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Progesterone inhibits glucocorticoid-induced murine thymocyte apoptosis

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Abstract

Sex and sex hormones modulate immune development and responses. A primary target of their effects is the structure and cellularity of the thymus; therefore, we examined the effects of sex and sex steroids on thymocyte apoptosis. We demonstrate initially that male DBA mice have a significantly higher percentage of glucocorticoid-induced apoptotic thymocytes ($46.1 \pm 3.8\%$) than their female counterparts ($31.6 \pm 3.1\%$; $P = 0.012$). We postulated that this gender difference was due to differential modulation of glucocorticoid-induced apoptosis by sex hormones such as estrogen, testosterone or progesterone. Both estrogen and testosterone increased in vitro thymocyte apoptosis. In contrast, progesterone not only inhibited spontaneous in vitro thymocyte apoptosis, but also prevented in vitro glucocorticoid-induced apoptosis. Progesterone administration also suppressed glucocorticoid-induced in vivo thymocyte apoptosis. These results suggest that anti-apoptotic effects of progesterone may influence T cell development and subsequent immune responses.

Keywords: Thymocyte; Apoptosis; Corticosteroid; Progesterone

1. Introduction

Immune response and the development of autoimmunity are sexually dichotomous. The reasons for sex-based differences in immune responses are complex and multifactorial [1–6]. A potentially key step in the differential development of the immune system is thymic T cell

Abbreviations: CD, Cyclodextrin; DEXA, Dexamethasone; PROG, Progesterone; PI, Propidium iodide.

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education and repertoire development. Apoptosis of thymocytes is a regulatory step in T cell selection, immune regulation and development of tolerance or autoimmunity [7–9]. Thymocyte apoptosis is regulated by the interplay of several factors, including avidity of the T cell receptor for self-antigen, recognition of major histocompatibility (MHC) determinants and the activity of endogenous glucocorticoids [7–11]. Glucocorticoids regulate thymic development through direct induction of thymocyte apoptosis or antagonism of T-cell receptor activation mediated apoptosis [10,11].

Glucocorticoids appear to induce murine thymic atrophy with a sex-based bias: female mouse thymocytes being more resistant to glucocorticoid-induced apoptosis than male thymocytes [12]. Sex steroids, at high concentrations, are also known to influence the structure and cellularity of the murine thymus [13–15], but their potential role, either agonistic or antagonistic to thymic apoptosis, has not been clearly defined. Estrogen has been shown to induce thymic atrophy similar to that of glucocorticoids [13,16] and testosterone has been shown to have pro-apoptotic effects on murine thymocytes [17]. In contrast, progesterone has either not had any effect on thymocytes [13] or has been weakly pro-apoptotic [18–20]. During fetal development, high concentrations of several steroids, including estrogens, progestins, and glucocorticoids are found in maternal serum, amniotic fluid and the fetal circulation [21–23]. For example, maternal production of progesterone is ≈ 250 mg (0.8 mmol) per day during the third trimester of pregnancy [21,22] and progesterone concentrations at the materno–fetal interface reach 3 μg/g of placental tissue [23]. A complex array of sex steroid receptors exists on thymocytes and thymic stromal cells (reviewed in Ref. [4]), thereby facilitating both direct and indirect sex steroid modulation of thymic structure and cellularity. Furthermore, thymic stromal cells have been shown to produce steroid hormones (e.g. pregnenolone and deoxycorticosterone) locally [24], a paracrine hormonal microenvironment that may be locally agonistic or antagonistic to endocrine effects on thymocytes. In this investigation, we verify sex-based differences in glucocorticoid-induced thymic apoptosis, examine differential effects of high concentrations of estrogen, testosterone and progesterone on murine thymocyte apoptosis and demonstrate that progesterone inhibits glucocorticoid-induced apoptosis in murine thymocytes.

2. Materials and methods

2.1. Animals

DBA/2 mice (Harlan Sprague–Dawley, Indianapolis, IN) were bred in the animal facility at the G.V. 'Sonny' Montgomery Department of Veterans Affairs Medical Center. Male mice, aged 4–5 weeks, were used for these experiments unless otherwise indicated.

