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Studies on bronchus associated lymphoid tissue (BALT) of black Bengal goat

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Abstract
Bronchus Associated Lymphoid Tissue (BALT) were collected from clinically healthy zero day and month wise from one month to fifth months old Black Bengal goats (*Capra hircus*). Bronchus associated lymphoid tissue (BALT) was not found macroscopically; however from date of birth to till the last experimental age group, it was identified microscopically in caparine lung. At birth the small lymphocytic aggregation was found near the respiratory pathway. The aggregation of lymphocyte was found in diffused form rather than in the nodular form. Mostly they were found in 1st and 2nd order of bronchus and at the respiratory bronchioles. From 1st month onward typical lymphonodular tissue was observed in the same area as described at the date of birth. The sizes of lymphnodules were increased according to advancement of age. BALT showed CD4+ T lymphocyte, MHC- II + expressing cells, IgM positive cells and CD8+ T lymphocytes in all the experimental groups. CD4 positive cells population was found to be more as compare to the CD8 cells. B lymphocytes were identified scatteredly in the bronchus associated lymph nodes and also within the interalveolar spaces. MHC II Positive cells were found in the interalveolar spaces, within alveoli and also at BALT.

Keywords: Black Bengal goat, bronchus, lymphoid tissue

Introduction
The respiratory tract contains a considerable amount of lymphoid tissue in the form of nodules in the walls of the bronchi as well as lymphocytes distribute diffusely throughout the lung and the walls of the airways. All these lymphoid tissues function, at least in part, independently of the systemic immune system. Because primed B cells can travel between the intestinal lymphoid tissues as well as to the respiratory tract and lacrimal and mammary glands, they are considered to be part of a common mucosal immune system. The aggregations of lymphoid cells in the bronchial mucosa, morphologically resemble the gut associated lymphoid tissue (GALT), are refer to as the bronchus associate lymphoid tissue (BALT). The presence of follicular lymphoid aggregation in the mucosa of the rabbit bronchial tract with a peculiar epithelial relationship (Bienensstock *et al.*, 1973). The BALT is not present in neonatal goats, appeared in 50% of 1 month old goats and markedly increases between 1 month and 1 year (Barman *et al.*, 1996) [1]. The tonsils of Waldeyer's ring and GALT, BALT are not present before birth in cattle, sheep, goats, pigs and horses (Elisabeth and Pabst, 2006) [7]. The proliferation of follicular dendritic cells S-100$^+$ and MHC type II$^+$ in this lymphoid tissue, suggested that antigen presentation to T and B lymphocytes and consequent proliferation are facilitated in these areas, which may thus induce a better local immune response than that produce by the diffuse lymphoid infiltrate. The number of mononuclear cells expressing MHC class II antigen, show a significant increase in infection goats in relation to the controls. This activation can participate in the signal regulation of antigen-presenting cells, both through the humoral and cellular immunity (Mossmann and Coffman, 1989) [10]. The CD4$^+$ T lymphocytes, S-100$^+$ cells, MHC class II$^+$ expressing cells and IgG$^+$ B lymphocytes represent the main cellular types involve in the lymphoreticular hyperplasia of the BALT from goats infection with three of the most important mycoplasma species frequently involve in outbreaks of pneumonia in goats. The BALT may be acquiring the typical features of MALT in sheep and goats with lung infections (Rodríguez *et al.*, 2001).
Sporadic literatures are available in respect to the bronchus associate lymphoid tissue of goat.

However no literature found in regards to our present studies. Hence the present studies has been undertaken to elucidate Bronchus Associated Lymphoid Tissue (BALT) of Black Bengal Goat.

Materials and Methods

Bronchus Associated Lymphoid Tissue (BALT) were collected from clinically healthy zero day and month wise from one month to fifth months old Black Bengal goats (*Capra hircus*) from the farm maintained by AICRP (All India Coordinate Research project) on goat. The animals were maintained in the farm house according to the stipulated guideline and permission of Institutional Animal Ethical committee of faculty of Veterinary and Animal sciences; West Bengal University of Animal and fishery Sciences, Kolkata. Two goats of either sex were utilized for this experiment in each age group. The Bronchus Associated Lymphoid Tissue (BALT) samples were taken from all groups of experimental animals and were fixed in 10 per cent neutral buffered formalin. The fixed samples were processed for paraffin sections and routine staining was done as per Luna, 1968 [9].

