International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Age Related Histochemical Changes in Thymus, Spleen and Mesenteric Lymph Nodes in Mice, Rat and Guinea Pig

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Abstract: ***The study was designed to observe the histochemical changes in the thymus, spleen and mesenteric lymph nodes in prenatal and postnatal age groups of mice, rat and guinea pigs. Materials for the present study were collected from six animals each in all the age groups of all the species. Tissue pieces were collected and frozen sections of 15 to 20 microns were used for the present study. In thymus, the free reticulo-epithelial cells, those involved in formation of Hassall's corpuscles and macrophages were PAS positive. Some of the Hassall's corpuscles were Alcian blue positive only in mice. The reticulo-epithelial cells and macrophages showed a positive reaction for Acid phosphatase. Alkaline phosphatase activity was found to be absent in all the species. In spleen, the capsule, trabeculae and vascular network were PAS positive. Reticular cells and macrophages showed and Acid phosphatase reaction. In mesenteric lymph nodes, the macrophages were positive for PAS and Acid phosphatase activity in all the age groups studied.***

Keywords: Histochemistry - lymphoid organs - pre and postnatal - laboratory animals

1. Introduction

In all vertebrates, the immune system provides defense or immunity against infectious agents ranging from viruses to multicellular parasites. This freedom from disease is depends on the existence of a complex and highly sophisticated defense system, called lymphatic system [5, 14].

Thymus is a central lymphoid organ in which bone marrow-derived T-cell precursors undergo differentiation, maturation and eventually leading to migration of positively selected thymocytes to the peripheral lymphoid organs [24,4]. Thymus differs from other lymphoid organs as it undergoes involution as age advances [9].

The spleen is the largest secondary lymphoid organ in mammals and also the primary site for immune cell proliferation and differentiation and is a specialised organ to perform filtration of blood. The splenic parenchyma plays a crucial role in immune responses such as exposure to blood-borne antigens [3,13,26].

The lymph node is a critical cross road for encounters between the antigen presenting cells, antigenic substances from lymph and lymphocytes recruited into lymph nodes from the blood [7]. Intestinal wall being the heaviest area of antigenic load, the lymph node associated with the intestine is of special interest [15]. Hence, priority has been given to study the structure of mesentric lymph node in the present study.

2. Literature Survey

The theme of biomedical research is the elucidation of biological cause, as they pertain to human health. Lab animal models are often used as specific test system for this purpose. Choosing a right species of experimental animal which is closely related to that of human immune system is an important step in biomedical research using lab animal species [17].

Among the laboratory animals, the Guinea pig (*Cavia porcellus*) is one of the most valuable animal model related to human [12]. Next to guinea pig, mice is the most commonly used lab animal model similar to immune system of human [8]. In this order, rats are also the most commonly used animal model mainly for long term test, nutritional and cancer research.

Although, there are several detailed reports available on histoarchitecture of lymphoid organs in laboratory animals, the histochemical study on both prenatal and postnatal age groups is very limited. A typical mammalian immune system is constantly changing throughout its life [21]. Hence, the present study is aimed to observe and compare the developmental changes that takes place in the histochemistry of thymus, spleen and mesentric lymph node of guinea pig, mice and rat during prenatal and postnatal period.

3. Materials and Methods

The laboratory animals for the study viz., mice, rat and guinea pigs were procured from the Laboratory Animal Medicine unit, Madhavaram Milk Colony, Tamil Nadu Veterinary and Animal Sciences University, Chennai-6000051, where they were maintained at controlled conditions of temperature (23^0C), relative humidity (50 to 55%). At the time of collection, the animals were apparently healthy.

The tissue pieces were collected from all the species of prenatal and postnatal age groups as given in Table 1. Animals were euthanized by using chloroform instead of CO_2 asphyxiation [6].

Cryosections of 15-20 µm thickness were obtained from fresh and formol-calcium fixed tissues for different histochemical studies. Chilled Formol calcium(4^0C) fixed frozen sections were used for localisation of alkaline phosphatase and acid phosphatase. Frozen sections from fresh unfixed tissue were used for localisation of lipids. The frozen sections were subjected for the following histochemical techniques:

1) Periodic acid-Schiff's (PAS) method for neutral mucopolysaccharides [1]
2) Combined Alcian blue-PAS method for acid and neutral mucopolysaccharides [2]
3) Oil red 'O' method for lipids [1]
4) Naphthol AS-BI phosphate method for acid and alkaline phosphatases [2]

Table 1: Collection of materials (thymus, spleen and mesenteric lymph node) for the study (Number of animals used for sample collection is in parenthesis)

Species	Prenatal (embryo)	Postnatal	
		Pre-pubertal	Post-pubertal
Mice	12 days (6) 16 days (6)	1 week (6) 3 week (6) 8 week (6)	4 months (6) 8 months (6) 12 months (6)
Rat	14 days (6) 18 days (6)	1 week (6) 3 week (6) 8 week (6)	4 months (6) 8 months (6) 12 months (6)
Guinea pig	35 days (6) 55 days (6)	1 week (6) 3 week (6) 8 week (6)	4 months (6) 8 months (6) 12 months (6)

