

Red in Translation

Bronchus-Associated Lymphoid Tissue

An Entry Site for Antigens for Successful Mucosal Vaccinations?

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An accumulation of lymphoid cells with a typical localization of B lymphocytes preferentially in a follicle and T lymphocytes, more peripherally around high endothelial venules in the wall of bronchi, is called bronchus-associated lymphoid tissue (BALT). A further structural component is a cap-like accumulation of lymphoid cells partly bulging into the lumen of the bronchus, called the dome area. The epithelium covering the dome lacks goblet cells, is infiltrated by lymphocytes, and contains cells specialized for antigen uptake—M cells. BALT is not present in all species and age groups and can be classified as a tertiary lymphoid organ. A hypothesis is proposed for a two-step vaccination protocol: first, BALT is induced and activated, and second, an antigen—the vaccine—is applied locally. BALT is part of the integrated mucosal-associated lymphoid tissue system.

Keywords: bronchus-associated lymphoid tissue; age dependence; functional anatomy; antigen uptake

After we published our observations on the lack of bronchus-associated lymphoid tissue (BALT) in the lungs of healthy adult humans (1), it took some time before BALT was no longer described as a constitutive lymphoid structure like Peyer's patches (PP) in reviews and textbooks of pneumology and immunology. In 2000 we summarized the data on human BALT in health and disease (2). The present update focuses on more recent findings, and on the concept of stimulating the development and activity of human BALT as an entry site for vaccines, in particular for infectious diseases of the lung.

AGE DEPENDENCE OF HUMAN BALT

After the absence of BALT in the lungs of adults without pulmonary diseases had been documented 20 years ago, we reported the presence of typical BALT in lungs of children who had died of sudden infant death syndrome (in 36%) or of other causes such as trauma (in 44%) (3), as shown in Figures 1 and 2. These frequencies were correlated to those of larynx-associated lymphoid tissue (LALT) (4) and nose-associated lymphoid tissue (NALT). The frequency of BALT in the human lung in health and diseases was summarized in 2009 (2).

In human fetal lungs BALT has also been described in a series of 74 fetuses and prenatal deaths. BALT was found as early as 16 weeks of gestation. The frequency of BALT in

newborns and fetuses with choriomeningitis was 100% (5). Thus, in a prenatal amniotic infection the lungs can react by forming BALT. This is in contrast to PP, which are regularly found prenatally in humans. Gould and Isaacson (6) documented BALT in many fetal lungs with choriomeningitis, while in lungs of fetuses without an obvious infection the frequency of BALT was 10%. In the 17 infant lungs studied, BALT was found in 77%, but a well-developed lymphoepithelium was present in only 24%. It remains to be clarified why BALT disappears with age, in contrast to PP, which are found in the human gut up to old age.

A further interesting approach is the transplantation of human fetal tissue in SCID mice, as only the co-implanted peribronchial lymph nodes induced BALT formation in contrast to bone marrow, thymus, or liver. This BALT was able to mount a robust T cell immune response to *Pseudomonas aeruginosa* (7). Moussallem and coworkers (8) quantified lymphocyte subsets as well as cytokine production in the lungs of 42 children (mean age 29 mo) who had died of measles, and described a loss of immune cells in BALT. However, none of the figures document structures that fulfill the criteria of BALT. Heier and colleagues (9) analyzed biopsy material of 45 children (age 9–23 mo) and documented a high frequency of tolerogenic dendritic cells (DC) and regulatory T cells (Tregs) in BALT not related to the atopic status of the individual.

Kawamata and coworkers (10) published recently an interesting paper on BALT in lungs of patients undergoing lobectomy due to lung carcinoma. They found BALT in 35% of the specimens, which might be due to the tumor and/or other noxious factors.

Furthermore, lymphocyte subsets in these human BALT have been characterized: most T cells were of memory phenotype, and nearly all B cells expressed $\alpha 4$ and L-selectin in contrast to T cells.

