Acta Veterinaria (Beograd), Vol. 46. No. 5-6, 271–276, 1996.

UDK 619:612.616.1

DIFFERENTIAL EFFECTS OF CASTRATION IN SEXUALLY IMMATURE AND MATURE RATS ON THE PHENOTYPIC PROFILE OF THYMOCYTES

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(Received, 6 November 1996.)

In order to assess the influence of gonadal hormones on the process of intrathymic T cell maturation, peripubertal (30 days old) and young adult (60 days old) rats were castrated and the total thymic cellularity as well as the cellularity of the main thymocyte subpopulations defined by the expression of CD4 and CD8 molecules, and TCRab were analyzed in adult (75 days old) rats. The total yield of thymocytes was significantly increased in adults castrated at day 30 over the controls. Also, the total number of CD4+/CD8+ double positive (DP) thymocytes as well as the total number of both the CD4+/CD8- and CD4-/CD8+ single positive (SP) and TCR$\alpha\beta$ positive thymocytes were significantly increased in these rats. However, castration performed in young adult rats affected neither the total thymic cellularity nor the cellularity of the main thymocyte subpopulations. Results clearly indicate that: a) castration performed before sexual maturity affects the process of intrathymic T cell maturation in adults and b) the process of intrathymic T cell development is not altered in adults of the same age castrated in very early adulthood.

Key words: rat thymus, thymocyte phenotypic characteristics, sex hormones, sexual maturation.

INTRODUCTION

It is well known that maturation of the hypothalamopituitary-gonadal axis and thymo-lymphatic axis are interrelated (Grossman, 1985). Pre-pubertally performed orchidectomy prevents thymic involution while orchiectomy of adult animals results in remarkable enlargement of the regressed thymus (Grossman, 1985; Greenstein et al. 1986). Also, it has been shown that androgens can affect the process of intrathymic T cell maturation (Grossman, 1985). In support of the specificity of androgen action it should be added that receptors for androgens have been demonstrated in both the reticuloepithelial cells and thymocytes (Leposavić and Mičić, 1992). However, data describing intrathymic changes during the process of T cell maturation in relation to gonadal deprivation performed at different stages of sexual maturity are still lacking.

Having that in mind, we orchidectomized rats during the peripubertal period when the most dramatic changes in gonadal development occur and analyzed thymic cellularity and expression of major antigens of differentiation on the thymocytes in adults. The effects of peripubertal gonadectomy were compared to the effects of the same treatment performed in early adulthood.

MATERIALS AND METHODS

Animals. Male rats of the inbred AO strain were bilaterally orchidectomized under Nembutal anaesthesia (Serva, Heidelberg, 40 mg/kg) at the ages of 30 or 60 days. Sham-operated and intact animals were used as controls. Since the sham-operated group was indistinguishable from the non-operated, the data from these two groups were pooled. Each experimental group consisted of at least 6 animals. All animals were sacrificed at the age of 75 days by decapitation. Their thymuses were carefully removed 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. Then the single-cell suspension was washed three times in ice-cold PBS (pH 7.3) containing 2% fetal calf serum (GIBCO Laboratories, 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%.

Flow cytometry. Immunofluorescence staining of thymocytes wasperformed using two independent systems: (a) direct two-color staining with fluorescein isothicyanate (FITC)-conjugated anti CD4 (clone W3/25, Serotec Ltd, Oxford, UK) and phycoerythrin conjugated (PE) anti CD8 (clone MRC OX-8, Serotec Ltd, Oxford, UK) mAbs and (b) indirect onecolor staining with biotin-conjugated mAb, most likely directed at a constant determinant of the rat α,β heterodimeric T cell receptor (TCR) (clone R73, Serotec Ltd, Oxford, UK), as primary reagent followed by FITC conjugated streptavidin (Becton Dickinson, Mountain View, CA).

Aliquots of 1 x 106 lymphoid cells in 100 μl of PS medium were dispensed into conical microcentrifuge tubes, centrifuged to yield a pellet, and the supernatant decanted. For direct two-color FCA the cells were incubated for 30 min on ice with both mAbs simultaneously. Antibodies were previously titrated to optimal concentrations at which no aggregation was detected. After the incubation the cells were washed with three changes of PS medium.

