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AGE-ASSOCIATED RESHAPING OF THE THYMOCYTE DIFFERENTIATION MODEL IN MALE RATS

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In order to elucidate the features of putative age-related changes in the intrathymic T cell maturation sequence, the thymus weight, thymocyte yield and relative proportions of thymocyte subsets were analyzed at distinct maturational stages (delineated by analysis of the expression of CD4/CD8 coreceptor molecules and TCR molecular complex) in male AO rats, from the peripubertal period (1 month of age) until 10 months of age. In 2-month-old rats both the thymus weight and lymphoid content were greater than in 1-month-old rats. The values of both parameters in 4-month-old rats were reduced to the corresponding values in 1-month-old rats. These levels were sustained until 10 months of age. However, the thymocyte composition was subjected to substantial changes during the whole period studied, probably due to alterations in action of both extrinsic and intrinsic factors which influence the ability of the thymus to support T cell maturation and/or feedback regulatory action of intrathymic CD4+ T cells on thymocytopoiesis (between the age of 7 and 10 months).

Key words: aging, male rats thymus, thymus size, T cell differentiation

INTRODUCTION

The involution of the thymus, beginning after puberty, is believed to represent one of the main factors contributing to the phenomenon of immunosenescence. The immunological aging that is a synonym for immunosenescence is currently viewed as part of a continuum of developmental processes, encompassing complex reorganizational events, compensatory mechanisms and qualitative alterations in the function of the immune system (Bodey *et al.*, 1997; Quaglino *et al.*, 1998, Effros and Globerson, 2000; Globerson and Effros, 2000). Namely, it has been widely accepted that the thymus involution-evoked restructuring of the T cell compartment plays a critical role in inducing changes, not only in cell-mediated, but also in humoral immune response occurring during the course of aging (Cossarizza *et al.*, 1997; Pawelec *et al.*, 1999; Linton and Thoman, 2001). Whether these changes in the immune

system are linked to the increased frequency of various diseases usually associated with aging, such as autoimmune phenomena, cancer and infection diseases remains to be definitely answered, although a temporal relationship has been shown (Barnet, 1970).

The thymus provides a microenvironment within which the bone marrow-derived progenitors proliferate, differentiate and undergo stringent selection processes to create a fully functional population of major histocompatibility complex (MHC) restricted, self tolerant T cells. The microenvironment is constituted mainly by an extracellular matrix and by different stromal cells (i.e. epithelial cells, macrophages, dendritic cells, fibroblasts) which influence the developing thymocytes via cell surface and secreted molecules (Boyd *et al*, 1993; Anderson *et al*., 1996; Bleul and Boehm, 2000). Due to their heterogeneity these cells, create a range of different microenvironmental niches through which the developing T cells pass receiving signals for positioning, proliferation, expression of cell surface molecules such as CD4 and CD8, T cell receptor (TCR) gene rearrangement, positive and negative selection and functional maturation (Boyd *et al*, 1993; Anderson *et al*., 1996).

Although it has been assumed that the changes within the intrathymic T cell maturation process reflecting alterations within the thymus microenvironment, are mainly responsible for remodeling of the T cell compartment which occurs with advancing age (Fabris *et al*, 1988; Globerson and Effros, 2000; Linton and Thoman, 2001), the nature of these alterations has not yet been fully elucidated.

Following the reasoning presented, and having in mind that the process of intrathymic T cell maturation can be monitored (since thymocytes at distinct stages of development express characteristic surface constellations of differentiation antigens) the thymocyte composition (i.e. relative proportions of the main thymocyte subsets delineated by expression of CD4/CD8 coreceptor molecules and TCR) was analyzed in male AO rats from the peripubertal stage (1 month of age) until 10 months of age in order to identify putative changes in T cell maturational sequence

MATERIAL AND METHODS

Experimental animals. Male inbred AO rats aged 1, 2, 4, 7 and 10 months were used in the present experiment. The animals were kept under routine laboratory conditions and provided with food and water *ad libitum*. The rats were killed by decapitation and their thymuses were removed carefully, freed from extraneous tissue, weighed and further processed for flow cytometric analyses. Each group consisted of at least 5 rats.

Flow cytometric analysis: Thymocyte suspensions for flow cytometric analysis were prepared by passing the relevant tissue through stainless steel mesh into ice-cold phosphate-buffered saline PBS (pH 7.3) containing 5% fetal calf serum (Gibco, Grand Island, NY, USA) and 0.01% sodium azide (PS medium). Following three washes in PS medium, the thymocyte single-cell suspensions were counted in a standard haemocytometer, adjusting the cell concentration to 1×10^7 cells/ml. The viability of thymic cell preparations, as determined by trypan blue exclusion, was routinely greater than 95%.