INDUCTION OF BALT IN THE LUNGS OF ADULTS

In a study on 44 elite cross-country skiers who were nonsmokers but obviously chronically exposed to air temperatures below 0°C, the authors described small BALT-like structures in biopsies from second- and third-generation bronchi (11). However, these structures consisted of neither germinal centers, high endothelial venules (HEV), nor a real lymphoepithelium. In a retrospective evaluation of lung sections of operation specimens ($n = 100$) with chronic pulmonary inflammation as one pathology diagnosis, only in 8% was typical BALT identified partially beyond an occlusion of the respective bronchus (12). In chronic obstructive pulmonary disease (COPD) there are obvious BALT structures, in particular in patients with a more severe form (GOLD stage 3 and 4), as documented by Hogg and colleagues (13). Meanwhile, it has been considered whether these accumulations of lymphoid tissue are beneficial or harm-

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TABLE 1. LIST OF AGENTS KNOWN TO INDUCE OR INHIBIT BALT

BALT Induction (Ref. no.)	BALT Inhibition
Cold air (12)	Steroids?*
Chronic inflammation (13)	
COPD (14)	
Bacteria (46, 50, 51, 55)	
Viruses (45, 58)?	
MALP-2 (TLR2/6-agonist) (56, 57)	

Definition of abbreviations: BALT, bronchus-associated lymphoid tissue; COPD, chronic obstructive pulmonary disease.

* No data are available whether BALT development or induction can be inhibited.

ful for the patient (14). In a recent study it was shown that the chemokine receptor CXCR3 is highly expressed by CD4⁺ CD8⁺ T and CD20⁺ B lymphocytes in BALT structures in COPD lungs (15). The functional and potential therapeutic relevance is still open to discussion.

LYMPHOID AGGREGATIONS WITHOUT CONTACT TO A BRONCHUS ARE NOT BALT

In several autoimmune diseases such as rheumatoid arthritis, Sjögren syndrome, and interstitial pneumonitis, aggregations of lymphoid cells in follicle-like structures (16) have been described, and these can be called tertiary lymphoid tissue (for review see Ref. 17).

Several such structures were found in the subpleural space or without contact to a bronchus, and therefore the term inducible BALT is misleading, as they cannot have the function of antigen uptake from the bronchus lumen (18–20). These lymphoid structures are of great interest in immune reaction of the lung and the role of chemokines. The loss of the chemokine ligand 13 (CXCL13) had minimal effects on the development and function of inducible BALT (iBALT) (21). It remains to be documented whether lymphoid aggregations induced in different parts of the lung are of interest in their response to inhaled antigens.

STRUCTURAL DETAILS OF BALT: HEV, LYMPHATICS, NERVES, TREGS

In the BALT of rats the HEV and the different lymphocytes in their wall have been studied using electron microscopic techniques including surface markers. It has been documented that the majority of lymphocytes in the wall of HEV are T lymphocytes (55%), with immunoglobulin (Ig)M+ (29%), IgA+ (27%), and IgG+ (2%).

B lymphocytes were also identified (22). Thus, the HEV are used as entry sites into the BALT as in the lymph nodes. In most species lymphocytes leave lymph nodes via efferent lymphatics. Applying the same technique, Otsuki and co-workers (22) documented T and B lymphocytes in the lymphatics in the BALT of rats. Recently, the expression of adhesion molecules on HEV in human BALT was studied: surprisingly, the peripheral lymph node addressin (PNAd) and not the mucosal addressin MAdCAM-1 (10) was expressed, documenting the unique structure and function of BALT. Interestingly, in NALT of mice a preponderance of PNAd over MAdCAM-1 was found (23). In a recent study, transferred T cells adhered preferentially in the BALT during asthma in CD26-deficient rats (24). Future studies should clarify the integration of BALT in the route of migrating lymphocytes. This would be of relevance for vaccination protocols.

