Postnatal Development of Thymus in Male Swiss Mice

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Abstract:
Puberty is one of the most important stages in the postnatal development of mammals. There is paucity of age related histological studies in developing thymus. Due to the variation in the onset of pubertal development there is some basic difference in the development of thymus in male and female mice so present study deals with the developmental changes which occur in thymus of male Swiss mice. To evaluate the developing changes in the thymus healthy pregnant female Swiss mice were used. After parturition the thymus of their pups were removed on 1st, 21st, 35th and 49th day, fix, embed and sections prepare for the histological study. At the time of birth the components of thymus are very clear but no differentiation between the cortical and medullary components are apparent. On 35th day Cortical and medullary epithelial cells got their maximum number, largest size and appropriate shape. Different developing T-lymphocytes are present but immature and apoptotic cells are also found due to thymic involution on 49th day. We conclude that Right from birth to puberty there are progressive architectural changes in the thymic component but after puberty retrogressive changes noted due to age associated thymic atrophy. We also conclude that the gonadal hormones are responsible for this type of age related thymic involution.

Keywords: Cortical and medullary epithelial cells, postnatal development, Swiss mice, thymic involution, thymus.

1.0 Introduction:
The body of mammalian organism is made-up by several systems such as integumentary system, nervous system, digestive system, circulatory system, respiratory system, excretory system, reproductive system and lymphoid system. All systems have their own importance for an organism but lymphoid system plays an important role as a solider against pathogens. Lymphoid system consists of several organs such as thymus, bone marrow, spleen and lymph nodes. Thymus is a main lymphoid organ where T-lymphocytes got there identification for proper functioning. Thymus has a wonderful historical view and one of the most mysterious organ in a mammalian animal body. The history of the thymus gland dates back more than 2000 years. The world thymus originates from the Latin derivation of the Greek word thymos, due to its resemblance with the flowers of the thyme plant (thymus serpyllum). The homonym thymos also translates as soul or spirit, and it is for this reason that the thymus was misrepresented as the seat of the soul by Greeks. The earliest known reference to the thymus is attributed to Rufus of Ephesus circa 100 AD (Jacobs et al., 1999). The term thymus was introduced in human anatomy by Claudius Galenos of Pergamon in the second century AD (Kachlik et al., 2008). Australian physician Jacques Miller recognized the function of thymus as a designer of immune system (Miller, 1961; Miller, 2002; 2004).

To understand the structure and function of such a mysterious organ as thymus one has to understand its phylogenetical and ontogenetical history. Most embryological evidence favours that the thymic epithelium is derived from both the ectoderm and the endoderm of the 3rd and the 4th branchial grooves and pharyngeal pouches. The rodent thymus develops from the endoderm of the 3rd and 4th pharyngeal pouches and surrounding mesenchyme (Dijkstra and Sminia, 1990; Ma et al., 2013). As development progresses, the thymus along with the thyroid and parathyroid, sharing the same pharyngeal pouch origin and migrate caudally. They separate around 15th day when the thymus migrates into the thorax. Embryonic thymic remnants can give rise to ectopic thymic
The mammalian thymus is located in the pericardial mediastinum, anterior to the major vessels of the heart, and ventral to the base of the heart and aortic arch, with variable extension of one or both lobes into the cervical region (Haley, 2003). Primarily it is an epithelial organ, containing many developing lymphocytes, that is surrounded by a mesenchymal capsule. Histologically the lobules of thymus can be broadly divided into two sub compartments, the cortex and the medulla each of which contains distinct populations of thymic epithelial cells, mesenchymal cells, endothelial cells and dendritic cells. Thus the thymus provides a unique microenvironment for the well-organized maturation of a diverse T cell repertoire. T cell progenitors originate in the bone marrow and, to complete a series of defined and coordinated developmental stages, enter in the thymus through blood stream, differentiate, undergo selection, and eventually mature into functional T cells (Koch and Radtke, 2011). Briefly, thymocytes are first specified to the T cell fate and then they proliferate and differentiate, undergoing positive selection for the ability to recognize self-MHC and negative selection to eliminate T cells that are potentially auto reactive. This process ultimately gives rise to various repertoires of peripheral T cells. Cortical and medullary thymic epithelial cells drive T cell differentiation, education, and selection processes (Romano et al., 2013). In this concern further studies are required to evaluate the step by step developmental changes in the thymus.

