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Differential Effects of Gonadectomy on the Thymocyte Phenotypic Profile in Male and Female Rats

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LEPOSAVIĆ, G., B. KARAPETROVIĆ, S. OBRADOVIĆ, B. VIDIĆ DANKOVIĆ AND D. KOSEC. *Differential effects of gonadectomy on the thymocyte phenotypic profile in male and female rats.* PHARMACOL BIOCHEM BEHAV **54**(1) 269-276, 1996. – As an organ responsible for generation of T-cell repertoire the thymus occupies a central position in establishment of mature immune response. To assess the potential role of the gonadal steroids in development and maintenance of immunological sexual dimorphism, the effects of gonadectomy pre- and postpuberty on the thymocyte profile of male and female rats were examined. Rats aged 30 days or 75 days were gonadectomized; 30 days later the thymic cellularity was estimated and the expression of the cell surface antigens (CD4 and CD8) and the T-cell receptor (TCR) $\alpha\beta$ was analyzed by flow cytometry. Regardless of age at surgery, the thymus weight and total thymocyte yield were greater in sham-operated males than females; this sexual dimorphism in thymic cellularity persisted after gonadectomy. Sexual dimorphism in the composition of thymocyte subsets was also evident in sham-operated rats, with males expressing a higher percentage of CD4 − 8 − cells, and remained after gonadectomy of adult rats. In male rats, gonadectomy at day 75 increased the percentage of CD4 + 8 − single-positive and TCR $\alpha\beta$ + cells. In contrast, in females, ovariectomy decreased the percentages of CD4 + 8 − single-positive, CD4 − CD8 − double-negative, and TCR $\alpha\beta$ + cells and increased the percentage of CD4 + CD8 + double-positive cells. In the immature rats gonadectomy increased the percentages of CD4 + 8 − single-positive and TCR $\alpha\beta$ + thymocytes and decreased the percentages of double-positive and double-negative cells in males, while in the female it increased the percentage of CD4 + 8 − single-positive thymocytes. Gonadectomy at that age abolished the sexual dimorphism in the expression of accessory molecules (i.e., CD4/CD8), but facilitated gender-specific expression of TCR $\alpha\beta$. In conclusion, the results suggest that the gonadal steroids are more important for the development than for the maintenance of the sexual dimorphism in the thymocyte composition.

Sexual dimorphism Thymic cellularity Thymocyte phenotypic characteristics Sex steroids Sexual maturation

EVIDENCE from clinical and experimental studies has established that both the cell-mediated and humoral immune responses are more efficient in females than in males (4,12,21, 22,25). However, the mechanisms controlling the development and maintenance of this immunological sexual dimorphism have not been fully elucidated, although it has been suggested that hormones of the gonads are involved (22). The data showing that skin allograft rejection time is shorter in inbred females than male mice and that gonadectomy shortens the rejection time in males (18) support this hypothesis. Furthermore, in the F1 NZB/NZW mice, the onset and course of autoimmune lupus can be altered by changing sex steroid environment (49). Normally, such females develop the disease and die, while males are not as susceptible. However, these females will survive if treated with androgens (49).

Several observations indicate that the immunological dimorphism develops during sexual maturation. For example, in males, depression in the primary and secondary immune responses occurs shortly after the sexual maturation when testosterone level is increasing (7), while in humans, a significant elevation in the circulating levels of IgM occurs in females as compared to males at 6 years of age (8). Furthermore, the onset of juvenile rheumatoid arthritis, a disease more prevalent in females (26), usually occurs before 5 years of age (1).

Finally, it has been hypothesized that: a) immunological dimorphism may be established early in the course of lymphocyte maturation, and b) the nature and concentration of gonadal hormones within the supporting microenvironment are major factors in the development of this dimorphism (21). The latter hypothesis is supported by evidence that both the

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thymocytes and thymic epithelial cells (TEC) express receptors for estrogens (20,27) and androgens (19,31,32), and that TEC also contain progesterone receptors (14,15). Our previous results have shown clear sexual dimorphism in the composition of thymocyte subsets defined by the expression of CD4, CD8 cell surface antigens, and the TCR $\alpha\beta$ complex (33).