For immunohistochemical examination the collected samples were fixed in 10 % neutral buffer formalin (NBF) and were dehydrated through graded alcohols before being embedded in paraffin wax. The sections (horizontal & Vertical) of 5µm thickness were obtained from each specimen.

The different lymphocyte subpopulations were visualized using the avidin-biotin peroxidase complex technique (ABC; Vector Laboratories, U.S.A). Paraffin-embedded samples were dewaxed with xylene and hydrated. Then dip into antigen unmasking (Vector Laboratories, U.S.A) solution for 15 min at 95°C. After washing the slides in PBS for 5 min, the samples were pre-treated with normal rabbit serum (Dako, Denmark) diluted 1:100 in PBS for 60 min at room temperature to block the non-specific binding sites. They were then incubated with the ready-to-use of each primary antibody (Table 1) in a moist chamber for 120 min at room temperature. Next, the slides were again washed in PBS and then incubated with a biotinylated rabbit antimouse IgG or horse antirabbit IgG (Vector Laboratories, U.S.A) according to primary antibody diluted 1:250 for 60 min. Before being washed in PBS and incubated again with ABC for 40 min, according to the instructions of the maker (Vectastain ABC Elit® Kit, Vectror Laboratories). Color was developed by a final incubation with 3.3' diaminobenzidine tetrahydrochloride (DAB, Peroxidase substrate kit Vector Laboratories) in buffer with hydrogen peroxide in distilled water for 5 min at room temperature. The reaction was stopped by rinsing the slides in water. The some slides were counterstained with haematoxylin, dehydrated and mounted. Most slides were mounted without counterstained.

Table 1: Primary Antibodies (ready- to- use) used in the Immunohistochemical Technique

Antibodies	Specificity	Isotype	Supplier
4B12	CD4*	IgG1	Dako Denmark
C8/144B	CD8*	IgG1	Dako Denmark
	IgM**		Dako Denmark
CR3/43	HLA-DP, DQ, DR*	IgG1	Dako Denmark

*****Monoclonal Antibody, ******Polyclonal Antibody

Result

Morphological and cellular organization of BALT

Bronchus associated lymphoid tissue (BALT) was not found macroscopically; however from date of birth to till the last experimental age group, it was identified microscopically in caparine lung (Fig. 1 & 2). At birth the small lymphocytic aggregation was found near the respiratory pathway. The aggregation of lymphocyte was found in diffused form rather than in the nodular form. Mostly they were found in 1st and 2nd order of bronchus and at the respiratory bronchioles. But it was noticeably observed that in all the samples there was huge lymphocytic aggregation within the interalveolar connective tissue. In higher magnification it was revealed that the lymphoid tissue was composed of small to medium size lymphocytes. The aggregation of lymphoid tissue mostly occurred near the basement membrane of the bronchioles and towards the outer most muscular layer of bronchus. From 1st month onward typical lymphonodular tissue was observed in the same area as described at the date of birth. The sizes of lymphnodules were increased according to advancement of age. The nodules were encapsulated by thin covering of connective tissue fibre. The lining epithelium revealed pseudo stratified to low cuboidal epithelium in case of bronchus and bronchioles respectively. Lymphocytic aggregation was found within the epithelium. Under higher magnification it was revealed that the lymphocyte migrated from the lymphoid follicle towards the surface epithelium of the bronchus or bronchioles. Therefore this epithelium may be considered as lymphoepithelial layer. Some metachromatic granular cells were found in the epithelium also.

More of less the lymphoid follicle showed no staining differences. It appeared as basophilic and likewise it could not be differentiated as cortex or medulla. From 1st month onward the lymphoid follicle were thickly populated with lymphocytes and few macrophages, plasma cells were occasionally found in same of the follicles.

At 5th month of age the size of lymphoid tissue was recorded maximum and numbers of lymphoid follicles were also more as compare to the other age groups of animals. From 3rd months few alveolar macrophages were identified in some sections.

Functional and immunological aspect of BALT

CD4 positive cells were identified within the lung parenchyma mostly CD4 positive cells were distributed near the bronchioles associated lymph nodes. But scatteredly CD4 positive cells were also identified within the interalveolar spaces and within the lymphoepithelial tissue (Fig. 3). The CD4 positive cells were found right from date of birth till 5th month of age. The number of CD4 positive cells was increased with increment of age. CD4 positive cells population was found to be more as compare to the CD8 cells (Fig. 4). Similarly CD8 cells were also identified in all the experimental animals and they were found to be localized in the same place like that of CD4 cells. But numbers of CD8 cells were less as compare to CD4 cells.