For indirect one-color FCA aliquots of 1 x 106 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 40C in the dark until analysis. All samples were analyzed on the same day on

a FACScan flow cytometer (Beston Dickinson, Mountain View, CA). A total of 10^4 flow cytometric events for the two-color and $5x10^3$ 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, Mountain View, CA).

Statistical analisis. The results were expressed as mean ± S.E.M. The significance of differences between means was analyzed by Student's t-test.

RESULTS

The total yield of thymocytes in the rats castrated at day 30 was significantly increased ($84 \times 10^7 \pm 0.12 \times 10^7$ vs. $47 \times 10^7 \pm 0.08 \times 10^7$ in the controls; $p<0.01$). However, in the rats subjected to the surgery at day 60 no significant changes in the total thymocyte number ($47 \times 10^7 \pm 0.06 \times 10^7$ vs. $47 \times 10^7 \pm 0.08 \times 10^7$ in the controls; $p>0.05$) were detected.

The flow cytometric analysis showed a significant increase in both the total number (Figure 1) and percentage(6.93% ± 0.24% vs. 5. 5.36% ± 0.54% in the

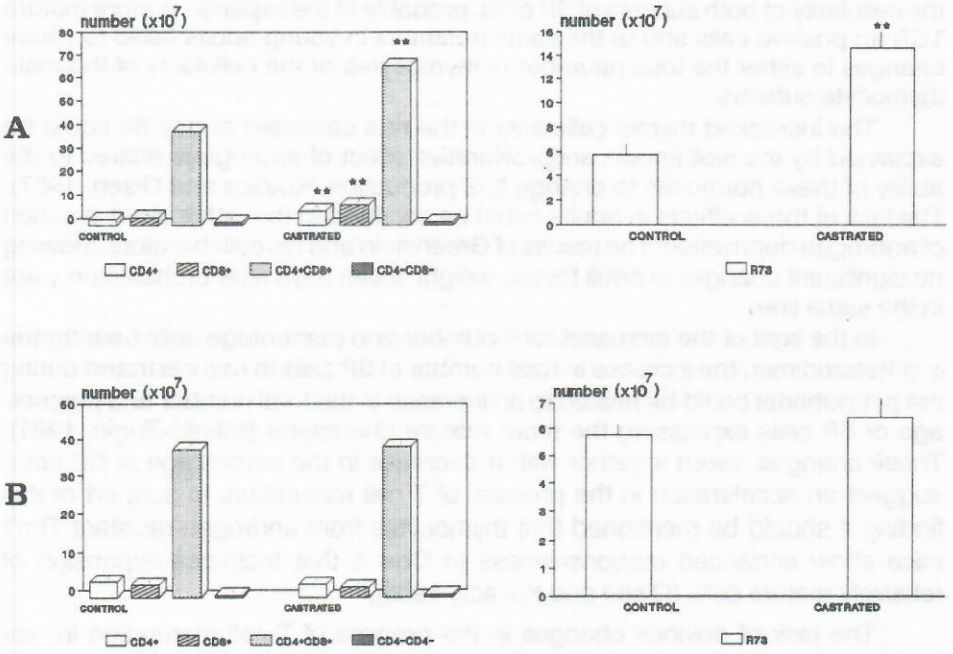

Figure 1. Total number of cells belonging to $CD4^+/CD4^-/CD8^+$ SP, $CD4/CD8^+$ DP, $CD4^-/CD^-$/DN and R73+ subpopulations in: a) rats castrated at 30 days old and B) rats castrated at 60 days old.
** $p < 0.01$

controls; $p<0.01$) of the $CD4^+/CD8^-$ SP cells, as well as an increase in the cellularity (Figure 1) and percentage (9.95% ± 1.02% vs. 6.98%± 0.61% in the

controls; $p<0.05$) of $CD4^-/CD8^+$ SP thymocytes in rats castrated at day 30. In the same animals the cellularity of $CD4^+/CD8^+$ DP thymocytes was significantly increased (Figure 1), but the relative proportion of these cells was significantly reduced (81.41% ± 0.43% vs. 84.78% ± 0.22% in the controls; $p<0.01$).