Broadly speaking, thymocytes at various stages of development differ from one another with respect to the level of expression of the α and β chains of the TCR, the associated nonpolymorphic CD3 complex proteins, and coreceptors CD4 or CD8. Mature thymocytes express either helper (CD4+8−) or cytotoxic/suppressor (CD4−8+) phenotype, and, hence, are termed single-positive cells. These cells comprise approximately 15% of thymocytes and are derived from CD4+8+ double-positive intermediates (roughly 80% of thymocytes belong to this pool), approximately 50% of which express the CD3-TCR $\alpha\beta$ at low levels. The precursors to the double-positive cells are the CD4−8− double-negative immature cells, which in general, do not express a complete TCR (3). Because the differentiation of T lymphocyte precursors into functionally mature cells proceeds in distinct stages that are identifiable by characteristic constellations of phenotypic markers, the significance of the composition of thymocyte subsets for monitoring T-cell maturation is clear.

In the present study we have examined the effects of gonadectomy before and after puberty on total thymic cellularity and on the various T-cell subsets in rats of both sexes in an attempt to assess significance of the sex steroids to the development and maintenance of sexual dimorphism in intrathymic T-cell maturation.

METHOD

Animals

AO rats of both sexes were obtained from the vivarium at Military Medical Academy, Belgrade. Immature (30-day-old) and adult (75-day-old) rats were bilaterally ovariectomized, orchidectomized, or sham-operated under surgical ether anesthesia. On day 30 after surgery, the animals were sacrificed by decapitation. The thymus glands were removed, carefully dissected free of parathymic lymph nodes and adherent membranous tissue, and weighed.

Preparation of Thymic Cell Suspensions

The thymic lobes were excised and placed in individual Petri dishes containing ice-cold phosphate-buffered saline (PBS). The thymocyte suspension was prepared by grinding the thymic tissue between the frosted ends of microscope slides and passing the resultant suspension through a fine nylon mesh. The single-cell suspension so obtained was washed three times in ice-cold PBS (pH 7.3) containing 2% fetal calf serum (Gibco, Grand Island, NY) and 0.01% sodium azide (PS medium). The cells were then counted in a standard hemocytometer and resuspended in an appropriate volume of PS medium. The viability of such cell preparations, as determined by Trypan blue exclusion, was routinely greater than 95%. Fluorescein isothiocyanate (FITC)-conjugated anti-CD5 monoclonal antibody (mAb) that recognizes a determinant expressed on all thymocytes and peripheral T-cells, but does not bind to β-cells, macrophages, NK cells, mast cells, or other cell types was used to determine purity of cell populations. The relative number of cells binding this mAb routinely exceeded 95%.

Flow Cytometry (FCA)

Immunofluorescence staining of thymocytes was performed using three independent systems: a) direct two-color staining with FITC-conjugated anti-CD4 (clone W3/25, Serotec, Oxford, UK) and phycoerythrin (PE)-conjugated anti-CD8 (clone MRC OX-8, Serotec) mAbs; b) indirect one-color staining with biotin-conjugated mAb, most likely directed at a constant determinant of the rat $\alpha\beta$ heterodimeric T-cell receptor (TCR) (clone R73, Serotec), as primary reagent followed by FITC-conjugated streptavidin (Becton Dickinson, Mountain View, CA); and c) direct one-color staining with FITC-conjugated anti-CD5 (clone MRC OX-19, Serotec) mAb.

Aliquots of 1×10^6 lymphoid cells in 100 μl PS medium were dispensed into conical microcentrifuge tubes, centrifuged to yield a pellet, and the supernatant fluid was decanted. For direct one- and two-color FCA, the cells were incubated for 30 min on ice with one or both mAbs simultaneously. Antibodies were previously titrated to optimal concentrations at which no aggregation was detected. After incubation, the cells were washed with three changes of PS medium.

For indirect one-color FCA, aliquots of 1×10^6 lymphoid cells were incubated with the first reagent for 30 min on ice, washed three times in PS medium, incubated for another 30 min on ice with the second reagent, and again washed three times in the same medium.

After labeling, the cells were fixed in 0.5 ml 1% paraformaldehyde and kept at 4°C in dark until analysis. All samples were analyzed on the same day on a FACScan flow cytometer (Becton Dickinson). Dead cells and debris were excluded from analysis by selective gating based on anterior and right angle scatter. Flow cytometric events (19^4) for the two-color and 5×10^3 flow cytometric events for one-color FCA were analyzed. The analyses were carried out with respect to appropriate isotypic and fluorochrome-matched controls, with Consort 30 and Lysis software (Becton Dickinson).

Statistical Analysis

Data were evaluated by one-way analysis of variance, followed by Fisher's test for comparison of different mean values.

RESULTS

Effects of Gonadectomy on the Thymic Weight

The thymic weight was significantly greater in sham-gonadectomized male rats at both day 75 ($p < 0.01$) and 30 ($p < 0.05$), as compared with that of age matched sham-operated females (Tables 1 and 2).