2.0 Materials and methods:
The proposed experiments were conducted in the Environmental and Developmental Toxicology Research Laboratory, Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India to observe the developing thymus in pups of Swiss mice.

2.1 Animals
Healthy adult female Swiss mice 8-10 weeks old and 30 gms average body weight were used for this study. Animals were obtained from the animal house of our department. Male and female mice in the ratio (1:4) kept in the cage for mating. Female mice were examined every day in the morning and female showing vaginal plug were isolated and their gestation period were recorded. Presence of spermatozoa in the vagina the following morning was considered day one of gestation. Confirmed pregnant females were housed in polyvinyl chloride cages (290×320×390 mm) wrapped with rice husk bedding, and maintained under standard laboratory conditions. The laboratory animals were kept in well ventilated animal room with relative humidity of 70-80%. The room lighting consisted of alternate 12 hours light and dark periods.

The animals had free access to food (Amrut R & M Pallet purchased from Pranav Agro Industries Ltd. Plot No. 19, 20, Virat Estate, Near Samrat Petrol Pump, National Highway No. 8, Waghadia Chokadi, Vadodara, Gujarat, India) and water. The maintenance and handling of the animals were done as per the guidelines of Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University (No. CS/Res/07/759).

2.2 Experimental Protocol
Females showing vaginal plug were separated and their gestation period were recorded. After parturition the thymus of their pups were removed on 1st , 21st , 35th and 49th day and these were subsequently fixed in Bouins solution for 24 hours and then transferred to 70% alcohol for prolonged washing to remove excess of picric acid from the tissues. Tissues were dehydrated by treating with a series of different grades of alcohol, cleared in xylene and embedded in paraffin wax following routine procedure of block preparation (Carleton et al., 1967). After wax impregnation, a solid block of paraffin wax containing the tissue was prepared using Leuckhart’s L pieces, placed on a metal plate serving as the base of the mould. The paraffin block was trimmed and mounted on the block holder. Routine 6 µ thick sections were cut with a rotator microtome and fixed on clear and albumenized slides. These slides containing sections were stained with haematoxylin and eosin. Appropriate sections were observed under the microscope. Photomicrographs of the desired section were obtained using digital research photographic microscope.

3.0 Results and Discussion:
Thymus is a bilobed organ and situated anterior to the pericardial membrane. Evidence for a functional cervical thymus in mice has been reported (Terszowski et al., 2006). Both lobes of thymus are connected to each other by a connective tissue known as isthmus. Each lobe is covered by a thin connective tissue capsule and in
most species lobes are divided into several lobules of different shape, size and orientation. There is no sub-lobulation in the mouse. Each lobe is divided into two regions, outer one is cortex and inner one is medulla. Cortical region is denser to epithelial cell in comparison to medullary region. Both regions are separated by vascular corticomedullary region. The cortical epithelial cells contain predominantly T lymphocytes, smaller populations of B lymphocytes, plasma cells and scattered endocrine cells. The medulla also contains reticular cells and the unique “Hassall’s Corpuscle” which are spherical structures composed of concentric layers of spindle shaped cells whose function is unknown (Pearse, 2006) but according to Watanabe et al., (2005) in human thymus they instruct dendritic cells to induce CD4+ and CD25+ regulatory T-cells.

Many researchers have investigated in mammals that during pregnancy the thymus loses its weight and cellularity with a marked involution of the cortex (Pepper, 1961; Clarke and Kendall, 1994; Kendall and Clarke, 1994). This occurs due to hormones of pregnancy (Phuc et al., 1981; Kendall et al., 1994). However, it is clear that the thymus of pregnant mammals are affected by pregnancy-associated hormones, maternal response to fetal antigens (Clarke, 1979, 1984) and events directed by the neuroendocrine system (Kendall et al., 1994). Kendall and Clarke (2000) reported that changes occur during pregnancy due to variation in high hormone levels. They also reported extreme loss of cortical thymocytes but medullary thymocyte number increased in mid to late pregnancy and reduced in late pregnancy. Thymus is covered with a thin connective tissue capsule that is composed of collagenous connective tissue fibers (Williams et al., 1995). According to Hamilton and Mossman (1976), the epithelial cells of the developing thymus becomes more loosely arranged to form a reticulum in which small lymphocytes soon appear at about 9th week. The vascular mesodermal tissue invades the gland in such a way as to produce its lobulation. At the time of birth the components of thymus are very clear but no differentiation between the cortical and medullary components are apparent. Well-developed epithelial cells are arranged in regular manner. Well-developed capsule cover is present. With advancing days of development like 21st and 35th day, well developed capsule and the increase number of cells in cortex, medulla, cortex-medullary zone and sub capsular region of thymus is observed till day 35th but after it reduced number of cells are seen on the day 49th (Plate 1. Fig 1 to 4).