4. Results and Discussion

Thymus

In the present study, the mesenchymal reticular cells of prenatal thymus and the free reticulo-epithelial cells present in both cortex and medullary portions were found to be positive for PAS reaction. Similarly, in Hassall's corpuscles, the central homogenous mass and the reticulo-epithelial cells around the corpuscles were positive for PAS [Fig-1]. This indicated that the secretory material has been made of neutral mucopolysaccharide as reported by [23]. However, in mice, some of the Hassall's corpuscles were Alcian blue positive indicating the presence of acid mucopolysaccharide as in Children and adult [10]. A similar observation was made in the pre-pubertal guinea pigs [Fig-2]. The macrophages which were found to be more in number as age advanced in post-pubertal age groups also showed PAS positive reaction.

The reticulo-epithelial cells of thymic parenchyma were acid phosphatase positive in all the age groups studied [Fig-3] which is contradict to the findings of [23] in thymus of Murrah buffalo calves. The acid phosphatase activity was found to be higher in mice when compared to rat and guinea pig in the present study which could be due to hte presence of lysosomes within these cells that attributed the phagocytic property as per [19].

No alkaline phosphatase activity was observed in all the age groups in all the species of the studied. Lipid accumulation was more in the post-pubertal age groups [Fig-4] when compared to prenatal and pre-pubertal age groups which indicated the age related involutory changes of thymus indicated by invasion of adipose tissue, loss of lymphocyte population ad formation of small pits [20].However, lipid accumulation was less evident in guinea pig when compared to rat and mice in the present study.

Spleen

In the present study, the capsule and trabeculae were rich in glycogen in all the species [Fig-5] . In mice, the reticular cells, macrophages and also erythrocytes were PAS positive in all theage groups studied. Whereas, in rat and guinea pigs, the spleenic erythrocytes didn't show PAS reaction as observed in rabbits [25].

In mice, the reticular cells of spleenic nodule and macrophages showed positive reaction for acid phosphatase in all the age groups. Whereas, in rat, the reticular cells showed strong acid phosphatase activity and macrophages situated at the periphery of the nodules alone positive for acid phosphatase reaction [Fig-6] . In guinea pig, the acid phosphatase activity in the reticular cells and macrophages was found to be stronger in post-pubertal age group than pre-pubertal and prenatal age group in rat [22] and dog and cat [11].

In all the species, there was no evidence of alkaline phosphatase activity observed in all the age groups.

Mesenteric lymph node

In mice, rat and guinea pig, capsule and macrophages were PAS positive in all the age groups studied. Acid phosphatase activity was very mild in the macrophages of mice and stronger activity was observed in the macrophages of rat and guinea pig. Alkaline phosphatase activity was found to be absent in all the species in all the age groups studied which were similar to the findings of [16] in rabbit and [11] in rats.

5. Conclusion

The present study describe the age related changes in the histochemistry of the lymphoid organs in lab animals in different age groups. In thymus, free reticulo-epithelial cells and those forming the Hassall's corpuscles were PAS positive in all the age groups in all the species. Some of the Hassall's corpuscles in the mice alone were Alcian blue positive. Acid phosphatase activity was evident in reticulo-epithelial cells and macrophages. In spleen, the capsule, trabeculae and vascular network of the red pulp were PAS positive and acid phosphatase activity was noticed in the reticular cells and macrophages. Macrophages of mesenteric lymph node was positive for PAS and acid phosphatase activity in all the species studied.

6. Future Scope

Though, the present study described the histochemical localization of mucopolysaccharide, lipid and phosphatases in the parenchyma of thymus, spleen and mesenteric lymph

nodes, quantitative and qualitative estimation of the T-lymphocyte subsets viz., CD-4 and CD-8 cells in these lymphoid organs will help in better understanding the immune function in lab animals. These observations will form a basis for further immunohistochemical and flow cytometric studies in the lymphoid organs in lab animals.

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List of Plates and Legends

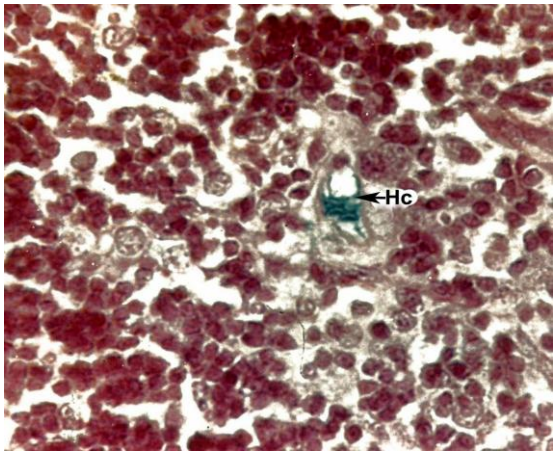

Figure 1: Photomicrograph of thymus of an eight month-old mouse showing the presence of acid mucopolysaccharide Hc - Hassall's corpuscles PAS-Alcian blue x 500