TABLE 1

EFFECTS OF GONADECTOMY (AT 75 DAYS OF AGE) ON THE THYMIC WEIGHT AND TOTAL THYMOCYTE YIELD

Groups	Thymic Weight (g)	Total Thymocyte Yield ($\times 10^7$)
Sham ovariectomized	0.32 ± 0.01*[a]	1.34 ± 0.02*[a]
Ovariectomized	0.74 ± 0.05*[b]	4.15 ± 0.37*[b]
Sham orchidectomized	0.52 ± 0.02*[c]	6.31 ± 0.16*[c]
Orchidectomized	0.81 ± 0.02	8.29 ± 0.07*[d]

Each value represents × ± SEM (n = 4–10).
*$p < 0.01$.
[a]Sham ovariectomized vs. sham orchidectomized.
[b]Ovariectomized vs. sham ovariectomized.
[c]Sham orchidectomized vs. orchidectomized.
[d]Orchidectomized vs. ovariectomized.

TABLE 2

EFFECTS OF GONADECTOMY (AT 30 DAYS OF AGE) ON THE THYMIC WEIGHT AND TOTAL THYMOCYTE YIELD

Groups	Thymic Weight (g)	Total Thymocyte Yield ($\times 10^7$)
Sham ovariectomized	0.36 ± 0.01*[a]	12.58 ± 0.61†[a]
Ovariectomized	0.82 ± 0.05†[b]	24.13 ± 0.33†[b]
Sham orchidectomized	0.60 ± 0.11†[c]	47.40 ± 3.75†[c]
Orchidectomized	0.95 ± 0.12	83.80 ± 5.38†[d]

Each value represents × ± SEM (n = 4–10).
*$p < 0.05$; †$p < 0.01$.
[a]Sham ovariectomized vs. sham orchidectomized.
[b]Ovariectomized vs. sham ovariectomized.
[c]Sham orchidectomized vs. orchidectomized.
[d]Orchidectomized vs. ovariectomized.

As expected from previous studies (21,54), gonadectomy of rats of either sex aged 30 or 75 days caused within 30 days a significant increase ($p < 0.01$ vs. sham-operated) in the thymic weight (Tables 1 and 2). In the rats of both age at gonadectomy, the thymic weight did not differ significantly between orchidectomized and ovariectomized animals (Tables 1 and 2).

Effects of Gonadectomy on the Total Thymocyte Yield

Similar to the thymic weight, the total thymocyte yield in sham-operated rats was significantly greater ($p < 0.01$) in males than females of the same age (Tables 1 and 2). Gonadectomy significantly increased the total thymocyte yield in the rats of both sexes, irrespective of their sexual maturity at the time of surgery (Tables 1 and 2). Contrary to thymic weight, sexual dimorphism in this respect remains after gonadectomy. Thus, in both the groups of orchidectomized rats the total thymocyte yields were significantly higher ($p < 0.01$) than those from age matched gonadectomized females (Tables 1 and 2).

Effects of Gonadectomy on the Expression of CD4, CD8 Antigens, and TCR αβ

According to the expression of CD4 and CD8 accessory molecules, rat thymocytes are divided into four main populations (CD4+8−, CD4−8+, CD4+8+, CD4−8−). The relative proportions of the four major thymocyte subsets were estimated by two-color FCA and, therefore, the cellularity of each subset was calculated.

Comparison of male and female rats subjected to sham-gonadectomy at 75 days of age revealed a marked sexual dimorphism. Thus, 30 days after the operation the percentage of the thymocytes expressing both accessory molecules (CD4+8+) was significantly ($p < 0.01$) higher, while the percentage of the thymocytes bearing neither molecule (CD4−8−) was significantly ($p < 0.01$) lower in the male as compared to the female (Fig. 1). In the male, gonadectomy resulted in a significant increase in the percentage of CD4+8− single-positive cells ($p < 0.01$ vs. corresponding sham-operated control) (Fig. 1). Gonadectomy of adult females had a different effect in this respect. In these rats a significant decrease in the percentage of CD4+8− single-positive ($p < 0.05$ vs. sham-operated controls) and CD4−8− double-negative cells ($p < 0.01$ vs. sham-operated controls) was accompanied by a significant increase ($p < 0.01$ vs. sham-operated controls) in the percentage of CD4+8+ double-positive cells (Fig. 1). After gonadectomy, the percentage of CD4+8− single-positive cells was significantly higher ($p < 0.01$), while the percentage of CD4−8− double-negative cells was significantly lower ($p < 0.05$) in male compared with female rats (Fig. 1).