2.2. Reagents and antibodies

Phenol-free RPMI 1640, minimal essential media (MEM), vitamins, nonessential amino acids (NEAA), L-glutamine, sodium pyruvate and antibiotic/antimycotic solution were purchased from Gibco/BRL (Gaithersburg, MD). In all experiments utilizing sera supplementation, fetal calf serum (Sigma, St. Louis, MO) was stripped of endogenous steroid hormones by adsorption with activated charcoal. Estrogen and progesterone were not detectable in the stripped sera by EIA (data not shown). 2β-mercaptoethanol (2-ME), activated charcoal, sesame oil, dexamethasone, progesterone, water-soluble hormones (dexamethasone, 17β-estradiol, progesterone, testosterone) and the solvent vehicle, cyclodextrin, were obtained from Sigma. Cyclodextrin is a cyclic oligosaccharide that incorporates hydrophobic molecules into a torus cavity, rendering the molecule water-soluble [25,26]. In preliminary studies, the proportion of apoptotic thymocytes (hypodiploid staining of DNA by propidium iodide) was similar for cells cultured for 24 or 48 h with cyclodextrin ($45.8 \pm 10.4\%$) or in media alone (40.6 ± 8.0; $n = 3$ at 24 h). In order to detect potentially significant differences in sex steroid effects on apoptosis, in vitro concentrations of gonadal steroids were used in a range of 10^{-4} to

10^{-6} M on the basis of previous studies which have demonstrated minimal [20] to maximal effects [17] effects on murine thymocyte apoptosis, cytokine production [27], cell differentiation [28] and stimulation of estrogen receptor transcriptional activity [29]. In the current study of pro- and anti-apoptotic effects of gonadal steroids, a concentration of 10^{-4} M was utilized, unless otherwise specified.

2.3. Thymocyte preparation and culture

Thymi were aseptically removed from mice and washed in RPMI 1640 media containing 5% charcoal stripped FCS and 6×10^{-5} M 2-ME. Cell suspensions were obtained by gentle disruption of the thymus with scalpel and tweezers. Thymocytes were cultured at 1×10^{6} cells/ml in 60 mm flat bottom plates at 37°C in humidified air with 5% CO_2 in phenol-free RPMI 1640 containing minimal essential media (MEM), vitamins, nonessential amino acids (NEAA), L-glutamine, sodium pyruvate, antibiotic/antimycotic solution and 5% charcoal stripped fetal calf serum (FCS) for the indicated times in the presence or absence of hormones.

2.4. Flow cytometry

Thymocytes were analyzed using a Becton Dickinson FACS. For analysis of DNA content by propidium iodide (PI), the cells were fixed with 1% paraformaldehyde, permeabilized with 70% ethanol, treated with ribonuclease A and stained with PI prior to flow cytometric analysis. Hypodiploid regions correlate with DNA fragmentation and other measures of apoptosis [30,31]. Fragmentation of DNA in individual cells was analyzed by the TUNEL method (Apotag Kit, Oncor, La Jolla, CA) according to manufacturers' instructions. Briefly, cells were fixed in 1% paraformaldehyde and permeabilized with 70% ethanol. 3′-OH DNA ends were extended with digoxigenin-labeled dUTP and the incorporated nucleotide was detected by the binding of FITC-conjugated anti-digoxigenin antibody. Cells were washed and fixed prior to flow cytometric analyses. For detection of cell surface phosphatidylserine, cells were washed twice with phosphate buffered saline (PBS) then binding buffer, and incubated with FITC conjugated phosphatidylserine binding protein (Annexin V, ApoAlert Kit, Clontech, San Diego, CA) for 15–30 min. Samples were analyzed immediately for Annexin V positivity.

2.5. Analysis of DNA cleavage by agarose gel electrophoresis

RNAse-treated genomic DNA, 3 μg per sample, was subjected to electrophoresis in 1% agarose gels containing ethidium bromide. Gels were photographed under UV illumination.

2.6. Measurement of estrogen and progesterone receptors

Estrogen and progesterone receptors (ER and PR respectively) in mouse thymocytes were assessed by the Abbott ER/PR EIA Monoclonal Kit (Abbott Park, IL) according the manufacturers' instructions. Results were compared to standard curves and samples, provided by the manufacturer and representing the absorption produced by known concentrations of ER and PR. Inter- and intra-assay variation was $\approx 10\%$; sensitivity of this system is ≈ 1.5 fmol ER or PR/mg protein.

2.7. Histopathologic examination

In vivo thymic apoptosis was examined by Annexin V staining and by histopathologic examination in representative mice. The right lobe of the mouse thymus was removed for mechanical disruption and Annexin V staining as described above. The left lobe of the thymus was fixed in 10% buffered formalin. Following routine processing, 5 μm sections were cut and stained with hematoxylin and eosin. Apoptotic cells were identified according to established criteria [32]. Proportions of apoptotic cells in thymic cortex and medulla were graded by the pathologist (AL) blinded to the nature of hormonal manipulation of the source animal.

