

Frequency and potential cause of bronchus-associated lymphoid tissue in fetal lungs

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Bronchus-associated lymphoid tissue consists of lymphoid follicles with or without a germinal center within the bronchial wall. Bronchus-associated lymphoid tissue is part of the integrated mucosal immune system and present in about 50% of healthy infants. We examined a series of 141 fetal and neonatal lungs and detected bronchus-associated lymphoid tissue in 100% of cases with amniotic infection while postpartum perinatal pneumonia did not elicit bronchus-associated lymphoid tissue formation. Only rarely and in low density, bronchus-associated lymphoid tissue was present in non-infected fetuses. The *in utero* formation of bronchus-associated lymphoid tissue seems to be a reactive phenomenon and – as has been shown in another study – does not portend an adverse prognosis.

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Bronchus-associated lymphoid tissue (BALT) consists of follicular-like accumulations of B lymphocytes with or without a classical germinal center and surrounding T lymphocytes in the bronchial wall. Small T lymphocytes infiltrate the overlying epithelium; sometimes a dome-like protrusion into the bronchial lumen is present. This area contains S100-positive, CD1a-negative dendritic cells, which are known as potent antigen-processing cells. There are no afferent lymphatics, instead, high endothelial venules (HEV) are the only entry site for lymphocytes to migrate into the BALT (1). BALT is part of the integrated mucosal immune system and was first described 30 yr ago in the lungs of rabbits (2). Its occurrence differs greatly between species.

Bronchus-associated lymphoid tissue is not a constitutive structure in the healthy adult lung. In contrast, several studies have shown that BALT can be demonstrated in about 50% of healthy infants and young children (3, 4). Thus, from about 0.5–5 yr of age it can be regarded as a regular element in the lung. Its presence has been related to the high amount of ‘unknown’ antigenic stimuli in the lungs at that age.

About 10 yr ago, BALT was reported in 45% of the lungs of fetuses born under the conditions of amniotic infection (AI) (5). We examined a series of 141 fetal and neonatal lungs and detected BALT in 100% of cases with chorioamnionitis while postpartum perinatal pneumonia did not elicit BALT formation. Only rarely and in low density, BALT was present in non-infected fetuses.

Materials and methods

The study included 141 fetuses and perinatal deaths from 1993 till 1999 with a gestational age of 16–41 wk. Death was mainly due to prematurity and mostly occurred within the first 24 h (Table 1). Permission for autopsy was granted in each individual case by the parents. This study was performed on routinely collected materials only; their use for scientific purposes was in accordance with ethical guidelines of the University Hospital Zurich at that time. Causes of prematurity and/or death were AI (n = 66), pre-eclampsia (PE) (n = 28), fetal malformation (n = 20), and premature labor (n = 12). Cause

Table 1. Clinical data, cause of premature birth and/or death and presence of bronchus-associated lymphoid tissue

	Patients	BALT
Died <24 h	108	63
Died >24 h	33	11
Chorioamnionitis	66	66
Pre-eclampsia	28	0
Fetal malformation	20	7
Other maternal causes	12	0
Placental causes	12	1
Birth asphyxia	3	0

of death of term newborns were abruptio placentae ($n = 12$) and birth asphyxia ($n = 3$). AI was diagnosed on the basis of maternal fever, elevated C-reactive protein levels, fetal tachycardia and chorioamnionitis on placental histology (all criteria had to be present). PE was diagnosed on the basis of edema, hypertension, proteinuria, exaggerated reflexes and elevated transaminases (all criteria had to be present) and/or acute atherosclerosis of spiral arteries on histology of the placenta or a placental bed biopsy.

All histological slides were obtained from formalin-fixed and paraffin-embedded tissue; 2- μ m-thick sections were cut from two blocks per case (upper and lower right lobe) and stained with hematoxylin-eosin. The detection of BALT is much easier when lymphocytes are specifically stained. Therefore, immunohistochemistry was performed with a standard peroxidase-anti-peroxidase technique with a primary anti-CD45 antibody from Dako (A/S Glostrup Denmark; clones 2B11 + PD7/26, cat-no. M0701; Fig. 1). The evaluation was carried out without knowledge of the clinical diagnosis. Quantitative measurements were taken by computer-assisted morphometry on a microscope equipped with a video camera (Zeiss AxioHOME, Oberkochen, Germany). Lymphoid aggregates were taken as BALT when they were in close contact with the bronchiolar epithelium. BALT was counted as lymphoid aggregates per mm^2 . Statistical analysis was performed with a non-parametric Mann-Whitney test and chi-square test for nominal data (Statview for Windows, version 5.01).

Results

Bronchus-associated lymphoid tissue was present in the slides of lung tissue of 74 fetuses and perinatal deaths; this included all cases with AI ($n = 66$). However, the density of BALT varied greatly between 0.07 and 4.7 aggregates per mm^2 . BALT was present as early as 16 wk gestational