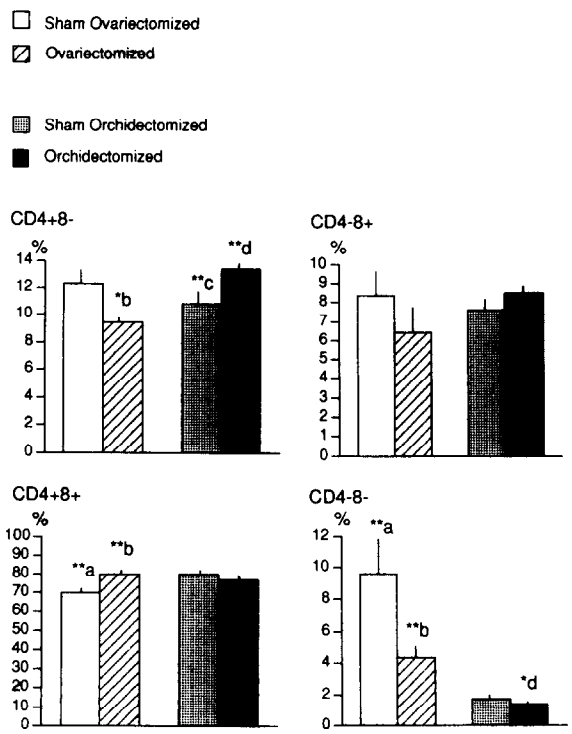

FIG. 1. Effects of gonadectomy at 75 days of age on the percentage of CD4+8−, CD4−8+, CD4+8+, and CD4−8− thymocytes. The results are expressed as mean ± SEM (n = 4–10). (a) Sham ovariectomized vs. sham orchidectomized. (b) Ovariectomized vs. sham ovariectomized. (c) Sham orchidectomized vs. orchidectomized. (d) Orchidectomized vs. ovariectomized. **$p < 0.01$; *$p < 0.05$.

In the male rats sham-operated at age of 75 days, the absolute numbers of CD4+8−, CD4−8+ and CD4+8+ thymocytes were significantly higher ($p < 0.01$) compared with those in sham-operated female rats (Table 3). The absolute number of cells in the each of these three subsets was increased significantly after gonadectomy in the adult rats of both sexes. Additionally, the cellularity of each of these three subsets was significantly higher in orchidectomized than in ovariectomized rats (Table 3).

The expression of TCR αβ was also analyzed. In adult rodents TCR αβ molecular complex is mainly expressed on the surface of more mature single-positive thymocytes or T-cells as an αβ heterodimer associated with the CD3 complex (10,37,39,48). Additionally, double-positive CD4+8+ thymocytes express also αβ heterodimer, but at low density (10,39). The expression of αβ TCR was evaluated using R73 mAb. In the adult rat thymus, mature medullary cells express the R73 determinant at the same level as peripheral T-cells, while only 6% of CD4+8+ are R73 positive. In the male rats sham-operated at 75 days of age the percentage of R73+ thymocytes was significantly lower ($p < 0.01$) compared with that in sham-operated females (Fig. 2). After gonadectomy the percentage of these cells significantly increased ($p < 0.05$) in males but decreased in females ($p < 0.01$) over the appro-

TABLE 3

EFFECTS OF GONADECTOMY (AT 75 DAYS OF AGE) ON THE ABSOLUTE NUMBER OF CD4+8− CD4−8+, CD4+8+, CD4−8−, AND TCR αβ+ THYMOCYTES

Groups	Absolute Thymocyte Number ($\times 10^7$)				
	CD4+8−	CD4−8+	CD4+8+	CD4−8−	TCR αβ+
Sham ovariectomized	0.17 ± 0.02*[a]	0.11 ± 0.02*[a]	0.93 ± 0.04*[a]	0.13 ± 0.03	0.30 ± 0.04*[a]
Ovariectomized	0.39 ± 0.03†[b]	0.25 ± 0.02*[b]	3.35 ± 0.40*[b]	0.16 ± 0.03	0.57 ± 0.05*[b]
Sham orchidectomized	0.69 ± 0.07*[c]	0.47 ± 0.02*[c]	5.04 ± 0.13*[c]	0.11 ± 0.01	1.16 ± 0.06*[c]
Orchidectomized	1.11 ± 0.02*[d]	0.71 ± 0.02*[d]	6.36 ± 0.03*[d]	0.12 ± 0.01	1.75 ± 0.01*[d]

Each value represents × ± SEM (n = 4–10).
*$p < 0.01$; †$p < 0.05$.
[a]Sham ovariectomized vs. sham orchidectomized.
[b]Ovariectomized vs. sham ovariectomized.
[c]Sham orchidectomized vs. orchidectomized.
[d]Orchidectomized vs. ovariectomized.

priate sham-operated controls. Therefore, in these rats, the percentage of R73+ cells was higher ($p < 0.01$) in orchidectomized than in ovariectomized rats (Fig. 2).

