ABSTRACT

Background: The thymus is considered to be a primary sex hormone-responsive organ, which plays an important role in maintaining a competent immune system.

Aim of the Work: This research was conducted to study the orchidectomy-induced changes in the lymphocytic as well as the non-lymphocytic contents of the rat thymus using histological, immunohistochemical and histometrical methods.

Material and Methods: Twenty male adult rats were randomly divided into two groups (control and orchidectomized). The animals were housed in cages with softwood granules as bedding. They had free access to standard diet and drinking water. Animals were sacrificed by cervical dislocation after four weeks of orchidectomy. The entire thymuses were dissected out, plot dried and weighed. Organ weights were expressed as absolute and relative weight (g/100g body weight). Five-μm sections were stained using Weigert’s haematoxylin and van Gieson’s stains for fibrous tissue, Periodic acid Schiff (PAS) for glycoproteins, methyl green-pyronin for plasma cells, Unna stain for mast cells and S-100 immunoperoxidase stain for interdigitating cells.

Results: In orchidectomized rats the absolute and relative thymic weight, the mean thickness of the cortex and the cortex/medullary ratio were significantly increased in comparison to the control rats. Using image analysis and color subtraction, the area% of the interdigitating cells in cortex and medulla was calculated, there was a significant decrease in the area% of the interdigitating cells both in the cortex and the medulla of the orchidectomized rats versus the control.

Conclusions: Thymus is an androgen – responsive tissue. The extract role of interdigitating cells in age induced thymic atrophy remain to be elucidated.

INTRODUCTION

Steroids can exert a profound influence over the immune response. In general, Estrogen has immunostimulatory effect (Ansar Ahmed, et al. 1989), while androgen has immunosuppressive effect (Schuurs and Verheul, 1990). Thus, females are more susceptible to autoimmune diseases like systemic lupus erythematosus (SLE) and Hashimoto thyroiditis, while men are significantly less affected by all autoimmune diseases. On the other hand, ablation of sex hormone function or production constitutes a common therapeutic strategy for the treatment of hormone responsive tumors such as prostate and breast cancer. Moreover, previous evidence suggests that androgen ablation therapy of prostate cancer patients induces elevations in circulating lymphocytes; a change which is associated with a more favorable prognosis (Oliver and Gallagher, 1995).

The thymus is a bilobed elongated lymphoepithelial organ located in the superior mediastinum that ensures T-cell differentiation and maturation (Gartner and Hiatt, 1997). The mammalian thymus consists of two distinct lobes connected by a connective tissue isthmus. A thin connective tissue capsule surrounds each lobe and, in most species, gives rise to septae, that partially subdivide the thymus into interconnecting lobules of variable size and orientation (Haley, 2003). It undergoes age-related (chronic, physiological) involution in the course of normal ontogenetic development (Haynes, 2000). In addition, it can undergo an acute
THYMUS HYPERTROPHY IN ORCHIDECTOMIZED RATS: AN IMMUNOHISTOCHEMICAL STUDY

(age-independent) regression, defined as spontaneous transient involution. This process is induced by either exogenous or endogenous factors, such as stress and malnutrition, or by pathological events, including some infections (Turke, 1997).

Several studies have suggested that thymus is a primary sex hormone-responsive organ (Verthelyi, 2001; Yao and Hou, 2004). It is confirmed by the fact that T-cell production by the thymus is maximal during young age and then decreases significantly after the onset of puberty under the influence of sex hormones. In addition, it has been shown that the thymus involutes extensively during human and animal pregnancy (Clark and Kendall, 1994). This involution is partially due to massive cortical thymocytes death and extensive phagocytosis of immature cortical thymocytes (Kendall, 1990).

Although the histological changes that occurred in the thymus gland after gonadectomy and after administration of various sex steroids (testosterone, estrogen, and progesterone) have been studied before (Wei, et al. 2001; Oner and Ozan, 2002), however, little information is available about structural changes that occur in the non-lymphoid component of the thymus that play an important role in T-cell development. The aim of this work is to study the histological, immunohistological and histometrical changes of the adult male rat thymus following orchidectomy.