Direct two-color or indirect one-color immunofluorescence labeling was performed for detecting thymocyte CD4/CD8 and TCR$\alpha\beta$ molecules,

respectively. Briefly, aliquots of 1 X 10^6 thymocytes in 100µl PS medium were dispensed into conical microcentrifuge tubes, centrifuged to yield a pellet and the supernatant fluid decanted. The cells were incubated for 30 min at 4°C either a) simultaneously with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (W3/25, Serotec, Oxford, UK) and phycoerythrin (PE)-conjugated anti-CD8 (MRC OX-8, Serotec) monoclonal antibodies (mAb) or b) with biotin-conjugated anti-TCRαβ (R73, Serotec) mAb as the primary reagent. After incubation, the aliquots were washed three times in PS medium, and then either a) fixed or b) incubated with the second step reagent FITC-conjugated streptavidin (Becton Dickinson, Mountain View, CA, USA).

After labeling, the cells were fixed in 0.5 ml 1% paraformaldehyde and kept at 4°C in the dark until analysis. All samples were analyzed on the same day on a FACScan flow cytometer (Becton Dickinson). Dead cells and debris were routinely excluded on the basis of forward light scatter and side light scatter. Usually, 5 x 10^3 or 2 x 10^4 flow cytometric events for one-color and two-color immunofluorescence staining, respectively, were analyzed. Appropriate isotypic and fluorochrome-matched controls were included and cell aliquors were analyzed using Consort 30 software (Becton Dickinson).

Statistical analysis. The data were expressed as mean SEM. One-way ANOVA followed by the LSD test for *post hoc* comparisons was performed for determining the differences between means, using the program SPSS 7.5 for Windows.

RESULTS

Thymus weight and thymocyte yield

In male AO rats, both the thymus weight and thymocyte yield significantly ($p<0.01$) increased between 1 and 2 months of age. Subsequently, the values of both parameters dropped markedly, so that in 4-month-old rats both the thymus weight and thymocyte yield were significantly ($p<0.01$) lower than in 2-month-old rats, but neither value was significantly ($p>0.05$) different from the corresponding value in 1-month-old rats. From the age of 4 months until the last time-point studied (10 months of age) the both thymus weight and thymocyte cellularity remained unaltered. (Table 1).

Expression of CD4/CD8 on the thymocytes

The analysis of the CD4/CD8 coreceptor molecule expression on thymocytes revealed that in 2-month-old male AO rats only the relative proportion of CD4-8- double negative (DN) cells was significantly ($p<0.05$) lower than in 1-month-old rats. (Figure 1).

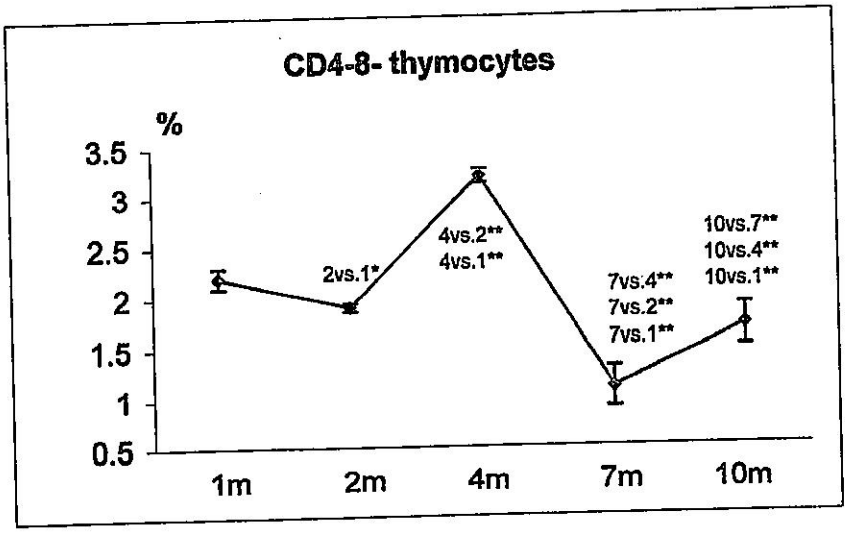
However, in 4-month-old rats, the percentage of CD4-8- DN cells and those of both CD4+8- and CD4-8+ single positive (SP) cells were significantly ($p<0.01$) higher than in 2-month-old rats. In contrast these rats the relative proportion of CD4+8+ double positive (DP) cells was significantly ($p<0.01$) reduced compared to that in 2-month-old animals (Fig. 1).

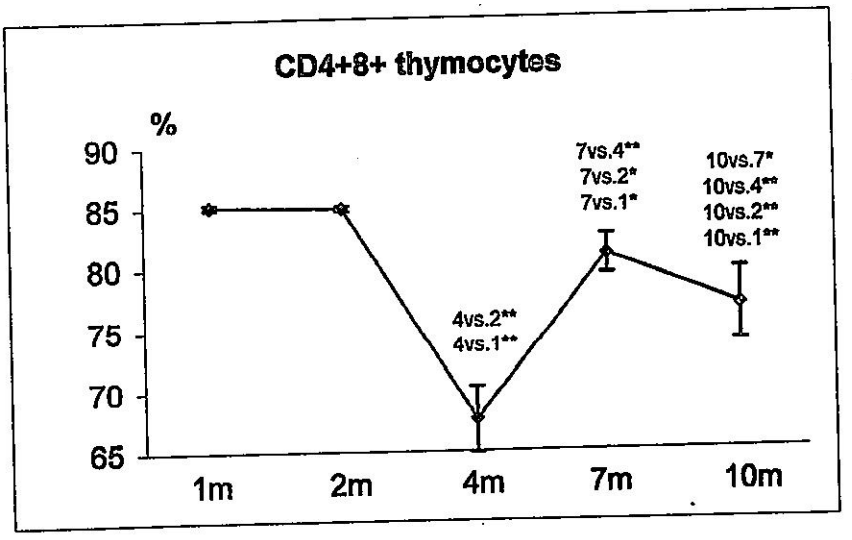
In 7-month-old rats the relative proportion of CD4-8- DN thymocytes was significantly ($p<0.01$) lower than the corresponding value, not only in 4-month-old, but also in 2-month-old rats. However, in the same animals, the percentage of CD4+8+ double positive (DP) cells was significantly ($p<0.01$) higher than that in 4-month-old rats, although it was still significantly ($p<0.01$) lower than the level in