IgM positive B lymphocytes were identified scatteredly in the bronchus associated lymph nodes and also within the interalveolar spaces but number of cells upto 2nd month of age was very few and in the later months number was increased proportionally (Fig. 5).

MHC II Positive cells were found in the interalveolar spaces, within alveoli and also at BALT (Fig. 6). MHC II polyclonal antibody some time positive for CD4 cells for this reason the MHC II + cell population was found towards the higher side within the BALT and also in the inter alveolar spaces. The number increased from 2nd months onwards.

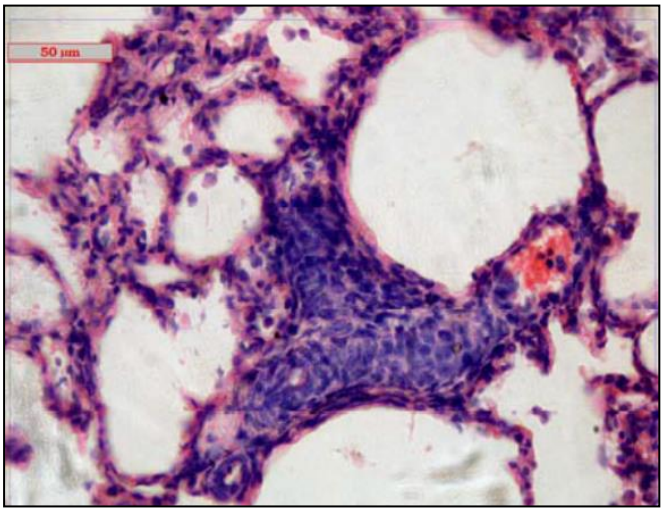

Fig 1: BALT (H & E) -1st Month

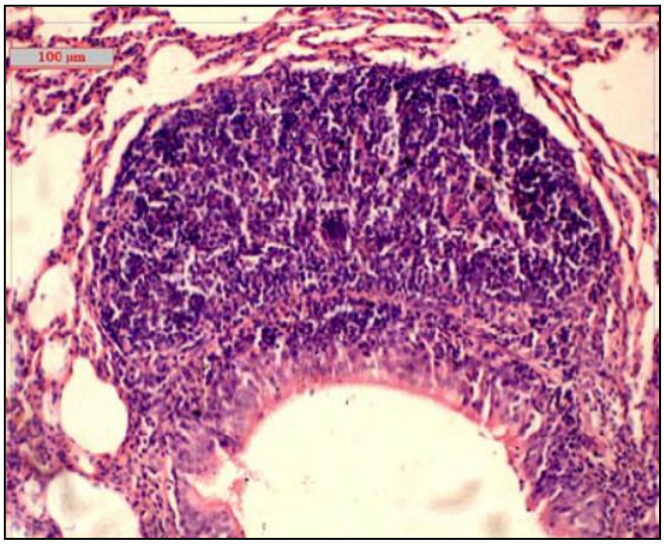