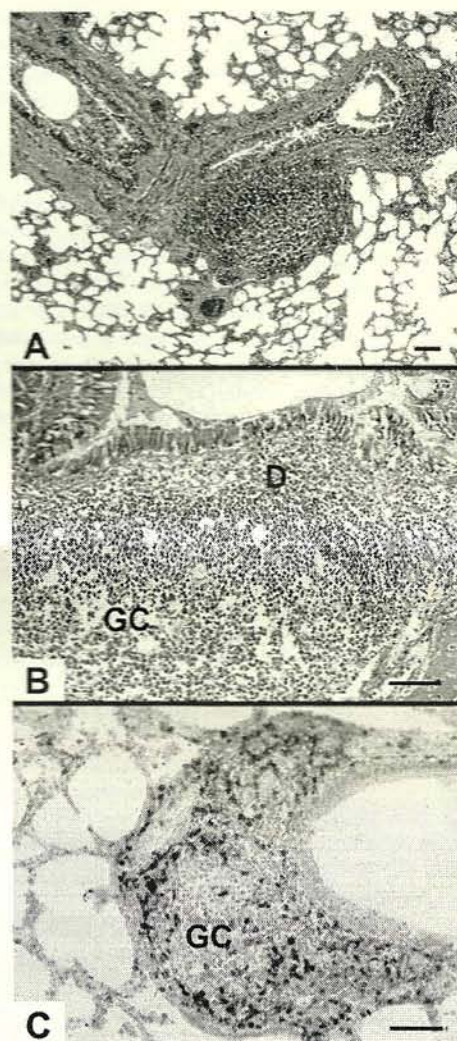


Figure 1. Histological section of the lung of young children documenting bronchus-associated lymphoid tissue (BALT). (A) Overview of BALT in an 11-month-old child (hematoxylin and eosin [H&E] staining). (B) BALT of a 10-month-old child, documenting the infiltration of the overlying epithelium by lymphocytes (H&E staining). D, dome area; GC, germinal center. (C) Immunohistology of a BALT structure of a child (8 mo old). T lymphocytes are shown in brown after applying an anti-CD2 antibody. GC, germinal center. Scale bars: 100 μ m.

BALT in rats (the microbial status had not been described) contained noradrenergic nerve fibers, which decreased with age (3 mo, 12 mo, and 24 mo of age) (25). In a previous study the same group had described a decrease with age of acetylcholinesterase and cholinacetyl transfer activity in rat BALT, as a marker for the parasympathetic system (26).

In a preliminary study on BALT from patients (without documentation of the case history), the same enzymes were shown, but the authors left it open whether this was a "neuronal" or a "non-neuronal" labeling (27). The functional role of the innervation pattern has to be studied in future in other species and in more detail.

In a recent paper, the concept was proposed that BALT formation may be provoked by the lack of CCR7 and that Tregs might be important in controlling BALT formation in mice (28). Interestingly, high numbers of Tregs have also been found in BALT of young children (9).

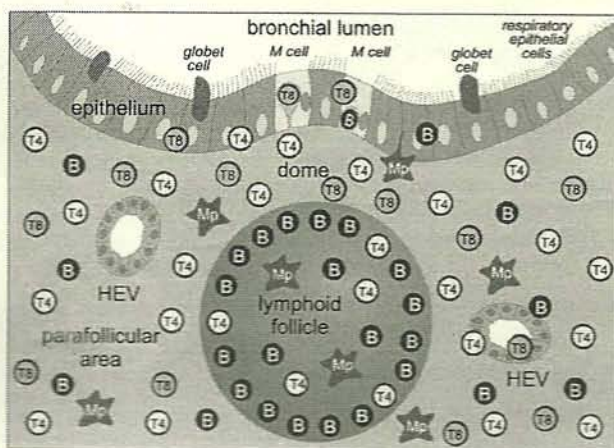


Figure 2. Schematic drawing of BALT with the main structural components (adapted by permission from Ref. 2).

M CELLS: A SITE OF ANTIGEN UPTAKE

A unique type of epithelial cell are microfold or membranous cells (M cells), which were found among the normal epithelial cells of the dome area of BALT in rabbits (Figure 2). The intraluminally applied, fluid-phase tracer horseradish peroxidase adhered to the apical membrane of these cells. Antibodies that identified M cells in PP also identified a subset of dome-covering epithelial cells. In rats it was shown that particularly antigen and virus were transported more avidly by M cells than by other epithelial cells. However, no clearly defined M cells have been characterized so far for BALT in humans (for review see Ref. 29). In rabbits the epithelium covering the dome of BALT did not express the secretory component for IgA (30), similar to the dome epithelium of PP, which facilitates the contact of luminal antigen with the M cells. Toyoshima and colleagues (31) documented BALT induction after approximately 10 repeated inhalations of *P. aeruginosa*, and the uptake of the fluorescently labeled bacteria with some strain dependence, and a higher uptake in sensitized mice. M cells in the lung have also been documented as entry sites for *Mycobacterium tuberculosis* in mice (32). The concept of oral immunization by a receptor-mediated targeting of M cells has been proposed (33, 34). By using an M cell-specific monoclonal antibody as a carrier for M cell-targeted mucosal vaccine, the antigen-specific immune reaction was documented by electron microscopy, immunohistochemistry, and antibody titres (35).