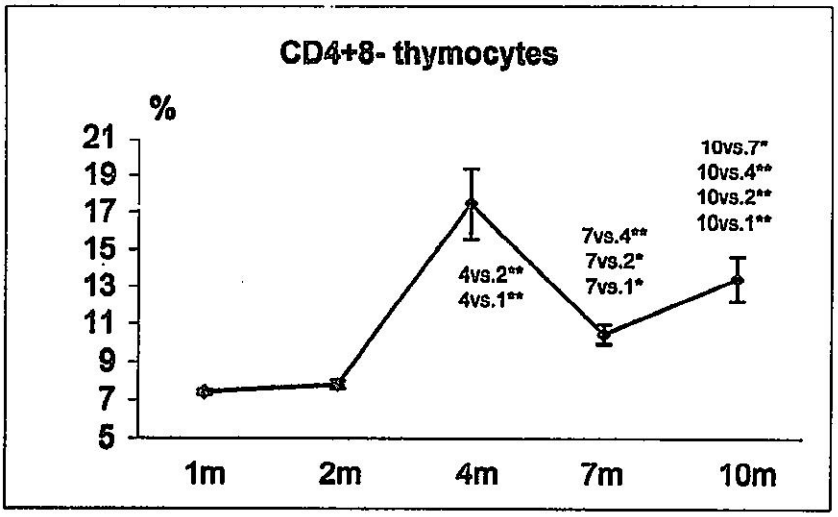
Table 1. Thymus weight and thymocyte yield in male AO rats of different age (from 1 month to 10 months).

	Male AO rats				
	1-month-old	2-month-old	4-month-old	7-month-old	10-month-old
Thymus weight (g) (X ± SEM)	0.29 ± 0.02	0.50 ± 0.02$^{2vs.1**}$	0.34 ± 0.02$^{4vs.2**}$	0.36 ± 0.04$^{7vs.2**}$	0.34 ± 0.05$^{10vs.2**}$
Thymocyte yield ($x10^7$) (X ± SEM)	51.65 ± 3.67	90.46 ± 6.90$^{2vs.1**}$	61.73 ± 4.71$^{4vs.2**}$	63.85 ± 4.87$^{7vs.2**}$	61.19 ± 4.67$^{10vs.2**}$

$^{2vs.1}$ 2- vs. 1-month-old rats; $^{4vs.2}$ 4- vs. 2-month-old rats; $^{7vs.2}$ 7- vs. 2-month-old rats; $^{10vs.2}$ 10- vs. 2-month-old rats
**p<0.01
n=5-7


CD4-8- thymocytes
%
3.5
3
2.5
2
1.5
1
0.5
2vs.1*
4vs.2**
4vs.1**
7vs.4**
7vs.2**
7vs.1**
10vs.7**
10vs.4**
10vs.1**
1m
2m
4m
7m
10m


CD4+8+ thymocytes
%
90
85
80
75
70
65
4vs.2**
4vs.1**
7vs.4**
7vs.2*
7vs.1*
10vs.7*
10vs.4**
10vs.2**
10vs.1**
1m
2m
4m
7m
10m