Fig 2: BALT (H & E) 5st Month

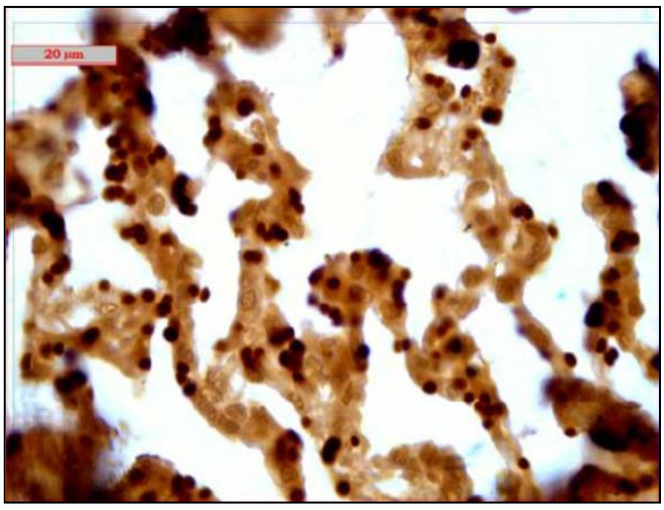

Fig 3: CD 4 Positive Cells - Day Old

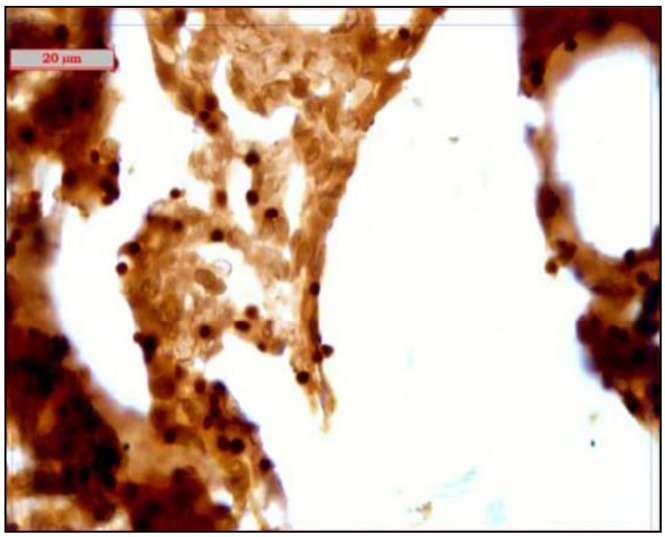

Fig 4: CD 8 Positive Cells – 5 Months

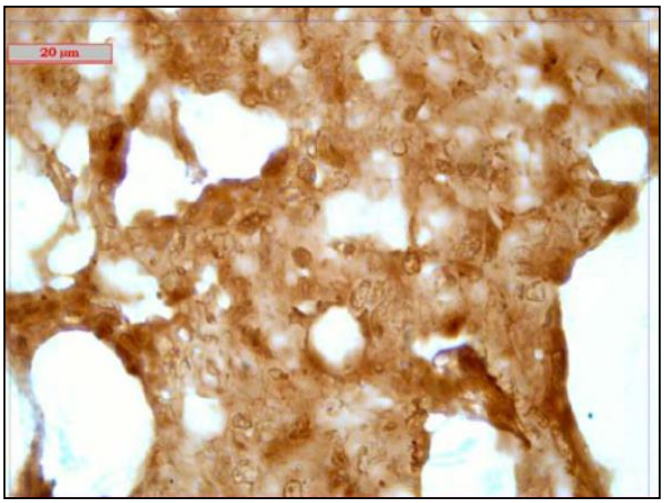

Fig 5: IgM Positive Cells – 3 Months

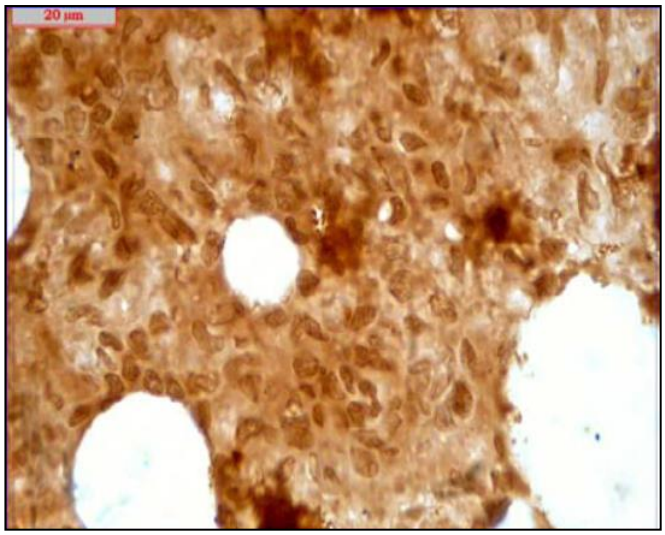

Fig 6: MHC-II Positive Cells – 4 Months

Discussion

Morphological and cellular organization of BALT

Elizabeth and Pabst (2006) [7] reported that in contrast to the tonsils of Waldeyer's ring and GALT, BALT was not present before birth in cattle, sheep, goats, pigs and horses. Barman *et al.* (1996) [1] examined that the BALT was not present in neonatal goats, appeared in 50% of 1 month old goats and

