SP thymocytes (39), while we took into consideration only the most mature TCR $\alpha\beta^{\text{high}}$ thymocytes.

Finally, it should be pointed out that withdrawal of circulating GCs might affect thymic cellularity and T cell differentiation, not only by acting directly on the thymic cells, but also indirectly, by affecting the action of other possible immunomodulators. In particular, GCs have been shown to influence catecholamine synthesis (58) and β -adrenoceptor expression (59). Catecholamines have been shown to influence T cell differentiation/maturation (41). Therefore, an altered catecholamine action in the absence of adrenal GCs might also contribute to the detected changes in Adx rats.

In conclusion, the present study indicates that adrenal GCs affect not only thymic cellularity, by increasing thymocyte apoptosis, but also T cell differentiation/maturation by influencing the TCR $\alpha\beta$ -dependent selection processes. It suggests that deprivation of adrenal GCs may lead, despite increasing thymic output (due to reduced “death by neglect”), to the narrowing of functional peripheral T cell repertoire, and to a reduced generation of the unwanted self-reactive repertoire. Furthermore, it indicates that adrenal GCs influencing post-selection maturation of thymocytes can play a regulatory role in controlling mature CD4+CD8-/CD4-CD8+ SP TCR $\alpha\beta^{\text{high}}$ cell ratio. Collectively, this data suggests that in states characterized by a lack of adrenal GC synthesis, or GC resistance, an altered T cell dependent immune response may be present, due to the altered intrathymic maturation of T cells.

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