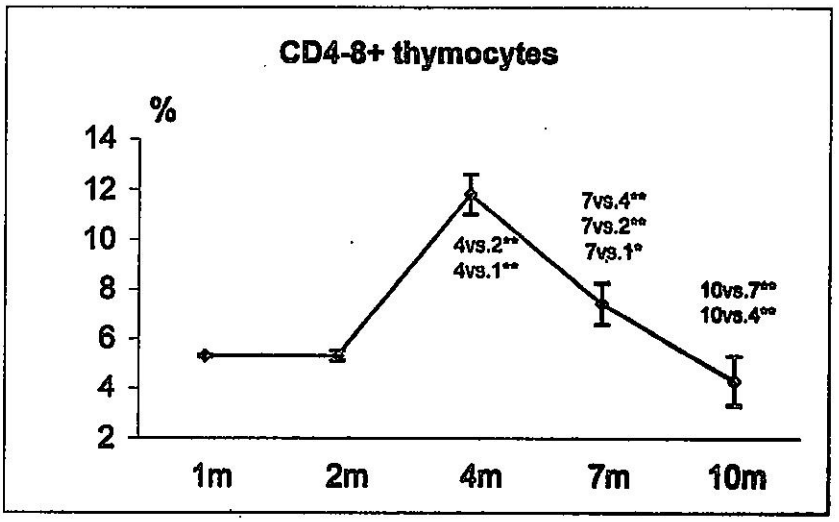


Figure 1. The relative proportions of CD4-8- (DN), CD4+8+ (DP), CD4+8- and CD4-8+ (SP) thymocytes in male AO rats of different age (from 1 to 10 months). The results are expressed as mean ±S.E.M. (n = 5-7)
* $p<0.05$; ** $p<0.01$
2vs.1 = 2 months of age vs. 1 month of age;
4vs.1 = 4 months of age vs. 1 month of age; 4vs.2 = 4 months of age vs. 2 months of age;
7vs.1 = 7 months of age vs. 1 month of age; 7vs.2 = 7 months of age vs. 2 months of age;
7vs.4 = 7 months of age vs. 4 months of age;
10vs.1 = 10 months of age vs. 1 month of age; 10vs.2 = 10 months of age vs. 2 months of age; 10vs.4 = 10 months of age vs. 4 months of age; 10vs.7=10 months of age vs. 7 months of age

2-month-old animals. Furthermore, in 7-month-old rats the percentages of both CD4+8- and CD4-8+ SP thymocytes were significantly ($p<0.01$) lower than in 4-month-old rats, but still higher than in 2-month-old rats (Fig. 1).

In 10-month-old rats the percentage of CD4-8- DN cells was significantly ($p<0.01$) higher than that in 7-month-old rats, so its value was not significantly ($p>0.05$) different from that in 2-month-old rats, although it still remained lower ($p<0.01$) than the level in 4-month-old rats. In addition, in 10-month-old animals, the relative proportion of CD4+8- SP thymocytes was significantly ($p<0.01$) higher than those in 7- and 2-month-old rats, but lower ($p<0.01$) than that in the 4-month-old rats. Furthermore, in 10-month-old rats, the percentage of CD4+8+ DP ($p<0.05$), and CD4-8+ SP ($p<0.01$) thymocytes were reduced compared to 7-month-old rats. The relative proportion of CD4+8+ DP cells still remained higher (p.01) than that in 4-month-old rats, although it was significantly ($p<0.01$) lower than the level in 2-month-old rats. However, the percentage of CD4-8+ SP cells was reduced to a value not significantly ($p<0.05$) different from the corresponding value in 2-month-old rats (Figure 1).

*Expression of TCR*αβ on the thymocytes

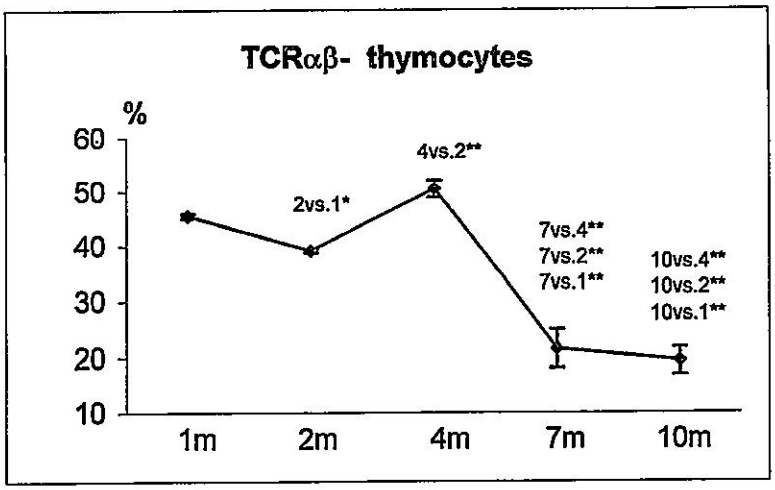
According to the intensity of fluorescence two subsets of thymocytes expressing TCRαβ were distinguished: a) the thymocytes expressing TCRαβ at a low level ($TCR\alpha\beta^{low}$) and b) thymocytes expressing high levels of TCRαβ ($TCR\alpha\beta^{high}$).

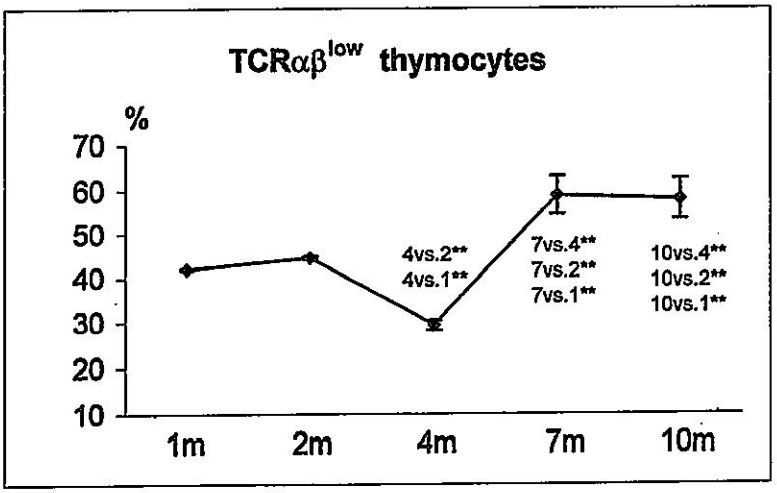
Compared with 1-month-old rats, in 2-month-old rats, a significant ($p<0.05$) decrease in the percentage of thymocytes without detectable TCRαβ on their surface (TCRαβ -) was revealed together with significant ($p<0.01$) increase in TCR $\alpha\beta^{high}$ (Figure 2).