MATERIAL AND METHODS

Animals used: Twenty male albino rats more than nine months old were used in this study. The animals were divided randomly into two groups (n = 10). Group I, sham control group. Group II, orchidectomized (castrated) rats. The animals were housed in cages with softwood granules as bedding and had free access to standard diet and drinking water.

Orchidectomy: Castration was performed using standard method of Azad et al. (1998). Male rats were anesthetized with intraperitoneal injection with 10% chloral hydrate (300 mg/kg). The scrotal sac was cleaned with antiseptic and an incision of approximately 2 cm was made midsagittally at the scrotal septum. The spermatic cords were dissected, tied and cut. The testes were carefully removed from the scrotal sac and the incision was then sutured by absorbable suture. In sham operations, the scrotal incisions were immediately sutured and the gonadal system was not manipulated. Animals were returned to the cages when they recovered from the anesthesia and were able to eat and drink.

Tissue processing: All animals were sacrificed by cervical dislocation after four weeks of castration. The entire thymuses were dissected out, plot dried and weighed. Organ weights were expressed as absolute and relative weight (g/100g body weight). The entire thymuses were then kept in boun's fixative for at least three days. Tissues were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin. Five-micrometer-thick sections were cut and stained with Weigert's haematoxylin and van Gieson’s stains for the fibrous tissue content of the thymus. Periodic acid Schiff (P.A.S) for glycoproteins. Methyl green-pyronin stain for plasma cells. Unna stain for mast cells. S-100 immunoperoxidase stain for interdigitating cells.

Morphometry: To quantitatively evaluate cortex/medullary ratio of the thymus, all functional lobules with an outer cortex and inner medulla were measured using ocular micrometer. In order to avoid errors resulting from tangential cuts that can occur within any given lobule, all lobules were examined and an overall ratio was determined.

Imaging: Tissue sections were examined and photographed using an Olympus microscope. Images were scanned using Acer 640P scanner, saved as RGB color TIFF files and used for the histomorphometrical analysis-using image processing computer software NIH ImageJ (http://rsb.info.nih.gov/ij/Java 1.5.1_06).

Image analysis: Images were calibrated against a calibrated stored image of a known value photographed with the same magnification as the non-calibrated images. The area% occupied by the interdigitating cells in cortex and medulla were calculated in a standard measuring frame using color subtraction. The color subtraction sequence removed the non-peroxidase background colors by replacing them with white. The area% occupied by the interdigitating cells was delineated, thresholded and calculated both in the cortex and in the medulla.

Statistical Analysis: Data were expressed as means ± SD. Differences between groups were determined using independent sample student t-
test after testing for normal distribution. The cortex and medulla thickness were log transformed to achieve normal distribution and justify the use of student t-test in comparison. Significant differences were attributed with $P \leq 0.05$.

RESULTS

Thymus of the control group appeared atrophied with distorted lobular pattern and loss of demarcation between cortex and medulla. There was widening of the interlobular spaces with increased amount of connective tissue within the capsule and interlobular septa and dilatation of the blood vessels (Fig. 1A). Thymic lobules consisted mainly of densely packed, small darkly stained thymocytes, which overshadowed the sparse epithelial cells (Fig. 2).

Thymus of the castrated rats showed restored lobular pattern and thymic histology. The cortex and medulla became more defined with clear demarcation between them (Fig. 1B). The darkly stained cortex contained more thymocytes with mitotically active lymphoblasts. Pyknotic thymocyte nuclei, which are normally abundant in the cortex, were rarely seen (Figs. 3, 4). The paler medulla consisted mainly of epithelial reticular cells and Hassal’s corpuscles (Fig. 4). The epithelial reticular cells were seen among thymocytes both in the cortex and medulla but were more abundant in the medulla. Their nuclei were rounded to oval, pale stained with one or more nucleoli. The cytoplasm of the epithelial reticulum cells was P.A.S. positive and had many cytoplasmic protrusions (Figs. 3, 4).