2.8. Statistical analyses

Data were summarized as mean ± S.E.M. Group means were compared using a paired or unpaired Student's t-test as appropriate, assuming equal variance. A P value < 0.05 was considered significant.

3. Results

3.1. Sexual dichotomy of dexamethasone-induced apoptosis in vivo

As shown in Fig. 1A, 24 h after in vivo administration of dexamethasone (50 μg/g body weight), the mean proportion of apoptotic thymocytes from male DBA/1 mice DNA was significantly higher (46.1 ± 3.8%) than the proportion of apoptotic thymocytes from female mice (31.6 ± 3.1%, $P = 0.012$, $n = 9$). Representative DNA histograms from control and dexamethasone-treated male and female DBA/2 mice are shown in Fig. 1, panel B. The majority of thymocytes in control animals were in G_0/G_1 phase of the cell cycle with only 1% of cells being apoptotic (hypodiploid) in both female and male mice (upper panels, Fig. 1B). Dexamethasone exposure increased the percentage of apoptotic thymocytes to 32% in this representative female mouse and to 47% in a representative male mouse thymocyte histogram (Fig. 1B, lower panels respectively). Total thymic cellularity was not significantly different between control groups of male and female mice. As would be expected, the increased apoptosis in male mice was associated with a trend towards reduced median number of total thymocytes in dexamethasone-treated male mice (1.7×10^7; $n = 7$) compared to that for female mice (3.5×10^7; $n = 7$, $P = 0.06$).

3.2. Effects of gonadal steroids on thymocytes in vitro

Gonadal steroids exhibiting pro- or anti-apoptotic effects on thymocyte apoptosis might account for the observed gender differences in glucocorticoid-induced thymic apoptosis. This possibility was tested in primary thymocyte cultures. Thymocytes from 5-week-old male mice were cultured in complete media with 10^{-4} M dexamethasone, estrogen, testosterone or progesterone. Apoptosis was assessed initially by agarose gel electrophoresis of DNA after 24 h of culture (Fig. 2, representative experiment; $n = 3$). As expected, ex vivo thymocytes (Lane 2) showed no DNA cleavage and cultured control thymocytes (Lane 3) showed modest DNA cleavage,

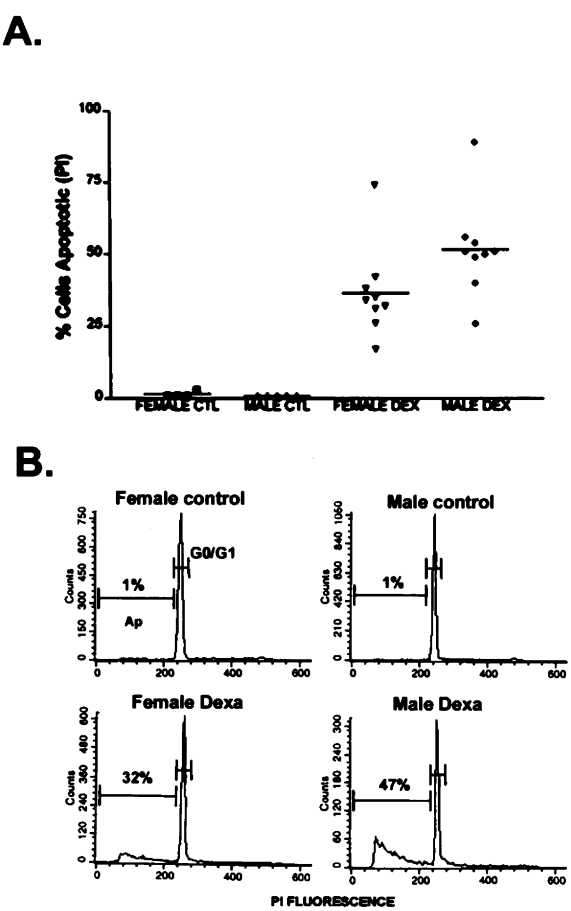

Fig. 1. Proportion of hypodiploid (apoptotic) thymocytes in control and dexamethasone-treated female and male DBA/2 mice as determined by PI staining. In treated mice, the mean percentage of apoptotic thymocytes in males ($n = 9$) was significantly higher than that in females ($n = 9$) ($P = 0.012$). (B) Representative PI histograms of thymocytes in control female and male and dexamethasone-treated female and male DBA/2 mice. Brackets indicate the hypodiploid region corresponding to apoptotic cells.