The percentage of TCRαβ - cells was significantly ($p<0.01$) increased in the 4-month-old compared to 2-month-old rats returning to a value not significantly different from the corresponding value in 1-month-old rats. In 4-month-old rats the percentage of $TCR\alpha\beta^{low}$ thymocytes was significantly ($p<0.01$) reduced, while that of $TCR\alpha\beta^{high}$ cells was significantly ($p<0.05$) increased compared to 2-month-old rats (Figure 2).

Conversely, between 4 and 7 months of age, there was a significant ($p<0.01$) drop in the relative proportion of TCRαβ - cells to a value significantly ($p<0.01$) lower than the value of corresponding parameter in 2-month-old rats. The decrease in the percentage of TCRαβ - cells in 7-month-old rats was accompanied by a significant ($p<0.01$) rise in the percentage of $TCR\alpha\beta^{low}$ cells, to a level higher ($p<0.01$) than that in 2-month-old rats. Since the percentage of $TCR\alpha\beta^{high}$ thymocytes did not significantly ($p>0.05$) alter between 4 and 7 months of age, the value of this parameter was also higher in 7-month-old rats ($p<0.01$) than in 2-month-old rats (Figure 2).

Between 7 and 10 months of age no significant ($p>0.05$) alterations in the relative proportions of any subset of cells delineated by the expression of TCR αβ was detected. Hence, similarly to 7-month-old rats, the percentage of TCRαβ - cells was significantly ($p<0.01$) lower, while that of $TCR\alpha\beta^{low}$ cells was significantly ($p<0.01$) higher in 10-month-old rats than the appropriate parameters in 4- and 2-month-old rats. In addition, also as for 7-month-old rats, the percentage of $TCR\alpha\beta^{high}$ cells was significantly ($p<0.01$) higher in 10-month-old rats than that in 2-month-old rats (Figure 2).


TCRαβ- thymocytes
%
60
50
40
30
20
10
1m
2m
4m
7m
10m
2vs.1*
4vs.2**
7vs.4**
7vs.2**
7vs.1**
10vs.4**
10vs.2**
10vs.1**


TCRαβ^low thymocytes
%
70
60
50
40
30
20
10
1m
2m
4m
7m
10m
4vs.2**
4vs.1**
7vs.4**
7vs.2**
7vs.1**
10vs.4**
10vs.2**
10vs.1**

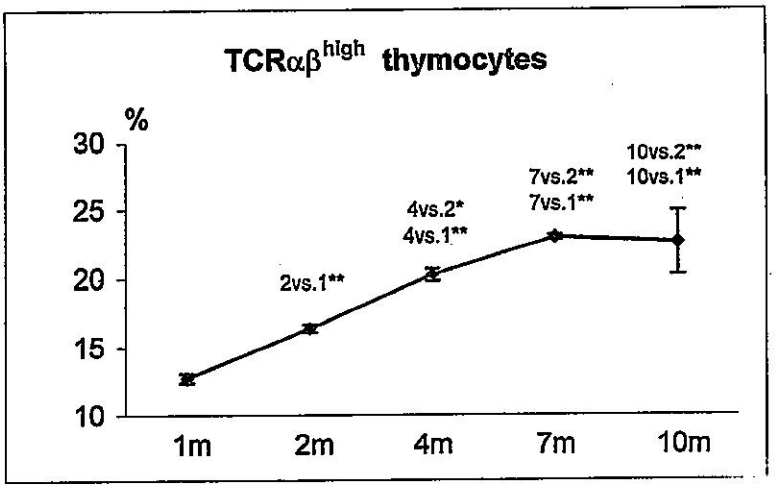


Figure 2. The relative proportions of thymocytes not expressing TRC (TCR -), expressing low levels (TCR ^{low}) and high levels of TCR (TCR ^{high}) in male AO rats of different age (from 1 to 10 months of age).The results are expressed as mean S.E.M. (n = 5-7)
* $p<0.05$; ** $p<0.01$
2vs.1 = 2 months of age vs. 1 month of age;
4vs.1 = 4 months of age vs. 1 month of age; 4vs.2 = 4 months of age vs. 2 months of age;
7vs.1 = 7 months of age vs. 1 month of age; 7vs.2 = 7 months of age vs. 2 months of age;
7vs.4 = 7 months of age vs. 4 months of age;
10vs.1 = 10 months of age vs. 1 month of age; 10vs.2 = 10 months of age vs. 2 months of age;
10vs.4 = 10 months of age vs. 4 months of age; 10vs.7=10 months of age vs. 7 months of age.