The absolute number of R73+ cells was significantly higher ($p < 0.01$) in rats sham-orchidectomized at age of 75 days than in the rats sham-ovariectomized at the same age (Table 3). Gonadectomy in the adult rats of both sexes significantly increased ($p < 0.01$) the absolute number of these cells over sham-operated controls; thus, this value remained higher ($p < 0.01$) in orchidectomized rats (Table 3).

On the other hand, in the rats sham-operated at day 30 of age, only the percentage of CD4−8− double-negative cells differed between males and females (Fig. 3). The percentage of these cells was higher ($p < 0.01$) in sham-operated males (Fig. 3). Gonadectomy of the male sexually immature rats caused a significant increase ($p < 0.01$) in the percentage of CD4+8− single-positive and concomitant decrease in the percentage of both CD4+8+ double-positive ($p < 0.01$) and CD4−8− double negative ($p < 0.05$) cells (Fig. 3). However, in the females, gonadectomy at that age evoked an increase ($p < 0.01$) in the percentage of CD4+8− single-positive thymocytes only. There were no significant differences, $F(3, 25) = 1.521$, between male and female rats gonadectomized at day 30 of age in the percentage of cells belonging to any of the four major thymocyte subsets (Fig. 3).

In rats sham-operated at age of 30 days, the absolute number of thymocytes within each of the main four subsets was significantly ($p < 0.01$) greater in the male than the female (Table 4). Orchidectomy at that age caused a significant ($p < 0.01$) enrichment in the number of thymocytes belonging to each of the four main thymocyte subsets (Table 4). However, in females, gonadectomy evoked a significant increase ($p < 0.01$) in the absolute number of CD4+8− single-positive and CD4+8+ double-positive thymocytes only (Table 4). The sexual dimorphism in the cellularity of the all four major thymocyte subsets remained after gonadectomy, with the absolute number of cells in the each of these subsets being higher in the males (Table 4).

The expression of R73 determinant did not differ between male and female rats sham operated at age of 30 days (Fig. 4). Orchidectomy at that age increased the percentage of R73+ thymocytes, and, thus, after gonadectomy the percentage of

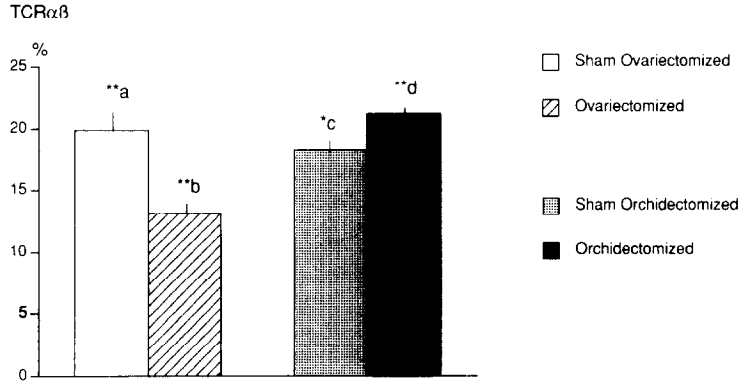

FIG. 2. Effects of gonadectomy at 75 days of age on the percentage of TCR αβ-positive thymocytes. The results are expressed as mean ± SEM (n = 4–10). (a) Sham ovariectomized vs. sham orchidectomized. (b) Ovariectomized vs. sham ovariectomized. (c) Sham orchidectomized vs. orchidectomized. (d) Orchidectomized vs. ovariectomized. **$p < 0.01$; *$p < 0.05$.