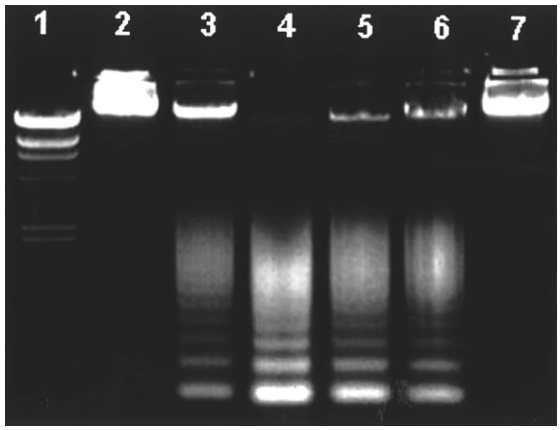

Fig. 2. Representative ($n = 3$) DNA ladder of mouse thymocytes. Lane 1: DNA molecular weight markers. Lane 2: Freshly harvested thymocytes ex vivo. Lanes 3–7 after 24 h culture. Lane 3, control; Lane 4, dexamethasone; Lane 5, testosterone; Lane 6, estrogen; Lane 7, progesterone.

consistent with a moderate level of spontaneous apoptosis. Dexamethasone (Lane 4) induced an accelerated pattern of internucleosomal DNA cleavage, typical of glucocorticoid-induced apoptosis. Testosterone (Lane 5) and estrogen (Lane 6) also induced internucleosomal DNA cleavage compared to cultured control thymocytes, but less than that seen in dexamethasone-induced cultures (Lane 4). In contrast, progesterone reduced the spontaneous apoptosis of cultured thymocytes (Lane 7). Similar results were obtained at 48 h incubation ($n = 3$, data not shown).

These results were confirmed by staining thymocytes with Annexin V-FITC. Representative histograms are shown in Fig. 3 ($n = 2$ experiments). Analyses of ex vivo thymocytes (Fig. 3A, 11%) and thymocytes cultured for 24 h (Fig. 3B, 35%) revealed modest apoptosis by Annexin V staining. Dexamethasone increased the proportion of apoptotic cells (Fig. 3C, 74%). Testosterone (Fig. 3D, 53%), or estrogen (Fig. 3E, 42%) also increased apoptosis at 24 h. In contrast, progesterone exposure (Fig. 3F, 21%) inhibited spontaneous thymocyte apoptosis compared to control thymocytes. Three additional experiments utilizing PI staining to assess apoptosis demonstrated that dexamethasone increased thymocyte apoptosis in vitro by a mean of 175% compared to control thymocyte cultures ($P = 0.024$). Progesterone significantly reduced in vitro apoptosis of DBA/2 thymocytes by 59% as assessed by PI staining ($P = 0.016$ versus control, data not shown). The pro-apoptotic effects of estrogen or testosterone and the anti-apoptotic effect of progesterone on in vitro murine thymocyte apoptosis as assessed by PI staining were concentration dependent over a range of 10^{-4} to 10^{-6} M (data not shown). No significant effects on thymocyte apoptosis were observed at concentrations $< 10^{-6}$ M. There were no qualitative or significant quantitative differences between the pro-apoptotic effects of estrogen and testosterone (data not shown).

3.3. Analysis of progesterone receptors

Thymocytes and thymic epithelial cells express glucocorticoid, androgen and estrogen receptors (reviewed in Ref. [4]). We compared progesterone receptor concentrations in 5-week-old male and female mouse thymi. Male mouse thymic tissue had a mean ($\pm$S.E.M.) progesterone receptor concentration of 27.9 ± 1.9 fmol/mg protein compared to 28.3 ± 2.7 fmol/mg protein in female mouse thymocytes ($n = 5$ each group). Thus, in this strain, male and female mice had similar thymic progesterone receptor expression at 5 weeks of age. Differential progesterone receptor expression is an unlikely explanation for the gender divergent results in apoptosis reported above.