DISCUSSION

The present study revealed that in male AO rats: a) age-associated changes in thymus weight paralleled those for thymocyte yield; b) the thymus weight and cellularity increased markedly between 1 and 2 months of age; c) the values of both parameters were significantly reduced at 4 months of age and d) they remained at that level until the last time-point studied (10 months of age).

These findings are in keeping with data showing that the thymus attains its maximum size at puberty, after which it starts progressively to involute (Grossman *et al.*, 1985; Fabris et *al.*, 1988; Utsuyama and Hirokawa, 1989). The age-associated changes in the thymus size can be related to findings suggesting that the thymus weight is influenced by two distinct groups of factors, one (GH, insulin like growth factor 1, thyroxine, triiodothyronine) causing thymic hypertrophy (Utsuyama and Hirokawa, 1989; Goya *et al.*, 1991; Hirokawa *et al.*, 1998, Globerson and Effros, 2000) and the other (mainly steroid sex hormones) producing thymic atrophy (Grossman, 1985; Utsuyama and Hirokawa, 1989; Kendall *et al*, 1990). The balance of their action changes during ontogenesis (Utsuyama and Hirokawa, 1989; Hirokawa *et al.*, 1998). Around puberty in most strains of rats the factors causing thymic atrophy become superior to those causing thymic hypertrophy, hence initiating thymus involution (Utsuyama and Hirokawa, 1989). Since the occurrence and rate of thymic involution substantially

differ among different strains of rats, e.g. rats of the Buffalo strain do not experience thymic involution (Hirokawa *et al*, 1994), genetic factors probably play a crucial role in determining the dynamics of changes and outcome of the interaction between these two groups of factors during ontogenesis. Accordingly, it seems obvious that, in rats of the AO strain, the balance between the factors exerting hypertrophy and those producing atrophic effects on the thymus: a) between 1 and 2 months of age shifts toward the former; b) then, between 2 and 4 months of age, moves in the opposite direction and c) between 4 and 10 months of age maintains a relatively steady level.

The present experiment also demonstrated that the composition of thymocyte subsets delineated by the expression of the main differentiational antigens in AO rats: a) undergoes substantial changes from 1 month of age until the last time-point studied and b) that changes occur even when the total thymic lymphoid content is stable (between 4 and 10 month of age). These findings confirm data showing that age-associated thymic structural alterations, size reductions, and changes in thymocyte development can be separated and that therefore they are causally unrelated (Lau and Spain, 2000). They are also consistent with the report that some factors (i.e. GH, insulin like growth factor 1) which influence the thymic lymphoid cell content do not affect T cell differentiation (Montecino-Rodriguez *et al.*, 1998).

The changes in thymocyte composition occurring between 1 and 4 months of age may partly be related to alterations in the concentrations of gonadal hormones occurring in the peripubertal and early adult stages of development. Thus, surgical removal of the gonads in the peripubertal and early adult stage has differential effects on thymocyte composition (Karapetrović *et al.*, 1996; Leposavić *et al*, 1996). In favor of this hypothesis are studies demonstrating, not only the presence of androgen-binding proteins in thymic tissue (Grossman *et al*, 1979; Viselli *et al*, 1995; Olsen *et al*, 2001), but also an important role of androgens in modulating thymus size and normal thymocyte development (Olsen and Kovacs, 1996; Olsen et al, 1998, 2001).

A reduced relative proportion of CD4-8- DN thymocytes, in conjunction with a lowered percentage of cells with non detectable levels of TCRαβ surface expression, in 2-month-old rats compared to those 1-month-old, suggests that the relative proportion of the most immature thymocytes, i.e. triple negative (TN) cells had decreased. This assumption further implies a decelerated thymocyte progenitor input and/or an accelerated transition of TN thymocytes to the next maturational stage in 2-month-old compared to 1-month old rats. Since in the same rats the percentage of $TCR\alpha\beta^{high}$ cells had increased, while the relative proportions of both CD4+8- and CD4-8+ SP cells was unaltered, it seems that the percentage of just positively selected $CD4+8+TCR\alpha\beta^{high}$ cells (Guidos, 1996) was increased, while that of the cells expressing both coreceptor molecules together with pre-Tα:βTCR and/or even TCRαβ but at a very low (not detectable by flow cytometry) level (CD4+8+TCRαβ - cells) (Jameson *et al.*, 1995; Guidos, 1996) was decreased. This assumption further implies that in 2-month-old rats (compared to 1-month-old rats) the transition rate of CD4+8+TCRαβ- cells to the next developmental stage (stage of $CD4+8+TCR\alpha\beta^{low}$ cell that are exposed to selection pressure) was increased, as well as the rate of positive selection, but not that of further maturation of the positively selected cells into mature SP cells.