Plate 1. TS of thymus in Swiss mice Fig. 1. on 1st day, 2. on 21st day, 3. on 35th day and 4. on 49th day, C= cortex and M= medulla. (10X, H&E)
On 1st day well-developed cortical cell covered with well-formed capsule is apparent. Shape, size and distribution of cells are appropriate. There are some differences in the distribution of cell in the regions of thymus. Some regions have denser cells in comparisons to others. The outer and denser region is cortex and inner and less dense region is known as medulla. There are no major differences found in the both regions on day 1st. According to the microscopic and gross histological findings of Solarovic et al., (2006) age dependent thymic changes in male rats of Wistar strain are similar with human thymus during aging (Plate 2. Fig. 1 and 2).

Many septa extend from capsule by which thymus of mouse is dividing into a large number of chambers known as lobules. Each lobule is divided into an outer cortex and an inner medulla. The cortex contains small lymphocytes in addition to macrophages. The medulla has abundant epithelial reticular cells, thymocytes and lymphocytes of medium and large size (Sakr and Lamfon, 2010). The cortex is composed primarily of lymphocytes (thymocytes), with a few epithelial and mesenchymal cells, whereas the medulla is composed of more epithelial cells but fewer lymphocytes. Epithelial cells compose the framework of the thymus which is functionally essential for the maturation of T-lymphocytes (Shimosato and Mukai, 1997). The ontogeny of T-lymphocyte is a refined process, which takes place within the thymus (Nunes-Alves et al., 2013) through a series of well-defined discrete stages and requires an appropriate lympho-stromal interaction (Romano et al., 2013).

![Plate 2. TS of Thymus in Swiss mice on 21st day Fig. 1. cortical region and Fig. 2. Medullary region. CP = Capsule, • = developing thymocytes in both regions, ◆ = Epithelial cells (in cortical region cortical epithelial cells and in medullary region medullary epithelial cells.) (40X, H&E)](image)

On day 21st cortical and medullary regions are clearly seen. Cortex is covered by capsule which is made up by connective tissue. Trabeculae arise from the capsule and pass into the cortex. The cortex contains cortical thymic epithelial cells. The medulla has abundant medullary thymic epithelial cells, thymocytes and lymphocytes of medium and large size (Plate 3. Fig. 1 and 2). At the end of development small groups of neutrophil cell precursors appeared in the interlobular connective tissue and the cortex. Cysts were not present up to day 21st after birth (bodey et al., 1987). The thymus provides a specialised microenvironment for the development of T-cell precursors that depends upon interactions with thymic epithelial cells, which provides signals for proliferation, survival and differentiation. It has been proposed that development of thymic epithelial cells themselves is regulated by signals produced by developing thymocytes (Jenkinson et al., 2005). Baik et al., (2013) reported that in the adult thymus, the development of self-tolerant thymocytes require interactions with thymic epithelial cells.
At the time of puberty (35th day) the sections of thymus shows infra structurally well-developed compartment of thymus. At this time cortical and medullary epithelial cells got their maximum number, largest size and appropriate shape. There is slight difference in cortical and medullary region cells (cortical region have more density of cells). Different stages of developing T-lymphocytes in cortical and medullary region are also found (Plate 4. Fig. 1 and 2). Normal development, histology, and function of the thymus have been reported previously (Kuper et al., 1992; Pearse, 2006). The use of standardized descriptive nomenclature with respect to thymic pathology is addressed (Haley et al., 2005; Elmore, 2006). The thymus develops gradually parallel to other organs till puberty but after puberty it goes to a dramatically changes known as thymic involution. In histological view involution is reduction in the size, decrease cortical lymphocyte, irregular cortex, increase in tangile body macrophages and demarcation of cortico medullary zone. The thymus body ratio is greatest perinatally but the organ continues to increase in absolute size until about puberty, after which it tends to gradually decline. According to Kuper et al., (2002) when there is a decrease in thymic size and cellularity one should use the term “reduced number of cortical lymphocyte and increased number of macrophages”. These changes may be identified as “atrophy or involution”. After puberty dramatically retrogressive changes occurs in size and cellularity of the thymus known as thymic involution or age associated thymic atrophy. (Sharma and kantwa, 2011).