Mast cells and plasma cells: Mast cells, in all thymuses examined, were selectively located in the capsule and septa but were more abundant in the control group in comparison to the castrated group (Figs. 5A, B). On the other hand, plasma cells in the castrated group were seen in clusters in the cortex, medulla, around blood vessels and in the connective tissue septa (Fig. 5C) while in the control group they were fewer and solitarily distributed in the medulla (Fig. 5D).

Immunohistochemistry: S-100 protein immunoperoxidase stain showed that S-100 immunoreactive cells were distributed mainly in the medulla and at the cortico-medullary border with some scattered elements in the cortex. The cells were dendritic in shape and embraced thymocytes with their branched processes; some of these thymocytes showed pyknotic nuclei. The staining was diffuse both in the nucleus and in the cytoplasm. Based on these morphological features, the immunostained elements were identified as interdigitating cells (Figs. 6, 7).

Morphometry and Image Analysis: The thymus underwent regeneration following castration with significant increase in the absolute and relative thymic weights as compared to the sham control group (Table 1). The cortex and medulla of the thymus were not affected to the same extent; the increase in thickness was significant in the cortex of the castrated group ($P = 0.002$) than in the medulla ($P = 0.81$). Moreover, the area% occupied by interdigitating cells was significantly decreased in the castrated group both in the cortex ($P = 0.02$) and the medulla ($P = 0.03$) (Table 2).

<table>
<thead>
<tr>
<th>Table 1: Effect of orchidectomy on body and thymus weight.</th>
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<tr>
<td>Control (mean ± SD)</td>
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<tr>
<td>Body weight (g)</td>
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<tr>
<td>Absolute thymus weight (g)</td>
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<td>Relative thymus weight (g/100g)</td>
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* $P < 0.05$ compared with orchitectomized control rats.

<table>
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<th>Table 2: Effect of orchidectomy on thickness of cortex and medulla, cortex/medulla ratio and area % occupied by IDC.</th>
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<tr>
<td>Control (mean ± SD)</td>
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<tr>
<td>Cortex Thickness (μm)</td>
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<tr>
<td>Medulla thickness (μm)</td>
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<tr>
<td>Cortex/Medulla ratio</td>
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<tr>
<td>Area % occupied by IDC in cortex</td>
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<tr>
<td>Area % occupied by IDC in medulla</td>
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* $P < 0.05$ compared with sham control rats.
**Fig. 1:** Photomicrographs of thymus:
A - sham control showing distortion of the lobular pattern of the thymus with loss of thymic tissue and difficulty to distinguish the boundary between cortex and medulla. There is widening of the interlobular spaces with connective tissue (CT) hyperplasia and dilatation of the blood vessels (V).

B - castrated showing an intact lobules with well demarcation between the darkly stained cortex (C) and lightly stained medulla (M). The lobules are partially separated by thin bands of connective tissue septa (S) containing blood vessels (V).

Weigert’s haematoxylin and van Gieson’s stain; X 40

**Fig. 2:** A photomicrograph of sham control thymus showing wide connective tissue septum (S) with occasional lymphocytes. Abundant small lymphocytes populate the cortex (arrows) clumped in between the dilated blood vessels (V). (PAS stain; X400)

Inset: high magnification of the cortex showing small lymphocytes (arrows) and PAS positive epithelial cell (arrowhead).

**Fig. 3:** A photomicrograph of thymus from castrated rat at the cortico/medullary junction showing the cortex (C) and the medulla (M). Abundant small lymphocytes overshadow the sparse epithelial cells (arrows). P.A.S positive Hassal’s corpuscle is seen in the medulla (arrowhead).

Inset: high magnification of Hassall’s corpuscle showing granular cytoplasm containing a small whorl of keratin appearing material and necrotic debris. The thymic tissue surrounding the Hassall’s corpuscles contains epithelial cells (arrow) and mitotic active lymphoblasts (arrowheads).

PAS stain; X1000

**Fig. 4:** A photomicrograph of thymus from castrated rat at the cortico/medullary junction showing the cortex (C) and the medulla (M).

Inset: high magnification of cortex showing epithelial reticulor cells (arrows) with P.A.S. positive cytoplasm, rounded to oval pale stained nuclei with one or more nucleoli. A small pyknotic lymphocyte is seen (arrowhead) among the lymphoblasts.