Compared to 2-month-old rats, a significant increase in the percentage of CD4-8- DN cells, as well as in that of both CD4+8- and CD4-8+ SP thymocytes,

accompanied by a proportional decrease in the percentage of CD4+8+ DP thymocytes was found in 4-month-old rats. Since the percentages of cells not-expressing TCRαβ was also increased in 4-month-old rats, it may be assumed that the proportion of the most immature TN (CD4-8-TCRαβ-) thymocytes had increased. This assumption further indicates that the thymocyte progenitor input was enhanced and/or that the transition of TN cells to the next DP developmental stage was decelerated in these rats. The reduced percentage of CD4+8+ DP cells, probably reflected a decrease in the percentage of $TCR\alpha\beta^{low}$ cells. If so, an increased rate of thymocyte positive selection in 4-month-old rats can be supposed. However, the reduction in percentage of $CD4+8+TCR\alpha\beta^{low}$ cells might also be related to increased apoptosis induced by exposure of these cells to the adult level of androgens (Guevara-Patino *et al*, 2000). The increase in the relative proportions of both subsets of thymocytes bearing only one coreceptor molecule, coupled with the increase in percentage of $TCR\alpha\beta^{high}$ cells most likely reflects accelerated maturation of the positively selected cells into both subsets of SP cells. However, a decreased rate of the SP mature thymocyte egress to the periphery cannot be excluded. An increased rate of thymocyte positive selection and accelerated maturation of the positively selected cells toward SP cells of both phenotypes, together with the decreased total number of thymocytes that was registered in the rats of this age, might be viewed as a developmental compensatory mechanism.

The reduction in relative proportion of CD4+8- SP in 7-month-old compared with 4-month-old rats, could be, at least partly, related to a diminishing capability of aging thymus to induce the maturation of CD4+ cells. In favor of this thesis are data that in both humans and mice the recovery of naive CD4+ cells (essentially dependent on thymic activity) after treatments producing a depletion of the peripheral T cell pool is inversely related to age (Mackall *et al.*, 1995, 1997; Rice and Bucy, 1995). The decline of thymic function with advancing age may be due either to intrinsically induced alterations in the epithelial cell population, so that they become less capable to synthesize and/or release thymic factors and their receptors (Fabris *et al.*, 1988; Garcia-Suarezl *et al*, 2000) or to alterations occurring in those extrinsic mechanisms that appear to control the epithelial cell function (Fabris *et al*, 1988; 1997). Experiments in mice have demonstrated that both intrinsic and extrinsic factors can play a significant role in this respect (Bach and Beaurain, 1979; Fabris *et al*, 1988). Moreover, there is also evidence that the age-dependent immune alteration is, to a large extent, not an intrinsic and irreversible phenomenon, but rather dependent on the age-associated neuroendocrine imbalance (Fabris *et al*, 1988; 1997). To further support this thesis are data indicating that the decrease in percentage of CD4+8- SP cells registered between 4 and 7 months of age, reflects a reduction in cellularity of the mature (expressing high levels of TCRαβ) subset of SP cells since: a) the percentages of immature SP cells with either nondetectable or very low levels of TCRαβ are generally very low in rats (Hunig *et al.*, 1989; Tsuchida *et al.*, 1994; Pejčić-Karapetrović *et al.*, in press), and b) in AO rats the relative proportions of these cells do not significantly alter between 3 and 7 months of age (Pejčić-Karapetrović *et al.*, in press). However, the decrease in the percentage of CD4+8- SP cells accompanied by a reduction in the percentage of CD4-8- DN, on one side, and an increase in the percentage of CD4+8+ DP thymocytes, on the other side, can be explained in the light of the proposed feedback regulatory role of CD4+8- cells on thymocytopoiesis (Mehr *et al.*, 1996, 1997). It has been

suggested that thymocyte development is subjected to regulation through 2 feedback loops. The mature CD4+ cells, whether newly generated in the thymus or re-entrants from the periphery, exert a negative feedback on the CD4-8- DN to CD4+8+ DP transition and on CD4+8+ DP cell growth, but a positive feedback on the CD4+8+ DP to CD4+8- SP transition (Mehr *et al.*, 1996, 1997).

The progressive decrease in the relative proportion of CD4-8+ SP cells demonstrated from 4 to 10 months of age suggests an age-related decrease of differentiation of CD4+8+ DP cells into the CD8 lineage.

Furthermore, the decrease in the percentage of TCRαβ- cells found in 7-month-old rats compared to 4-month-old controls could be related to a reduction in the percentage of CD4-8- DN thymocytes, that further supports the previous thesis. On the other hand, the increase in the percentage of $TCR\alpha\beta^{low}$ cells between 4 and 7 months of age probably reflected an increase in the percentage of CD4+8+ DP cells with low expression of this molecular complex. Taking into consideration changes within subsets delineated by expression of CD4/CD8 molecules, it can be assumed that the relative proportion of $TCR\alpha\beta^{high}$ cells remained unaltered between 4 and 7 months of age due to an increase in the relative proportion of CD4+8+ DP $TCR\alpha\beta^{high}$ cells which was accompanied by a proportional decrease in the relative proportions of both subsets of SP cells, that is also consistent with the presumed decelerated maturation of CD4+8+ DP into SP cells in 7-month-old compared with 1-month-old rats.