markedly increases between 1 month and 1 year. In this present study embryonic development of BALT was not considered however at birth BALT was found in diffused form and from first month onward typical lymphonodular tissue was found though out the post natal developmental phage which is in partial agreement of Barman *et al.* (1996) [1]. The aggregation of lymphoid cells in the bronchial mucosa had been named the bronchus associated lymphoid tissue. Likewise in sheep no lymphoid nodules were identified from 6 month to 9 years around bronchi and bronchioles only dense aggregation were identified Chen *et al.* (1989) [6]. These statements are not in accordance with our present findings. The BALT increased in size and also in numbers. No supportive literature was found in this aspect. The Bronchus-associated lymphoid tissue (BALT) was a constitutive mucosal lymphoid tissue adjacent to major airway in some mammalian species, including rats and rabbits, but not humans or mice (Randnall, 2010). Beside the lymphoid nodule in the wall of bronchi or bronchioles lymphocyte was distributed diffusely in the interalveolar space. Similar observation was reported by Tizard (2000) [15]. Lymphocyte was also noticed in the epithelium and some metachromatic granules in the cytoplasm giving the appearance of primitive mast cells (Bienestock and Johnston, 1976) [3]. Bienestock *et al.* (1973) reported that morphology of GALT and BALT was same. But Sminia *et al.* (1989) reported Peyer's patches and BALT was not covered by a capsule which is not in agreement with our present findings.

Functional and immunological aspect of BALT

BALT had major morphologic and functional similarities to peyers patches (Bienestock, 1980 and Rodriguez *et al.* 2001) [13]. BALT showed CD4+ T lymphocyte, MHC- II + expressing cells, IgM positive cells and CD8+ T lymphocytes in all the experimental groups. Earlier it was reported by Rodrignez *et al.* (2001), Mossamann and Coffman (1989) [10] and Beinestock and Befus (1984) [5]. Hunninghake and Fauci (1976) [8] studied that in case of pig the proliferated lymphoid tissue of the BALT showed a distribution pattern for T and B lymphocytes and follicular dendritic cells similar to that of lymph node cortex.

Racz *et al.* (1977) [11] examined that the rare plasma cells were seen to be scattered throughout the lymphoid follicles and occasionally plasma cells were found in the overlying lymphoepithelium. The follicles contained the high walled endothelium associated with post-capillary venules and this type of endothelium appeared to 'be more prominent following antigen challenge. But such type of plasma cell was not identified in the lymphoepithelium. No secondary follicle as reported by Rodriguezd *et al.* (2001) [13] in case of goat and no ectopic lymphoid tissue that was formed upon inflammation or infection in both mice and human as reported by Randall (2010) [12] were observed in any of the experimental group. Lymphocyte was found in the lymphoepithelial tissue in the experimental animal. This was in accordance with Simnia *et al.* (1989). But no M cell was found. This lymphoepithelium is reminiscent of the follicle associated epithelium overlying peyers patches in the gut. This may act as a sampler of the luminal antigen much or thus the follicle associated epithelium of MALT. Initially upto 2 months, the size of BALT was found small and even at birth it was resemble like a diffuse aggregation but size increased abruptly with age after 3 months. This may be due to the encounter of BALT with the air brone antigen which promotes complete BALT development.

The MHC-II + cells showed a significant increase from 2 months onward. This may be due to active participation in the signal regulation of APC both through humoral and cellular immunity (Mossmann and Coffman, 1989) [10]. CD8+ cells were not reported by any worker but their presence in low frequency in all experimental groups may indicate that the local cell mediated immunological role of BALT against antigen.

Conclusion

At birth the small lymphocytic aggregation was found near the respiratory pathway. The aggregation of lymphocyte was found in diffused form rather than in the nodular form. Mostly they were found in 1st and 2nd order of bronchus and at the respiratory bronchioles. From 1st month onward typical lymphonodular tissue was observed in the same area as described at the date of birth. The sizes of lymphnodules were increased according to advancement of age. BALT showed CD4+ T lymphocyte, MHC- II + expressing cells, IgM positive cells and CD8+ T lymphocytes in all the experimental groups. CD4 positive cells population was found to be more as compare to the CD8 cells. B lymphocytes were identified scatteredly in the bronchus associated lymph nodes and also within the interalveolar spaces. MHC II Positive cells were found in the interalveolar spaces, within alveoli and also at BALT.

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