PAS stain; X1000
Testosterone, the principal male sex hormone, is primarily secreted in the testes of males and in small amounts in the ovaries of females and the adrenal glands (Freeman, et al. 2001). The thymus weight and cellularity are influenced by two different groups of factors: one (GH, insulin-like growth factor 1, thyroxine, triiodothyronine, melatonin) causing thymic hypertrophy (Hirokawa, et al. 1998) and the other factor is mainly steroid hormones producing thymic atrophy (Utsuyama & Hirokawa, 1989). The balance of their action changes during postnatal life leading to thymic involution. Accordingly, it can be assumed that the changes in thymic structure observed in the present study may be related, directly or indirectly, to changes in testosterone levels after orchidectomy.

In the present study, the thymus of the control rats showed distorted lobular pattern with increased connective tissue content and depletion of the cortical thymocytes. These findings are consistent with age-related changes of the thymus in mammals where thymic involution is associated with a gradual decline in the size and the function of the thymus, which starts at adolescence and progresses with age (Aspinall and Andrew, 2000;
by the frequent observation of mitotic
by either increased thymopoiesis as evidenced
crease of the size of the thymus may be explained
crease in the rate of thymocytes destruction, or a
endogenous corticosteroids that lead to either in-
cytes are especially susceptible to the action of
cortical thymocytes. This
main bulk of lymphocytes (thymocytes). This
hyperplesia. These
results in regeneration
mus. Moreover, treatment of old male rats with a
sory sex organs with trophic effects on the thy-
in rats caused involutional effects on the acces-
previous studies which stated that orchidectomy
hyperplesia. These
endings of pyknotic nuclei.

In orchidectomized male rats, the large thymic
weight gain and the increase in cortex/medullary
ratio seen in this study can be attributed to cortical
hyperplesia. These findings are in agreement with
previous studies which stated that orchidectomy
in rats caused involutional effects on the access-
sory sex organs with trophic effects on the thy-
mus. Moreover, treatment of old male rats with a
able analogue of luteinizing hormone-releasing
hormone and the subsequent decrease in testos-
sterone has been shown to result in regeneration of
the thymus (Greenstein, et al. 1987). The in-
crease of the size of the thymus may be explained
by either increased thymopoiesis as evidenced by
the frequent observation of mitotic figures of
lymphoblasts or by decrease in their death rate as
evidenced by the rare findings of pyknotic nuclei.
It is also possible that inhibition of testosterone
synthesis and release following orchidectomy not
only removes its immunoinhibitory effect on the
thymus but also allows other immunoregulatory
substances to regenerate the thymus (Rai & Hal-
dar, 2003).

Immunohistochemical analysis and hormone
binding assays revealed that androgen effects on
the thymus could be exerted through conventio-
nal receptor-mediated mechanisms (Kumar, et
both through the classical intracellular andro-
gen receptor (iAR) and on membrane androgen
receptors (mAR) on cell surfaces (Benten, et al.
2002). Cells binding testosterone are localized in
the outer thymic cortex (thymocytes) as well as
in corticomedullary region and in the medulla
(thymic epithelial cells). These results indicate
that testosterone has influence upon the function
of these cells. Testosterone can modulate T-cell
proliferation and/or differentiation, not only di-
rectly acting on the T-cell population localized
in the outer thymic cortex, but also indirectly by
modulating the function of the thymic epithelial
cells that bind testosterone and may in turn act se-
condarily on cortical thymocytes, or their precu-
sors within the thymus (Leposavic & Micic, 1992;

In this study, thymic mast cells were selectively
localized in the capsule and septa and exhibited
a connective tissue phenotype and were never
observed in the thymic parenchyma. The less of-
ten observation of mast cells following orchidec-
tomy could be explained by decrease in volume
of the connective tissue content of the thymus as
a result of its cellular hyperplesia (Barbini, et al.
1981). On the other hand, plasma cells increased
following castration and were seen within the
parenchyma and around blood vessels. This is
is in agreement with previous reports which stated
that B-lymphopoiesis negatively regulated by
steroid sex hormones and that loss of androgen
production or function results in significant in-
crease in B-lymphopoiesis and in the number of
peripheral B-cells (Olsen, et al. 1991; Viselli, et
al. 1997).