However, between 7 and 10 months of age the percentage of CD4+8- cells increased slightly, but significantly, remaining still less than in 4-month-old rats, and this increase was followed by a rise in the percentage of CD4-8- DN thymocytes (to a value still less than in 4-month-old rats) and by a drop in the percentage of DP CD4+8+ cells (to a value still higher than that in 4-month-old rats). If we suppose that the increase in the percentage of CD4+8- SP cells reflected an increase in the percentage of mature cells of that phenotype, then it could be hypothesized that, in AO rats, between 7 and 10 months of age there is a checkpoint in the feedback control of T cell differentiation by the intrathymic CD4+ cells (i.e. that the CD4+ feedback regulatory mechanism becomes less effective). This hypothesis is in keeping with data obtained concerning the T cell development in mouse fetal stroma colonized with immature thymocytes and CD4+ T cells from young and old donors that strongly indicate that the mature CD4+ cells regulate thymocytopoiesis in an age-related differential manner (Fridkis-Hareli *et al.*, 1994; Mehr *et al.*, 1996).

No altered percentages of thymocyte subsets delineated by expression of TCRαβ between 7 and 10 months of age, coupled with the changes in thymocyte subsets defined by expression of coreceptor molecules, suggests that the percentage of TCRαβ- cells remained unaltered in spite of an increase in the percentage of CD4-8- DN cells. This probably reflects a decrease in the percentage of CD4+8+ DP cells expressing pre-Tα:βTCR and/or even TCRαβ at a very low (not detectable by flow cytometry) level (Jameson *et al.*, 1995; Guidos, 1996). This notion is consistent with the previously assumed decrease in CD4-8- DN to CD4+8+ DP differentiation produced by an attenuated CD4+ regulatory feedback action. The unaltered level of $TCR\alpha\beta^{high}$ cells might be explained by a decrease in the percentage of CD4+8+ DP and/or CD4-8+ cells expressing high levels of TCRαβ paralleled by a proportional increase in the percentage of CD4+8- SP $TCR\alpha\beta^{high}$ cells, that is not dissonant with the previously postulated decrease in the regulatory efficiency of CD4+ cells.

Although both the present and previously reported data (Fridkis-Hareli *et al.*, 1994; Mehr *et al.*, 1996) suggest that the efficiency of CD4+ cells in regulating thymocytopoiesis changes in an age-related manner, there is an obvious discrepancy in the nature of these changes. Namely, contrary to the present findings, an age-associated increase in the efficiency of CD4+ cell feedback control of thymocytopoiesis has been previously suggested (Fridkis-Hareli *et al.*, 1994; Mehr *et al.*, 1996). The discrepancy may be reconciled by data showing that age-associated changes in the composition of thymocyte subsets substantially differ among different strains of mice (Dubiski *et al.*, 1989), as well as by the fact that the thymocyte composition was not analyzed after 10 months of age in the present experiment.

In conclusion, the present findings have demonstrated that, after a marked decrease between age of 2 and 4 months thymus size and cellularity remain significantly unaltered until age the of 10-months in rats of the AO strain. Furthermore, the distribution of the main thymocyte subsets delineated by expression of CD4/8 and TCR$\alpha\beta$ undergoes substantial changes from 1-month of age until 10-months of age, most likely due to changes in the action of extrinsic and intrinsic factors modulating the ability of the thymus microenvironment to support T cell development and/or feedback regulatory action of mature intrathymic CD4+ T cells on thymocytopoiesis (between the ages of 7 and 10 months). Further investigations are underway in order to define precisely extrinsic factors influencing T cell maturation and thus, a possible route/s of intervention to ameliorate thymus function in advanced age.

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PROMENE U MODELU DIFERENTOVANJA TIMOCITA U MUŽJAKA PACOVA SA GODINAMA

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SADRŽAJ

U cilju definisanja karakteristika procesa sazrevanja T ćelija u timusu u funkciji uzrasta, u mužjaka pacova AO soja, različitog uzrasta (od 1 meseca do 10 meseci) posmatrane su: težina timusa, ukupan broj timocita, kao i relativna zastupljenost pojedinih timocitnih subpopulacija (koje su razdvojene na osnovu ekspresije CD4/8 koreceptornih molekula i antigena TCRαβ molekulskog kompleksa). U životinja uzrasta 2 meseca i težina timusa i ukupan broj timocita bili su značajno veći nego u životinja uzrasta od mesec dana. Međutim, u životinja uzrasta od 4 meseca vrednost ovih parametra nije se razlikovala od vrednosti odgovarajućih parametara sa mesec dana. Vrednosti oba ova parametra nisu se bitno menjale od 4. do 10. meseca života. Sa druge strane, naši rezulatati su pokazali značajne promene u relativnom odnosu timocitnih subpopulacija u ispitivanom periodu. Ove promene se mogu objasniti promenama u delovanju, kako spoljašnjih tako i unutrašnjih, faktora koji modulišu sposobnost timusne mikrosredine da podrži proces sazrevanja T ćelija i/ili utiču na efikasnost regulatornog delovanja intratimusnih CD4+ T ćelija, mehanizmom negativne povratne sprege, na proces sazrevanja T ćelija u timusu.