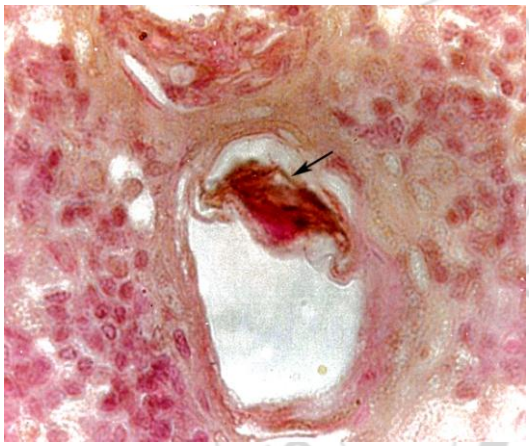

Figure 2: Photomicrograph of thymus of an eight week-old guinea pig showing positivity for PAS in Hassall's corpuscles (arrow) PAS x 500

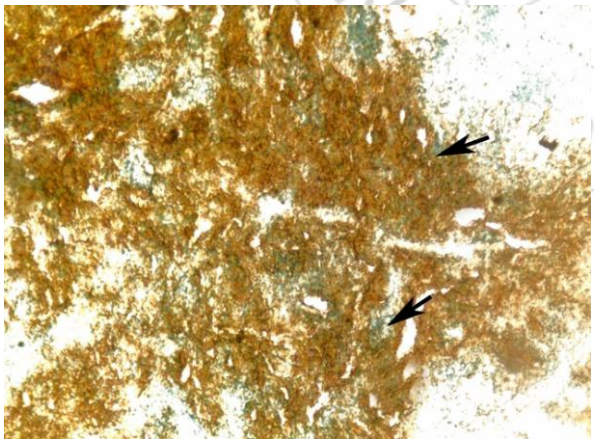

Figure 3: Photomicrograph of thymus of an eight month-old mouse showing acid phosphatase activity in the cells of medulla (arrows) Naphthol AS-BI phosphate x 80

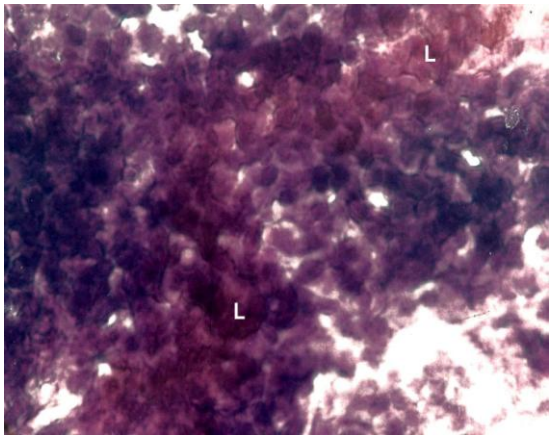

Figure 4: Photomicrograph of thymus of a twelve month-old rat showing lipid accumulation L - Lipid Oil Red 'O' x 500 Naphthol AS-BI phosphate x 80

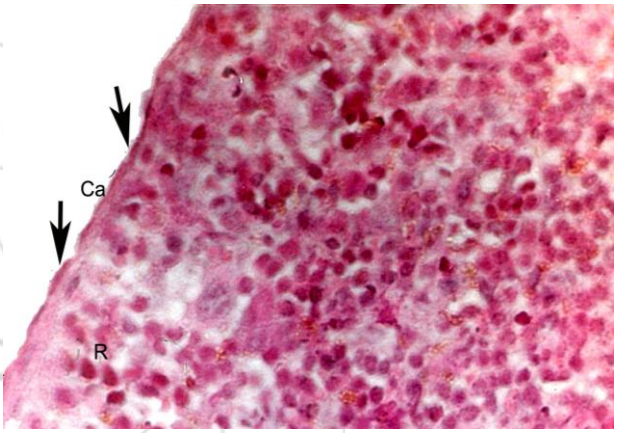

Figure 5: Photomicrograph of spleen of a four month-old mouse showing PAS positive areas R - Reticular cell Ca - Capsule PAS x 500 Naphthol AS-BI phosphate x 80

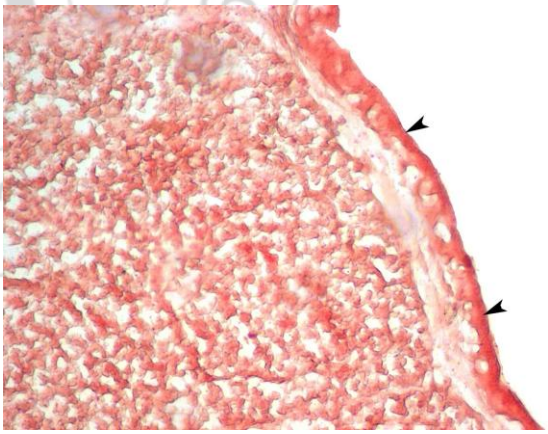

Figure 6: Photomicrograph of spleen of an eight week-old rat showing acid phosphatase activity (arrows) Naphthol AS-BI phosphate x 80 Naphthol AS-BI phosphate x 80