S-100 protein, first detected in the brain, is con-
sidered a useful immunohistochemical marker for
a subset of dendritic cells, the interdigitating cells,
which are mainly located in T-dependent areas of
lymphoid tissues (Ushiki, et al. 1984; Uccini, et
al. 1986). Thymic interdigitating cells are special-
ized antigen-presenting cells that play a role in
thymocytes negative selection by expression of
the major histocompatibility complex (MHC-I and
MHC-II). Only thymocytes adapted to self-MHC
molecule can survive (2%) and continue to mature
the rest will undergo an apoptotic death (Sprent &
Webb, 1995; Ardavin, 1997). In the present stu-
dy immunoreactivity for S-100 protein was de-
 monstrated in large cells with branched processes
mainly in the thymic medulla with some scattered
lements in the cortex. The decrease in the area%
occupied by the interdigitating cells in the castra-
ted rats observed in the present study may be rela-
ted to the increase of thymocytes which made the
interdigitating cells appeared less prominent and
not necessarily due to a decrease in the number or
size of these cells.

In conclusion, the present findings confirm that
thymus is an androgen-responsive tissue. A sig-
significant increase in thymus weight and cellularity after orchidectomy indicates a significant role for androgens in immune modulation. The exact role of interdigitating cells in age-induced thymic atrophy remain to be elucidated.

REFERENCES


تضخم الغدة الليموفية في الجرذان البيضاء المخصية: دراسة هستو كيميائية مناعية

DALIA SALEH

دراسة هستو كيميائية مناعية

داليا محمود صالح
قسم التشريح - كلية الطب - جامعة المنصورة

ملخص البحث

تلعب الغدة الليموفية دور هام في عمل الجهاز المناعي وتعتبر من الأعضاء الأولوية التي تتأثر بالهرمونات الجنسية، وعليه فإن الهدف من هذا البحث هو دراسة تأثير الإخصاء على التركيب الليموفوي وغير الليموفوي للغدة الليموفية وذلك باستخدام الفحص الهستولوجي والهستولوجي المناعي.

وقد استخدم في هذا البحث عشرون من ذكور الجرذان البيضاء البالغة. وقسمت الجرذان عشوائيا إلى مجموعتين (مجموعة ضابطة وأخرى أخرى لها عملية إخصاء). وقد أُقيمت الحيوانات في أقفاص وراقوا دخول حز في حوض معياري وملح للترب. وبعد أربعة أسابيع من الإخصاء تم التضحية بالحيوانات عن طريق كسر فرط الدم. شُرح الغدة الليموفية ووزنت وتتم حساب الوزن المطلق والوزن النسبي (جم/100 جم من وزن الجسم). واستخدم قطاعات من الغدة تحت حسا (إيكوراون) للصبغة بصباغة (أيبروند هيماتوكسيلين و فن جسن) لصبغ الأنسجة الليفية، (أيبروند أسيد برينسي) للبروتين المكرري، و (أيبروند أسيد برينسي) لاصبغ الخلايا البلازمية، وصبغة (قوة) لصبغ الخلايا البلازمية.

البدينية و صباغة (1000) الهستو كيميائية المناعية لصبع الخلايا المتناقلة.

كان هناك زيادة في الوزن المطلق والوزن النسبي للغدة الليموفية في الجرذان التي تعرضت للإخصاء، كما كان هناك زيادة بشكل ملحوظ في متوسط سمك طبقة القشرة وزاد في نسبة متوسط سمك طبقة القشرة إلى سمك طبقة اللب وذلك مقارنة بال مجموعة الضابطة. واستخدام برنامج تحليل الصورة كان هناك نقص في النسبة المئوية للمساحة التي تشغلها الخلايا المتناقلة في كل من القشرة واللب للغدة الليموفية للمجموعة التي تعرضت للإخصاء وذلك مقارنة بالمجموعة الضابطة.