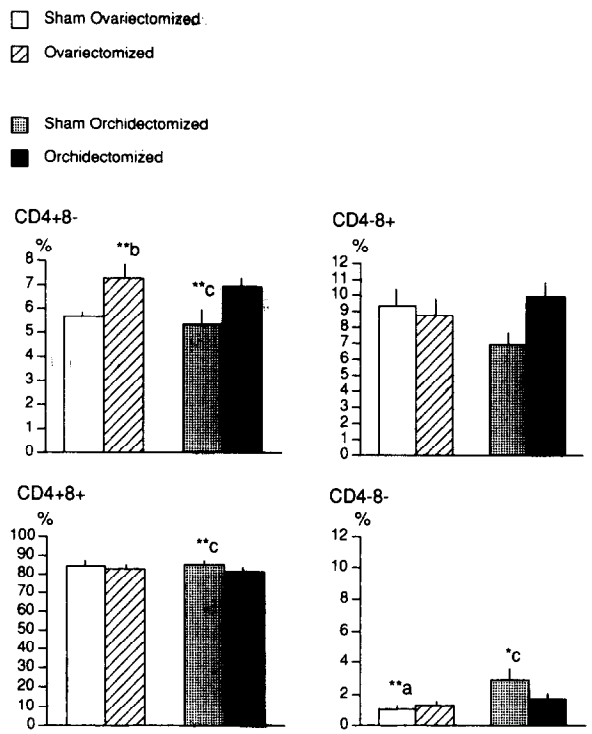

FIG. 3. Effects of gonadectomy at 30 days of age on the percentage of CD4+8−, CD4−8+, CD4+8+, and CD4−8− thymocytes. The results are expressed as mean ± SEM (n = 4-10). (a) Sham ovariectomized vs. sham orchidectomized. (b) Ovariectomized vs. sham ovariectomized. (c) Sham orchidectomized vs. orchidectomized. **p < 0.01; *p < 0.05.

these cells was significantly higher (p < 0.01) in males than females (Fig. 4).

Following sham gonadectomy at 30 days of age the absolute number of R73+ cells was higher (p < 0.05) in males than females (Table 4). Because orchidectomy at that age further increased the percentage of cells expressing R73 determinant, the absolute number of these cells remained significantly higher (p < 0.01) in orchidectomized rats (Table 4).

DISCUSSION

The results described in this study confirmed previous reports (6,21,43,45) that gonadectomy increases the total thymocyte yield in rats of both sexes, regardless of their sexual maturity at the surgery. They also show sexual dimorphism in the total thymic cellularity in both control (sham-operated) and gonadectomized (at 30 and 75 days of age) rats. In addition, the study revealed that: a) sexual dimorphism in the composition of thymocyte subsets exists in the control rats of both age; b) gonadectomy exerts marked effects on different thymocyte subsets, which depend on both the sex and maturity of animal at the time of surgery; c) sexual dimorphism in the composition of the four major thymocyte subsets persists after castration of adults, but is abolished following gonadectomy of sexually immature rats. However, sexual dimorphism in the expression of TCR $\alpha\beta$ persists after gonadectomy of both the adult and immature rats.

The expansion of the thymocyte population described in the all gonadectomized rats could be accomplished by one or more of the following mechanisms: a) the increased rate of prothymocyte entry into thymus and/or decreased rate of thymocyte egress to the periphery; b) the increased proliferation of thymocytes; and c) the decreased rate of thymocyte apoptosis, because a classic observation in endocrinology indicates that involution of many tissues is dependent on sex steroids (53).

Because gonadectomy in adults did not affect the absolute number of double-negative cells, it seems that, in these rats, neither the entry of the precursor cells into the thymus nor the proliferation of CD4−8− thymocytes was enhanced. By contrast, our finding that gonadectomy in immature rats increased the absolute number of double-negative cells, suggests that, in prepubertal rats, the entry of precursor cells and/or the rate of double-negative cell proliferation was altered. Our findings in adults are in agreement with a previous result that, following gonadectomy of adult mice, double-negative subset is not affected in this respect (43). On the other hand, other experiments in adult male mice have revealed that androgens exert a tonic inhibitory effect on the thymocyte proliferation acting probably via the specific androgen receptors (6,31,43, 45) to induce the synthesis of factors such as transforming growth factor (42,43), which is involved in inhibition of cellular proliferation or downregulating expression of a gene product critical for cell cycle entry (43). Moreover, the evi-

TABLE 4

EFFECTS OF GONADECTOMY (AT 30 DAYS OF AGE) ON THE ABSOLUTE NUMBER OF CD4+8−, CD4−8+, CD4+8+, CD4−8−, AND TCR $\alpha\beta$+ THYMOCYTES

Groups	Absolute Thymocyte Number ($\times 10^7$)				
	CD4+8−	CD4−8+	CD4+8+	CD4−8−	TCR $\alpha\beta$+
Sham ovariectomized	0.71 ± 0.02*[a]	1.18 ± 0.13*[a]	10.56 ± 0.57*[a]	0.13 ± 0.02*[a]	1.34 ± 0.02†[a]
Ovariectomized	1.75 ± 0.10*[b]	2.13 ± 0.25	19.94 ± 0.19*[b]	0.31 ± 0.01	3.09 ± 0.28
Sham orchidectomized	2.21 ± 0.24*[c]	3.68 ± 0.13*[c]	40.35 ± 0.30*[c]	0.77 ± 0.13*[c]	4.07 ± 0.22*[c]
Orchidectomized	5.82 ± 0.12*[d]	8.36 ± 0.80*[d]	68.38 ± 0.21*[d]	1.44 ± 0.20*[d]	14.78 ± 1.14*[d]

Each value represents × ± SEM (n = 4-10).
*p < 0.01; †p < 0.05.
[a]Sham ovariectomized vs. sham orchidectomized.
[b]Ovariectomized vs. sham ovariectomized.
[c]Sham orchidectomized vs. orchidectomized.
[d]Orchidectomized vs. ovariectomized.

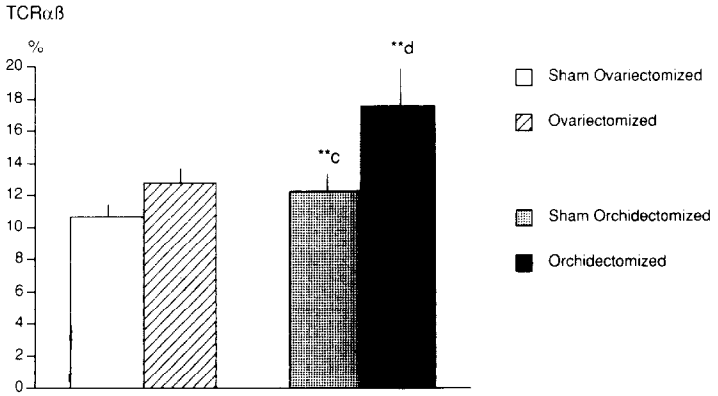

FIG. 4. Effects of gonadectomy at 30 days of age on the percentage of TCR $\alpha\beta$-positive thymocytes. The results are expressed as mean ± SEM (n = 4–10). (c) Sham orchidectomized vs. orchidectomized. (d) Orchidectomized vs. ovariectomized. $^{**}p < 0.01$.

dence that the TEC also bear androgen receptors (32,50) opens the possibility of an indirect modulation of the thymocyte proliferation. The present results are consistent with the antiproliferative androgen action on the thymocytes, because, on the one hand, in the orchidectomized rats the total number of CD4+8+ cells was increased, and on the other hand, it has been shown that CD4+8+ and CD3− or $CD3^{low}$ thymocytes are responsive to the proliferative signals that are transmitted in the absence of androgens (43).

The removal of gonads in females also increased the total thymocyte yield. However, as in the male, gonadectomy did not alter the total cellularity of double-negative subset in the female suggesting the same implications. On the other hand, substantial evidence indicates that estrogens, like androgens, have an antiproliferative effect on the thymus. Thus, it has been reported that: a) estrogen receptors are selectively localized in the large thymocyte subpopulation (α-cells) represented by lymphoblastoid cells (23,24); b) estrogen receptor expression in thymocytes increases after Con A stimulation (24); and c) estrogen decreases the [^{3}H]thymidine incorporation into thymocytes in vitro (24). In addition, the most sensitive cells to the estrogen treatment in vivo are the cortical thymocytes (36). Because the CD4+8+ double-positive cells represent the majority of the cortical thymocytes (28), our observation that the cellularity of double-positive thymocyte subset is increased in both (i.e., immature and adult) groups of ovariectomized rats is in line with the previous findings.

Because both testosterone and estrogen have been shown to exert antiproliferative effects on thymocytes (24,43) and, regardless of age, sexual dimorphism in the total thymic cellularity is evident not only in control, but also in gonadectomized rats, it seems likely that the mechanisms responsible for the immunological dimorphism are not related to the presence of the gonadal steroid hormones. There are several possibilities that may explain this finding. First, a direct control of the proliferative capacity is exerted by X or Y chromosomal gene(s). Second, the thymocyte proliferation is under indirect modulatory influence of some other hormones and/or factors. Putative candidates are adrenal glucocorticoids and pituitary hormones such as GH and PRL, for at least two reasons: they are known to modify thymocyte proliferative response (5,16,30,38), and there are overt differences in the circulating levels of these hormones between males and females (11,17). Additionally, catecholamines may contribute to the sexually dimorphic responsiveness of the thymic cells to steroids and peptides because gender-specific changes in the density and distribution of thymic β_2-adrenoceptors have been shown during ontogeny (35), and catecholamine modulation of the thymocyte proliferative activity has been documented (13). Third, having in mind that: a) cells with morphological features associated with steroid secretion have been identified in the thymus (9); b) steroid hormones can be produced locally in the mouse thymus (29); and c) the ontogeny of thymus steroid production is inversely related to that of adrenal (18), it might be hypothesized that: a) a reduction in gonadal steroid production is accompanied by increased production of sex steroids by the thymus, and if so, b) the sex steroids originating from the thymus are responsible for the persistence of sexual dimorphism in the size of thymocyte population after gonadectomy.