In the rats subjected to the surgery at day 30 the changes in SP subsets were accomparied by an increase in the total number (Figure 1) and percentage (17.60% ± 2.36% vs. 12.40% ± 0.91% in the controls; $p<0.05$) of thymocytes binding R73 mAb.

In the rasts castrated as adults neither the total number (Figure 1) nor percentage of cells belonging to the four major thymocyte subsets was altered. Also neither the total number (Figure 1) nor percentage of R73 positive thymocytes was significantly affected.

DISCUSSION

The present results clearly indicate that: a) removal of gonads during the peripubertal period significantly increases the total thymic cellularity as well as the cellularity of both subsets of SP cells, probably at the expense of more mature TCR ab positive cells and b) the same treatment in young adults failed to cause changes in either the total naumber of thymocytes or the cellularity of the main thymocyte subsets.

The increased thymic cellularity in the rats castrated at day 30 could be explained by the well-known antiproliferative effect of androgens related to the ability of these hormones to change IL-2 production (Kovacs and Olsen, 1987). The lack of these effects in adults could be ascribed to the rather short duration of androgen deprivation. The results of Greenstein and his collaborators showing no significant changes in adult thymic weight seven days after orchidectomy are in the same line.

In the light of the increaset total number and percentage cells bearing the α, β heterodimer, the increase in total number of SP cells in rats castrated during the peripubertal could be related to an increase in the total number and percentage of SP cells expressing the more mature phenotype (Nikolić-Žugić, 1991). These changes, taken together with a decrease in the percentage of DP cells, suggest an acceleration in the process of T cell maturation. In support of this finding it should be mentioned that thymocytes from androgen-resistant TfmY mice show enhanced responsiveness to Con A that indicates expansion of relatively mature cells (Olsen and Kovacs, 1989).

The lack of obvious changes in the process of T cell maturation in rats orchidectomized in early adulthood could be ascribed either to the different sensibility of this process to androgen action at distinct periods of sexual maturity or to the rather short time (15 days) allowed for expression of the effects caused by androgen deprivation.

To exclude on of these two possibilities the effects of long-term gonadal deprivation in adults should be investigated.

Acknowledgement

This work was supported by the Ministry of Science and Technology of the Republic of Serbia.

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RAZLIČITI EFEKTI KASTRACIJE POLNO NEZRELIH I ZRELIH PACOVA NA FENOTIPSKI PROFIL TIMOCITA

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SADRŽAJ

U cilju ispitivanja uticaja gonadnih hormona na proces sazrevanja T ćelija u timusu kastrirani su peripubertetni (30 dana stari) i odrasli (60 dana stari) pacovi. U jednih i drugih, 75. dana života, određivan je ukupan broj timocita, kao i broj timocita unutar osnovnih timocitnih subpopulacija, koje su izdvojene na osnovu ekspresije eksesornih CD4 i CD8 molekula i α, β T ćelijskog kompleksa (TCR). Ukupan broj timocita bio je značajno povećan u pacova koji su kastrirani 30. dana života u poređenju sa kontrolnim, "lažno" operisanim životinjama. U ovih životinja došlo je i do značajnog povećanja ukupnog broja: $CD4^+/CD8^+$ dvostruko pozitivnih (DP), $CD4^+/CD8^-$ i $CD4^-/CD8^+$ jednostruko pozitivnih (SP), kao i TCR α β pozitivnih timocita. Međutim, kastracija adultnih pacova nije uticala na ukupnu celularnost timusa, kao ni na celularnost glavnih timocitnih subpopulacija. Rezultati jesno pokazuju da: a) kastracija pre sticanja polne zrelosti značajno menja proces sazrevanja T ćelija u timusu pacova i b) proces sazrevanja T ćelija u timusu nije bitno poremećen 15 dana nakon kastracije mladih polno zrelih pacova.