3.4. Antagonism of dexamethasone-induced apoptosis by progesterone

We next questioned whether progesterone could inhibit glucocorticoid-induced thymocyte apoptosis in vitro. In three PI staining experiments (Fig. 4), dexamethasone increased mean in vitro thymocyte apoptosis to $49 \pm 9\%$, compared to $29 \pm 7\%$ in control cultures ($P < 0.024$, DEX versus CD). Progesterone, as shown previously, reduced spontaneous in vitro thymic apoptosis to $12 \pm 9\%$ ($P < 0.05$ PROG versus CD). Progesterone also significantly prevented glucocorticoid-induced apoptosis in vitro, reducing the mean percent of apoptotic cells from 49 to 18% ($P < 0.020$; DEX

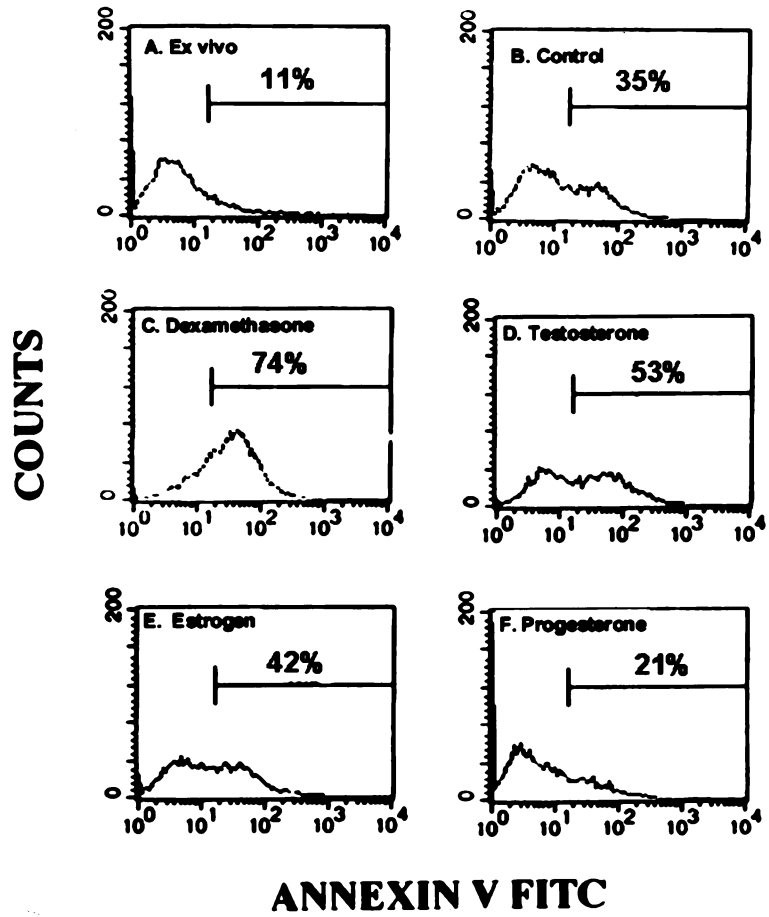

Fig. 3. Representative ($n = 3$) Annexin V staining of mouse thymocytes with percent Annexin V^+ thymocytes. (A) Thymocytes ex vivo. After 24 h in vitro: (B) Control. (C) Dexamethasone. (D) Testosterone. (E) Estrogen. (F) Progesterone.

versus P + D). Results obtained at 48 h of culture (data not shown) were similar to the 24-h data. Therefore, progesterone significantly antagonized dexamethasone-induced apoptosis.

Progesterone's antagonism of dexamethasone-induced thymocyte apoptosis was verified by TUNEL staining and DNA electrophoresis (data not shown). Additionally, the temporal relationship of progesterone's anti-apoptotic action was analyzed. Dexamethasone-induced apoptosis was markedly inhibited when murine thymocytes were stimulated with progesterone either concomitantly or 24 h before, but not after, dexamethasone stimulation (data not shown). These results suggest that progesterone can modify the effects of glucocorticoid-induced thymocyte apoptosis but not 'rescue' thymocytes once committed to a cell death pathway.

We extended our investigation to in vivo anti-apoptotic actions of progesterone on glucocorticoid-induced thymocyte apoptosis. Twelve hours after subcutaneous injection of either 0.5 or 5 μg/g mouse body weight of dexamethasone, ex vivo thymocytes and thymi from control and progesterone-treated mice were analyzed by Annexin V staining and histopathologic examination. Compared to control mice, progesterone alone slightly reduced in vivo thymocyte apoptosis, whereas dex-