The study also revealed that gonadectomy of adult females significantly affects the composition of thymocyte subsets. The effects of ovariectomy were opposite to those (a significant decrease in number and proportion of CD4+8+ double-positive cells followed by a parallel increase in the percentage of the CD4+8− single-positive and CD4−8− double-negative cells) evoked by treatment of thymocytes in vitro with 17β-estradiol (51). Thus, present study accords with reports that CD4+8− single-positive and CD4+8+ double-positive thymocytes are the most sensitive to the estrogen action. It has also been proposed that the double-positive thymocytes sensitive to the estrogen action are in an intermediate state of differentiation (47,51) and/or in a terminal phase of life being committed physiologically to intrathymic death (51). Therefore, it has been suggested that estrogens accelerate the physiological process of intrathymic selection and/or death (37,51). Our finding that in the adult rats ovariectomy reduced the percentage of both CD4+8− single-positive and TCR $\alpha\beta$+ cells, supports the latter hypothesis. It is also in keeping with the assumption that estrogen treatment may create a functional imbalance by increasing both the percentage of TCR $\alpha\beta$+ thymocytes (44,51) and the ratio of CD4+8− to CD4−8+ cells in the mature thymocyte subset (51).

Gonadectomy of adult males produced effects on percentage of both CD4+8− and TCR $\alpha\beta$+ cells that were opposite to those observed after gonadectomy in the female. However, it has been reported that in adult C57BL/6 mice orchidectomy produces a significant decrease in the relative proportion of CD4−8+ single-positive thymocytes and, thus, shifts the T-

cell balance toward the CD4+ helper subset (40). This discrepancy might be ascribed to the fact that sequence of events leading to maturation of T-cells differs even between different strains of mice (37) and, therefore, most probably, between mice and rats, as well. To support this is finding that castration of adult male BDF1 (C57BL/6 × DBA/2)F1 mice induces a decrease in the percentage of CD4+8− single-positive thymocytes (2).

The present results also indicate that, in the rats of both sexes, the effects of gonadectomy on the thymocyte maturational sequence depend on sexual maturity of animal at the time of surgery. They, thus, confirm our previous findings in male rats (34). It has also been shown in adult rats that the effects of androgen deprivation on thymocyte phenotypic profile differ from those evoked by a genetically induced androgen resistance in Tfm/Y mice (41).

The results clearly show that sexual dimorphism in the composition of thymocyte subsets persists after the gonadectomy of adult rats. This finding might be explained by reasons similar to those stated previously to explain persistence of sexual dimorphism in the total thymocyte number.

According to the present results, gonadectomy of females at the age of 30 days elicited a significant increase in the percentage of CD4+8− single-positive cells, confirming that this subset is sensitive to estrogen action. However, in contrast to the adults, ovariectomy of immature rats had no effect on the percentage of CD4+8+ double-positive or CD4−CD8− double-negative cells. Similarly, the subset of TCR $\alpha\beta$+ cells remained unaltered. This observation suggests that sensitivity of thymocyte subsets to the estrogen action changes with sexual maturation. Gonadectomy of immature (30 days) male rats affected different thymocyte subsets. Thus, in these rats, like those castrated at 75 days of age, not only was there an increase in the percentage of CD4+8− single-positive thymocytes but, in addition, there were changes in double-positive and double-negative subsets. Furthermore, rats orchidectomized at 30 days exhibited an increase in both the percentage and total number of TCR $\alpha\beta$+ cells similar to that of adult castrates. These observations also support the hypothesis of thymocyte distinct sensitivity to sex steroids at different age.

Our results also indicate that gonadectomy at 30 days abrogates the sexual dimorphism in the expression of accessory molecules present in the control rats, but facilitates the development of gender-specific changes in the percentage of thymocytes bearing TCR $\alpha\beta$. Because it has been shown that dimorphic immune response is fully established only after the puberty (1,7,8,43), and as sexual dimorphism in the expression of TCR $\alpha\beta$ did not exist in the rats sham gonadectomized at age of 30 days, it seems logical to suppose that sexual dimorphism in the expression of accessory molecules and TCR $\alpha\beta$ is not fully accomplished at the same time during ontogenesis; once the dimorphism is fully established, it can be only modulated, but not abolished by deprivation of the gonadal hormones. If this is so, it might be hypothesized that the gonadal steroids are more important for the development than for the maintenance of the immunological sexual dimorphism.

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