In addition to M cells, there are DC in the air-conducting parts of the respiratory tract (36–38). The number and topographic distribution of CD11 c+ DC in the human trachea have been documented recently (39). Surprisingly, the DC number and function in the lung could be enhanced by a single intratracheal installation of the growth factor Flt3L in rats (40). This might be a future therapeutic approach to enhance antigen uptake by DC.

A further interesting aspect is to define which antigens are taken up by epithelial DC or by M cells in the BALT epithelium and whether both entry sites induce tolerance and/or protective immune reactions. The time kinetics seem to play a major role, as shown by an accelerated immigration of DC to the bronchial lymph node but a refractory later phase of this mobilization in spite of ongoing virus replication (41).

Zuercher and Cebra (42) compared the structure and function of putative mucosal inductive sites (NALT, BALT, and PP) after reovirus infection in rats, and stated that BALT is not

an inductive site for mucosal immune reactions. However, it is not known why the rats in these experiments (aged 6–10 wk) already had active BALT, which is not typical for conventionally housed rats.

WHAT CAN BE LEARNED FROM OTHER SPECIES?

It has been known for some time that mycoplasma infections in pigs induce enormous hyperplasia of BALT. Saradell and coworkers (43) characterized the leukocyte subsets in the hyperplastic BALT after *Mycoplasma hyopneumoniae* infection. As it is difficult to breed rabbits free of mycoplasma infections, such a nonvirulent infection might have been the reason for the description of BALT mainly in this species since 1974 (for review see Ref. 44). Interesting new aspects have been described for pigs infected with different bacteria. Surfactant protein D (SP-D) was documented in DC of BALT, indicating it as a link between the innate and adaptive immune system in the lung (45). However, in mice lacking SP-D the development of BALT was documented (46). There are numerous publications on BALT size and subset composition in different types of inflammation (e.g., induced by lipopolysaccharide, formaldehyde or xylene exposure, or pulmonary infections ranging from *Trichinella spiralis* [47] to *Mycobacterium tuberculosis* [48]). In these experiments with chronic infections of mice with mycobacteria, not only granuloma but also BALT-like structures were found with follicular DC and HEV. Thus, such animal experiments can only provide indications of mechanism and functions of BALT development. Future experiments should be performed in animals with a highly defined microbial status. Recently, Halle and colleagues documented (49) that a single intranasal dose of the replication-deficient vaccinia virus induced BALT with a typical BALT structure 8 days later. Intratracheally injected antigen-loaded DC immigrated into BALT and stimulated T lymphocytes. These data support the concept of BALT as a tertiary lymphoid organ.

THE TWO-STEP CONCEPT TO TAKE ADVANTAGE OF BALT IN MUCOSAL VACCINATION

Recently, the great interest in developing mucosal vaccines (50), and the urgent need for the development of targeted therapeutic strategies in manipulating lung immune responses and preventing immune-mediated damage, have been stressed (51).

A new concept would be to induce BALT (Figure 3) and/or increase its activity including M cells as a first step followed by inhaling (for example) killed bacteria as a vaccine in a second step. Repeated inhalation of heat-killed *P. aeruginosa* in mice

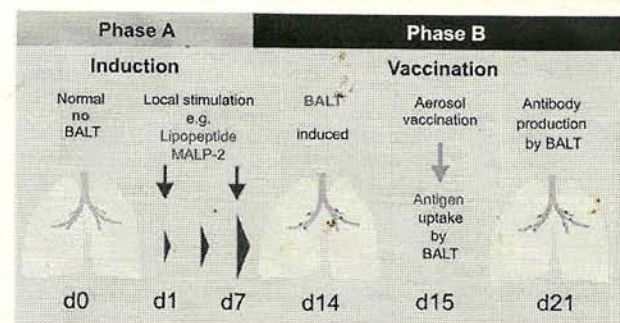


Figure 3. Schematic drawing of the two-step concept for inducing the development of BALT followed by intratracheal application of antigens as a vaccination strategy.

(31) or applying the TLR 2/6 agonist MALP-2 in rats (52, 53) increased and activated BALT. Agents known to induce or inhibit BALT was summarized in Table 1.

As the second step, the vaccine inhalation should result in increased protective immune reaction. Thus, BALT might be used as an entry site for vaccines in the lung (Figure 3).

However, it has to be clarified in other species and models whether BALT harbor virus (e.g., Epstein-Barr virus) as shown in CCR7^{-/-} mice (54), which could be dangerous when BALT is induced by a stimulation protocol.

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