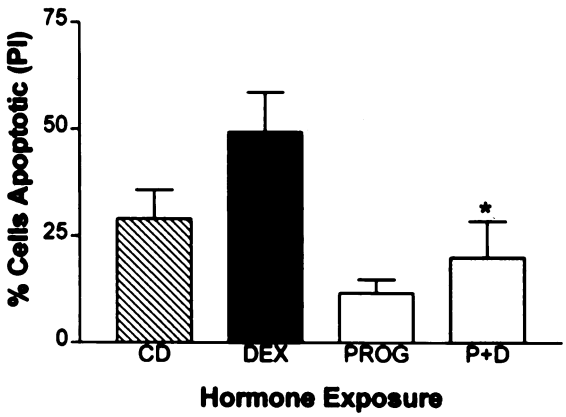

Fig. 4. Mean percent ($n = 3$) of apoptotic cells as determined by PI staining in equimolar amounts (10^{-4} M) of vehicle (CD), dexamethasone (DEX), progesterone (PROG), and progesterone and dexamethasone (P + D) after 24 h exposure. The induction of apoptosis by dexamethasone ($P = 0.024$ for DEX versus CD) was reduced by the presence of progesterone (*$P = 0.020$ for DEX versus P + D).

amethasone (D 0.5 and D 5) increased thymocyte apoptosis (Fig. 5A). Progesterone administration prior to dexamethasone administration (D 0.5 + P) prevented and reversed thymocyte apoptosis induced by 0.5 ug/g dose of dexamethasone (from $+35$ to -15%; $P < 0.05$, $n = 4$). However, progesterone could only reduce, but not prevent, the apoptosis induced by 5 μg/g dose of dexamethasone (Fig. 5A, from $+200$ to $+158\%$, $P =$ NS). Representative Annexin V thymocyte histograms (Fig. 5B) demonstrate the increase in apoptosis in response to dexamethasone, the absence of a significant effect of progesterone compared to control, and the inhibitory effect of progesterone on dexamethasone-induced thymic apoptosis in vivo. Histopathologic examination of thymi confirmed the inhibition of dexamethasone-induced thymic apoptosis by progesterone, especially at the lower dose of dexamethasone (data not shown).

4. Discussion

At high concentrations, sex steroids have been shown to alter thymic cellularity and architecture and glucocorticoids, estrogen, and testosterone have been shown to induce apoptosis in thymocytes [13–17]. We postulated that the sex steroids — estrogen, testosterone, and progesterone — have differential effects on thymocyte apoptosis. This possibility was supported by our initial results showing sex-specific differences in dexamethasone-induced thymic apoptosis. DBA/1 male mouse thymocytes had a higher percentage of glucocorticoid-induced apoptotic thymocytes compared to their female counterparts (Fig. 1A,B). A previous study had demonstrated strain-dependent differences in glucocorticoid-induced murine thymic atrophy. As important, a sex difference in glucocorticoid-induced thymic atrophy was suggested. The current study confirms this observation and extends it by suggesting that sex steroids may modulate the response to glucocorticoid-induced thymic apoptosis.

In the current study, estrogen and testosterone induced modest, concentration dependent (from 10^{-6} to 10^{-4} M, not shown) increases on murine thymocyte apoptosis in vitro. These results are consistent with reports that estrogen and testosterone are pro-apoptotic to murine thymocytes [13–17]. Others have shown that estrogen and testosterone in vitro had no effect on DNA fragmentation in cloned interleukin-2 dependent CTLL-2 T cells [18]. Olsen et al. reported a broad range of DNA fragmentation 6–97% (mean = $35 \pm 11\%$) above control in thymic organ cultures stimulated with 10^{-6} M dihydrotestosterone (DHT) [17]. Utilization of high concentrations (10^{-6} to 10^{-4} M) of a potent, non-physiologic corticosteroid, dexamethasone, was necessary to demonstrate to a concentration dependent increase in apoptosis of monocytes [33]. In vivo, 6 mg progesterone per day per mouse had no effect on thymus weight or cell number, whereas estrogen and testosterone induced variable degrees of thymic apoptosis [13]. In contrast, 12 days administration of high dose progesterone (20 μg/μl concentration; exact administration amounts not given) and estrogen (20 ng/μl; exact administration amounts not given) produced significant thymic atrophy [34], consistent with previous reports that in vivo estrogen or testosterone administration to Balb/c mice caused thymic atrophy, comparable to, but less than that produced by dexamethasone [13].

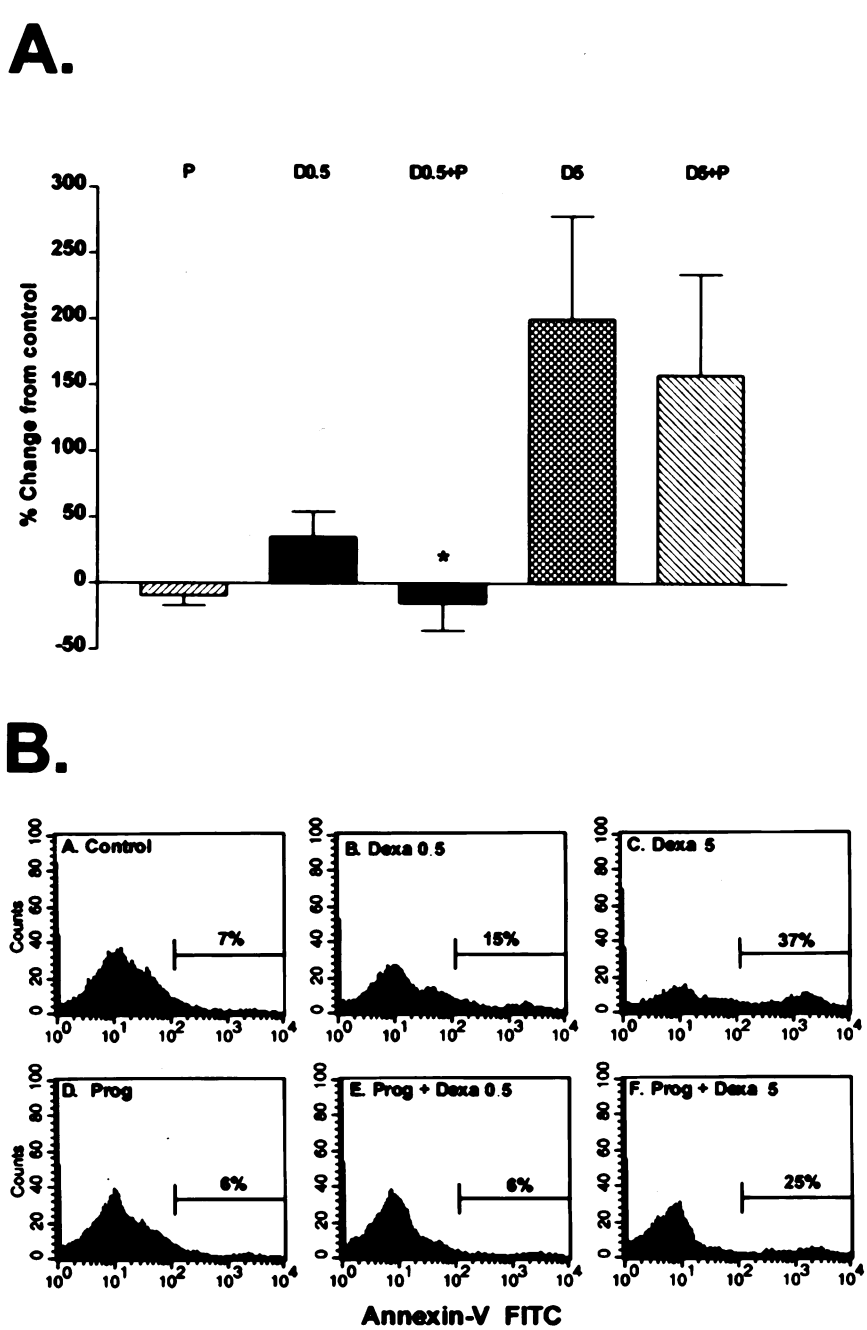

Fig. 5. (A) Thymocyte apoptosis by Annexin V staining of ex vivo thymocytes from male DBA/2 mice treated with progesterone (*P*; 50 μg/g per mouse), dexamethasone (D; 0.5 or 5 μg/g per mouse), or progesterone 12 h prior to dexamethasone ($P + D_{0.5}$ or $P + D_5$). Values represent mean percent of increase or decrease compared to control (oil-injected) animals ($n = 4$ mice/group). $^*P < 0.05$ versus $D_{0.5}$. (B) Representative Annexin V histograms of freshly harvested thymocytes from control, dexamethasone-treated (0.5 and 5), progesterone (50) and progesterone pre-treated/dexamethasone-treated mice. Percent Annexin V^+ cells are shown.

In interpreting the results of our study, it should be considered that other models of apoptosis have used high concentrations of a non-physiological glucocorticoid, dexamethasone, with greater potency than its physiologic counterparts [33]. Few, if any studies have demonstrated a dose response relationship of thymocyte apoptosis for sex steroids, especially for concentrations $< 10^{-6}$ M. The reasons for this are unclear, since gonadal organ manipulations clearly affect thymic structure and cellularity [4,17]. Perhaps thymic stromal cells (discussed below) alter the sensitivity of thymocytes sex steroid responses. In comparison to the current study, Iwata et al. demonstrated no effects of estrogen or testosterone and only weak effects of progesterone on in vitro DO-11.10 thymoma cell [19] or Balb/c mouse thymocyte apoptosis [20] at concentrations of 10^{-5} M. The variable effects observed over different concentrations are also likely influenced by the model employed and the steroid used, as well as a number of confounding factors including but not limited to steroid interconversions (e.g. progesterone to testosterone), relative potency issues, and receptor-dependent and independent actions. A major difference in the current study is the mouse strain used. As discussed above for glucocorticoid-induced apoptosis, genetic background may influence thymocyte apoptosis susceptibility, particularly as related to steroid hormones [11]. Supraphysiologic concentrations of estrogen, testosterone, and progesterone were utilized in the current study to maximize potential apoptotic actions [18–20] and steroid receptor transcriptional effects [29]. The current results show that estrogen, testosterone, and progesterone, at high concentrations, directly affect thymocyte apoptosis.

The sex difference in glucocorticoid-induced thymic apoptosis observed in this study could be due to the pro-apoptotic effects of testosterone. Castration of male mice reduces thymocyte apoptosis, and DHT replacement increases thymic apoptosis [17]. However, since estrogen and testosterone, in this study, had qualitatively similar pro-apoptotic effects on thymocyte apoptosis, it was considered that progesterone, through anti-apoptotic effects, could contribute to the sex-based disparity in glucocorticoid-induced thymic apoptosis. In the current study, progesterone reduced spontaneous thymocyte apoptosis in vitro (Figs. 2 and 3) and antagonized glucocorticoid-induced apoptosis both in vitro (Fig. 4) and in vivo (Fig. 5A,B). We believe this is the first report, albeit employing supraphysiological concentrations, of hormonal antagonism of glucocorticoid-induced thymic apoptosis.

In other studies, high concentrations of progesterone had weak pro-apoptotic effects on CTLL-2 cells [18]. Progesterone has also been shown to increase DNA fragmentation (apoptosis) in the DO-11.10 thymoma cell line [19] and Balb/c thymocytes [20], yet reduce apoptosis induced by T-cell receptor (TCR) stimulation [19]. Progesterone, when administered in vivo, has had no effect on murine thymic weight or cell number at a very high concentration [13], but, in combination with estrogen, induced significant thymic atrophy [34]. As noted above, varied hormonal and cell specific responses could be expected, based on cell specific promoter regions or proteins [29]. In the present study, in vivo progesterone receptors were expressed at similar concentrations in thymic tissue of male and female DBA/2 mice, and were unlikely to account for the sex-based differences in glucocorticoid-induced thymic apoptosis in females. Although some anti-glucocorticoid actions of progesterone have been postulated to occur through competition for steroid receptor binding [35], it has been shown that high concentrations of estrogen or testosterone do not occupy glucocorticoid receptors in vitro [13]. Several potential alternative mechanisms of glucocorticoid antagonism exist [36,37] and are currently under investigation.

Our findings suggest that progesterone may play a role in thymic selection processes, through antagonism of glucocorticoid-induced thymic apoptosis; however, the supraphysiological concentrations used in this study to observe a measurable effect make extrapolation to physiologic mechanisms difficult. Moreover, in vivo, thymic stromal cells modulate thymocyte development and homeostasis [24,34]. Tibbetts et al. have reported that thymic stromal cell progesterone receptor (PR) is required for complete hormone and pregnancy induced thymic involution (PR) [34].

PR, thus far, has been shown to be restricted to thymic stromal cells (reviewed in Refs. [17] and [34]). However, at the high concentrations used in this study, progesterone induced thymocytes, independent of their stromal elements, to resist glucocorticoid-induced apoptosis. Thus, the anti-apoptotic effects of progesterone may be a receptor-independent phenomenon. Delineation of this possibility is the focus of future work. Thymic epithelial cells also produce progestins and corticosteroids locally [24]. Although unknown, in vivo stromal paracrine effects of progestins may be additive, synergistic, or antagonistic to other endocrine effects. High concentrations of progestins present at the onset of puberty [38] or during pregnancy [21] may combine with progestins produced in the thymic microenvironment [24] to protect or prevent thymocytes from undergoing apoptosis. Further investigations will be needed to confirm the anti-apoptotic actions of progesterone at supraphysiological concentrations and determine any potential role of progestins modulating physiological thymic apoptosis in vivo.

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