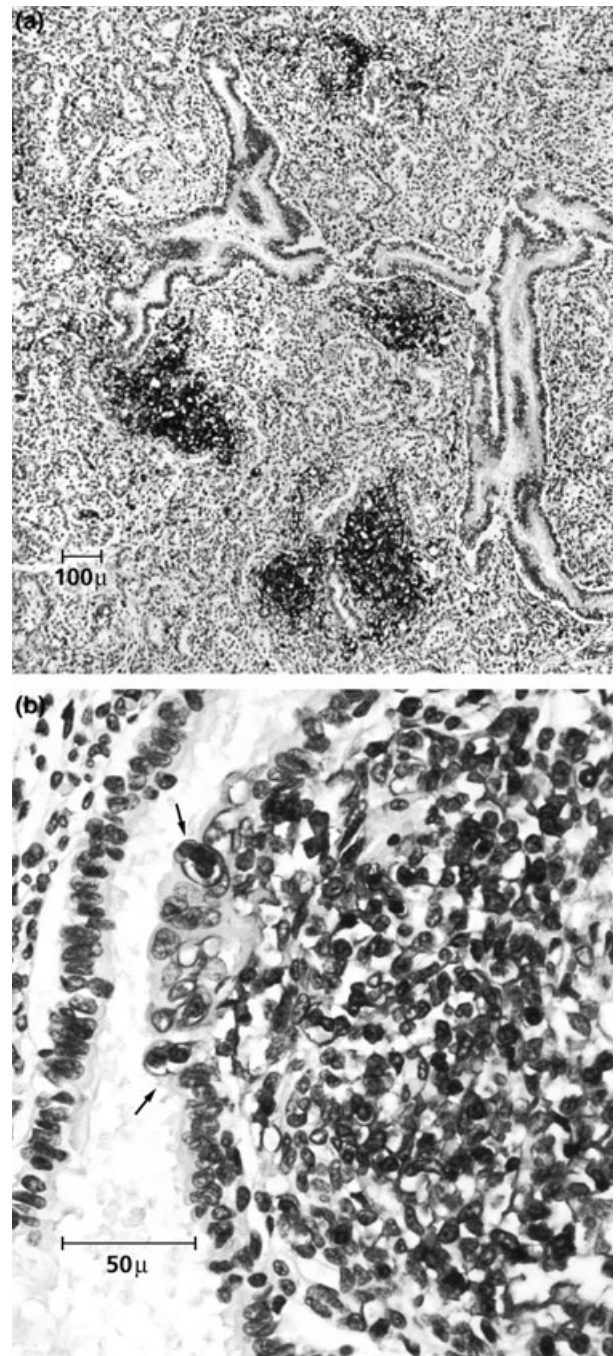


Fig. 1. Bronchus-associated lymphoid tissue highlighted with anti-CD45 immunohistochemistry in a fetus of 23 wk gestational age is preferentially found at the ramifications of bronchi (a); lymphocytes enter between epithelial cells (arrows) (b).

age, and there was no correlation of BALT density and gestational age.

Eight fetuses born between 18 and 21 wk gestational age without chorioamnionitis showed low-density BALT (0.05–0.21 aggregates per mm^2). Of those, one presented with severe intrauterine growth retardation due to maternal

hypertension, two had trisomy 21 without malformations, five showed a combination of several severe malformations and one had isolated hydrocephalus. None of the eight revealed signs of infection, oligohydramnion was not reported.

Twelve newborns that were born under the conditions of AI did not die right after birth but 1–4 days later. All revealed BALT. In contrast, four newborns surviving 2–5 days died from pneumonia. This was not preceded by chorioamnionitis and their lungs did not reveal BALT.

Morphometric analysis also evaluated the occurrence of single lymphocytes, which are diffusely scattered through lung tissue. The range was between 15 and 524 lymphocytes per mm² lung tissue. There was no significant difference between different fetal conditions. However, there was a significant increase in lymphocyte density with advancing gestational age.

Discussion

If BALT is searched for by immunohistochemistry which highlights even rare lymphoid aggregates, the occurrence is 100% in fetuses/newborns with chorioamnionitis. With the same sensitive approach BALT is present in <4% of fetuses/newborns emanating from other condition. All BALT detected in other conditions was of low density; the majority occurring in complex malformation syndromes with no common denominator as to its possible etiology. All eight cases had an intact integument and oligohydramnion as a possible cause of low clearing of antigens from amniotic fluid was not reported.

In children born under the conditions of chorioamnionitis, BALT remains visible for some time and can thus serve as an indicator of antecedent chorioamnionitis in perinatal autopsy cases. In contrast, early onset postpartum pneumonia seems not to be able to stimulate BALT formation within a few days. From this, it can be deduced that the formation of BALT is not a simple reaction to exogenous bacteria in the alveoli but rather a complex immunological response to bacteria plus a change in cytokines derived from swallowed amniotic fluid. With chorioamnionitis strong increases of interleukin-1, interleukin-8 and granulocyte-colony stimulating factor can be observed in amniotic fluid, particularly dramatic is the increase in interleukin-6 by two orders of magnitude (6, 7). In a mouse model, sustained interleukin-6 signaling led to the development of lymphoid-like structures in the lung (8).

Chorioamnionitis leads to some more adaptive changes in the fetus. We reported (9) a strong increase in granulopoiesis occurring in the sinusoids of the fetal liver with chorioamnionitis. The quantitation of this extramedullary granulopoiesis reveals a positive correlation with the density of BALT, which might indicate simply that both phenomena increase with duration time of AI.

The occurrence of BALT in later life has been attributed to periods of increased influx of antigens or chronic irritation of the respiratory epithelium. Beyond infancy, BALT was found in 82% of smokers and 14% of non-smokers (10). However, a considerable number of lymphoid follicles was associated with the ducts of bronchial glands or deep within the bronchial walls without contact to the epithelium. The latter two structures do not fulfill criteria of BALT in the strict sense.

It is also conceivable that the combination of antigens arriving via the airways and a distinct immunological predisposition favors the formation of BALT. In line with this, BALT was found in the lungs in all eight patients with rheumatoid arthritis, even when strict criteria were used (11). Also some chronic lung diseases with an immunological etiology show BALT formation; in this organ, infection can always be an additional parameter (12).

As far as life *in utero* is concerned, the formation of BALT seems to be an adaptive/reactive phenomenon and does not portend a bad prognosis as we have shown in groups of newborns surviving chorioamnionitis (13).

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