In young thymus (5 week old), the division between cortex and medulla is distinct (Danielle et al., 2008). In the mature thymus, thymocyte maturation depends on interaction with different thymic epithelial subtypes but the molecular mechanisms that generate these epithelial subtypes are not well understood. Evidence is accumulating that during fetal thymus development, epithelial cells differentiate by successive interactions with differentiating thymocytes (Manley, 2000). The thymus of three month old rat is characterized by a wide cortex rich in thymocytes, sharp corticomedullary junction, a thin thymic capsule and narrow interlobular connective tissue (Solarovic et al., 2006). Findings of Cavalliotti et al., (2008) demonstrate that all thymus compartments (subcapsular spaces, cortical and medullar thymus microenvironment) contain numerous thymocytes even after the thymus has aged. Endothelial cells of thymus microvessels contain numerous gaps. These are tight in young subjects and become loose with age. Thymocytes, in older subjects, are always found near these loose endothelial gaps of thymus microvessels. While thymus cortical microvessels are provided with pericytes and/or periarteriolar spaces, microvessels of the thymus medullar are free of such spaces.
Plate 4. TS of Thymus in Swiss mice on 35\textsuperscript{th} day Fig. 1. Cortical region and Fig. 2. Medullary region. $\blacktriangleleft$ = Developing thymocytes in both regions, $\triangleleft$ = Epithelial cells (in cortical region cortical epithelial cells and in medullary region medullary epithelial cells) and $\blacksquare$ = Hassal's Carps in medullary region. (40X, H&E)

Plate 5. TS of Thymus in Swiss mice on 49\textsuperscript{th} day Fig. 1. Cortical region and Fig. 2. Medullary region. TC = Thicker capsule, $\blacktriangleleft$ = Developing thymocytes in both regions, $\triangleleft$ = Epithelial cells (in cortical region cortical epithelial cells and in medullary region medullary epithelial cells), $\rightarrow$ = modified cortical epithelial cell and $\blacktriangle$ = modified medullary epithelial cells. (40X, H&E)

At the termination of experiment (on 49\textsuperscript{th} day) degeneration (become thicker) in capsule begins. Demarcation of cortex and medulla which seen on 35\textsuperscript{th} day starts disappearing. Now medullary region have more cells in comparison to 35\textsuperscript{th} day medulla and cortex have less number of cells in comparison to 35\textsuperscript{th} day cortex. Enlarged cells are found in both regions with lesser numbers as compared with 35\textsuperscript{th} day thymus. Different developing T-lymphocytes are present but immature and apoptotic cells are also found (Plate 5. Fig. 1 and 2).

In the thymus of 18 month old animals a marked reduction in the size of functional tissue (thymocytes and thymic epithelium), particularly in the cortex, is evident as well as the loss of clearly identifiable corticomedullary junction. The density and size of thymic epithelial cells are notably reduced in the medulla of old rats compared with young adult rats. These changes in lymphoepithelial tissue of aged thymus are accompanied by a marked increase in the content of inter-lobular connective and adipose tissue and thickening of the thymic capsule (Solarovic et al., 2006).
2006). During involution, the epithelial component atrophies, resulting in scattered small lymphocytes in abundant adipose tissue (Nishino et al., 2006). Further studies are required to define the precise developmental requirements of thymic epithelium which are involved in the generation and maintenance of mature thymic microenvironments.

4.0 Conclusions:
- Thymus develops progressively from birth to 35th day, after that it undergoes cellularity loss and adipose tissue deposition, this type of changes occur due to gonadal hormones.
- These changes are sex related as the female thymus involutes earlier in comparison to male thymus. This phenomenon depends on the concentration of gonadal hormones.
- From the finding of present work we conclude that thymus of male mice completely mature in between 5th to 7th week. With the onset of gonadal development the thymic involution takes place.
- In the present finding developing Hassall’s Corpuscles are observed on 35th day.
- Commencement time of puberty in mammals is species specific by which the development of thymus is also affected so further studies are required to pin point the precise developmental period and thymic involution in different mammalian species.

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References:


