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Impact of chorioamnionitis on the development of human fetal lung: an immunohistochemical study

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Summary

Purpose: Current studies suggest that changes of chorioamnionitis are associated with the appearance of bronchial-associated lymphoid tissue (BALT), during fetal development. The aim of this study was to examine and analyse apart from the appearance of BALT, the expression of structural proteins in the lung parenchyma during gestation. *Materials and Methods:* A series of 149 paraffin-embedded human fetal lung specimens at the second trimester of development were examined by immunohistochemistry using the monoclonal antibodies CD20, CD3, Tenascin-C, Vimentin, and Fibronectin. *Results:* The results of this study showed that 1) BALT does not develop in fetal period and 2) BALT which develops during fetal period is probably in response to antigenic stimulation where in the present cases occurs to be changes of chorioamnionitis which decreased the expression of filaments proteins in the intermediate cells of lung parenchyma in comparison with the normal ones. *Conclusion:* The expressions' pattern of intermediate filaments proteins in the lung parenchyma can be modified by the presence of chorioamnionitis in the fetal membranes.

Key words: Chorioamnionitis; Lung; Vimentin; Tenascin-C; Fibronectin; BALT.

Introduction

It is now generally accepted that histologic chorioamnionitis is the hallmark of ascending intrauterine infection, and inflammation in and between placental villi indicates blood-borne infection. Although chorioamnionitis constitutes the most common form of placental inflammation in humans and occurs in approximately 4% of otherwise non-complicated term births [1-4], very little is known about the biological and clinical pathological significance and consequences of chorioamnionitis in mesenchymal components of fetal lung parenchyma during the development. The expression status of proteins: Tenascin-C - a large glycoprotein synthesized by fibroblasts which is believed to have active functions in fetal lung branching morphogenesis [5], Vimentin - an intermediate filament protein, serving as modulator between extracellular influences governing calcium flux into the cell and stains mesenchymal components [6], Fibronectin - a cell adhesive extracellular matrix protein highly expressed in developing lungs [7], and in correlation with the appearance of bronchus-associated lymphoid tissue (BALT) - refers to the well-organized lymphoid tissue that is located under the respiratory epithelium in the bronchial wall [8] - expressing by the lymphocytic markers CD20, and CD3 have never been assessed.

Accordingly the aims of the present study were: i) to explore the immunohistochemical expression of Tenascin-C, Vimentin, and Fibronectin in fetal lung without changes of chorioamnionitis, and ii) to discover the expression of the above proteins in fetal lung with changes of chorioamnionitis. In the latter case, a concomitant variable degree of lymphocytic infiltrate was seen in the lung parenchyma especially around the bronchial tree.

Materials and Methods

Specimens of 149 post-mortem human fetal lung at the second trimester of development during the years 2005-2012 were obtained from the archives of the Department of Histology – Embryology, Medical Faculty of Democritus, University of Thrace, Alexandroupolis, Greece. 32 out of 149 specimens were with normal histology fetal membranes whereas 18, 25, and 45 were presented with mild, moderate, and severe chorioamnionitis, respectively. In addition, the authors examined 17 fetal membranes with hydropic degeneration of chorion villi, eight coming from embryos diagnosed with Down syndrome, two cases infected with Toxoplasma hominis, and also two infected with cytomegalovirus (CMV).

A series of four-μm thick sections were cut from the specimens. The histological features of all cases were reassessed on the basis of sections stained with haematoxylin and eosin. Representative paraffin blocks were available for immunohistochemical evaluation.

Immunohistochemistry

Immunohistochemical staining was performed on four-μm-thick deparaffinised sections. The sections were pretreated by heating in a pressure cooker containing a citrate buffer (pH 6.0), then washing with distilled water and cooking to room temperature with Tris buffer. Sections were incubated with primary antibodies for 30 min-

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Table 1. — *Vimentin expression in the interstitial tissue of lung parenchymal tissue in correlation with the histological changes of fetal membranes (amnion and chorion).*

Number of incidents	Histological changes of fetal membranes	Vimentin expression in intermediate cells of the lung parenchyma			
149		0+ (%)	1+ (%)	2+ (%)	3+ (%)
32	Normal histology fetal membranes				32 (100)
18	Mild changes of acute chorioamnionitis		10 (55.55)	5 (27.77)	3 (16.66)
25	Moderate changes of acute chorioamnionitis	13 (52)	5 (20)	4 (16)	3 (12)
45	Severe changes of acute chorioamnionitis	26 (57.77)	8 (17.77)	7 (15.55)	4 (8.88)
17	Hydropic degeneration of chorionic villi	2 (11.76)	2 (11.76)	3 (17.65)	10 (58.82)
8	Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis	4 (50)	2 (25)	1 (12.5)	1 (12.5)
2	Toxoplasmosis - severe changes of acute chorioamnionitis	2 (100)			
2	Cytomegalovirus - severe changes of acute chorioamnionitis	1 (50)	1 (50)		

Table 2. — *Fibronectin expression in the interstitial tissue of lung parenchymal tissue in correlation with the histological changes of fetal membranes (amnion and chorion).*

Number of incidents	Histological changes of fetal membranes	Fibronectin expression in intermediate cells of the lung parenchyma			
149		0+ (%)	1+ (%)	2+ (%)	3+ (%)
32	Normal histology fetal membranes				32 (100)
18	Mild changes of acute chorioamnionitis	5 (27.77)	6 (33.33)	4 (22.22)	3 (16.66)
25	Moderate changes of acute chorioamnionitis	10 (40)	7 (28)	5 (20)	3 (12)
45	Severe changes of acute chorioamnionitis	28 (62.2)	10 (22.22)	5 (11.1)	2 (4.44)
17	Hydropic degeneration of chorionic villi	5 (29.4)	3 (17.65)	2 (11.76)	7 (41.17)
8	Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis	4 (50)	3 (37.5)		1 (12.5)
2	Toxoplasmosis - severe changes of acute chorioamnionitis	2 (100)			
2	Cytomegalovirus - severe changes of acute chorioamnionitis	1 (50)	1 (50)		

utes. The primary monoclonal antibodies that were used were: anti-Vimentin (1:400 dilation in 10% Normal Rabbit Serum (NRS)/PBS), anti-Tenascin-C (1:100 dilation in NRS/PBS), and anti-Fibronectin (1:400 dilation in NRS/PBS).

After washing following primary antibody incubation, the bound antibody was visualized by the alkaline phosphatase anti-alkaline phosphatase (APAAP) method using the alkaline phosphatase detection system.

Diaminobenzidine was used as the chromogen substrate, producing a brown end-product and the sections were counterstained using haematoxylin. The specificity and the pattern of each antibody were tested on positive control tissue samples according the manufacturers' technical data.

For the immunohistochemical expression of lymphoid tissue (BALT), either B-lymphocytes or T-lymphocytes, the authors used the monoclonal pan-B cell antibody CD20, and the polyclonal pan-T cell antibody CD3 (ready to use).

The sections were scored using a semiquantitative system based on the frequency of immunohistochemical reactivity of individual parenchymal elements or vessels as follows: negative (0), weak (1+), moderate (2+), or strong (3+). Evaluation of immunohistochemical expression was based on the frequency of cytoplasmic reactivity.

Results

One hundred and forty nine specimens met inclusion criteria for performing Tenascin-C, Vimentin, Fibronectin immunohistochemistry.

The authors observed that 32 cases showing normal histology of fetal membranes, all 32 cases, showed strong positivity (3+) with Vimentin, Fibronectin, and Tenscin-C in the intermediate cells in the lung parenchyma.

On the contrary, the positive expression 3(+) of all Vimentin, Fibronectin, and Tenscin-C proteins were decreased accordingly to the severity of inflammation.

In more details, Vimentin expression ranged from 16.66% in mild to 8.88% in severe changes of acute chorioamnionitis. Same pattern presented Fibronectin and Tenascin-C expression that fluctuated from 16.66% in mild to 4.44 in severe inflammation and 11.11 to 6.66%, respectively.

Immunohistochemically staining for Vimentin, Fibronectin, and Tenacsin-C in 17 samples diagnosed with hydropic degeneration of chorionic villi showed positive expression (3+) in 58.82%, 41.17%, and 58.8%, respectively. In eight samples coming from embryos with Down Syndrome, diagnosed with severe changes of chorioamnionitis, the positive expression for the structural proteins is about 12.5%. Finally, none of the samples coming from infected by Toxoplasma hominis and CMV embryos presented positive expression for these proteins.

On the other hand, in 32 cases showing normal histology of fetal membranes, all of these presented no staining (0+) with CD20 and CD3 in the peribranchial tissue of the lung parenchyma.

The immunohistochemical reactivity data are summarized in Tables 1-5 and Figures 1 and 2. According to data that provided in Tables 4 and 5 the positive expression of

Table 3. — *Tenascin-C expression in the interstitial tissue of lung parenchymal tissue in correlation with the histological changes of fetal membranes (amnion and chorion).*

Number of incidents	Histological changes of fetal membranes	Tenascin expression in intermediate cells of the lung parenchyma			
149		0+ (%)	1+ (%)	2+ (%)	3+ (%)
32	Normal histology fetal membranes				32 (100)
18	Mild changes of acute chorioamnionitis	7 (38.88)	5 (27.77)	4 (22.22)	2 (11.11)
25	Moderate changes of acute chorioamnionitis	14 (56)	6 (24)	3 (12)	2 (8)
45	Severe changes of acute chorioamnionitis	27 (60)	10 (22.22)	5 (11.11)	3 (6.66)
17	Hydropic degeneration of chorionic villi	2 (11.76)	2 (11.76)	3 (17.65)	10 (58.8)
8	Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis	3 (37.5)	3 (37.5)	1 (12.5)	1 (12.5)
2	Toxoplasmosis - severe changes of acute chorioamnionitis	2 (100)			
2	Cytomegalovirus - severe changes of acute chorioamnionitis	2 (100)			

Table 4. — *CD20 expression in the peribranchial tissue of lung parenchyma in correlation with the histological changes of fetal membranes (amnion and chorion).*

Number of incidents	Histological changes of fetal membranes	CD20 expression in intermediate cells of the lung parenchyma			
149		0+ (%)	1+ (%)	2+ (%)	3+ (%)
32	Normal histology fetal membranes	32 (100)			
18	Mild changes of acute chorioamnionitis	1 (5.55)	2 (11.11)	5 (27.77)	10 (55.55)
25	Moderate changes of acute chorioamnionitis	2 (8)	2 (8)	6 (24)	15 (60)
45	Severe changes of acute chorioamnionitis	2 (4.44)	3 (6.66)	10 (22.22)	30 (66.66)
17	Hydropic degeneration of chorionic villi	15 (88.23)	1 (5.88)	1 (5.88)	
8	Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis	1 (12.5)	1 (12.5)	2 (25)	4 (50)
2	Toxoplasmosis - severe changes of acute chorioamnionitis			1 (50)	1 (50)
2	Cytomegalovirus - severe changes of acute chorioamnionitis			1 (50)	1 (50)

Table 5. — *CD3 expression in the peribranchial tissue in correlation with the histological changes of fetal membranes (amnion and chorion).*

Number of incidents	Histological changes of fetal membranes	CD3 expression in intermediate cells of the lung parenchyma			
149		0+ (%)	1+ (%)	2+ (%)	3+ (%)
32	Normal histology fetal membranes	32 (100)			
18	Mild changes of acute chorioamnionitis	2 (11.11)	3 (16.66)	5 (27.77)	8 (44.44)
25	Moderate changes of acute chorioamnionitis	2 (8)	4 (16)	6 (24)	13 (52)
45	Severe changes of acute chorioamnionitis	2 (4.44)	5 (11.11)	10 (22.22)	28 (62.22)
17	Hydropic degeneration of chorionic villi	12 (70.58)	3 (17.65)	1 (5.88)	1 (5.88)
8	Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis		1 (12.5)	1 (12.5)	6 (75)
2	Toxoplasmosis - severe changes of acute chorioamnionitis			1 (50)	1 (50)
2	Cytomegalovirus -severe changes of acute chorioamnionitis			1 (50)	1 (50)

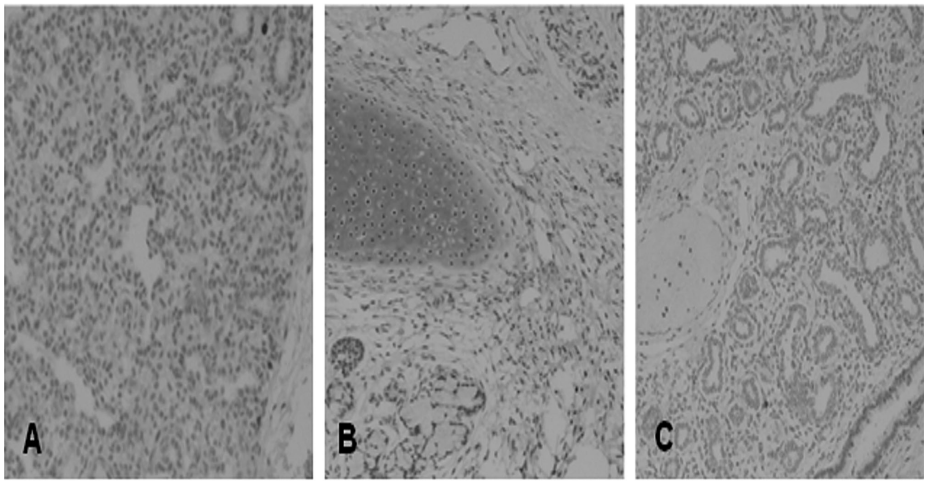

Figure 1. — Representative micrographs immunostaining for Vimentin (A), Fibronectin (B), and Tenascin-C (C) in intermediate cells of the lung parenchyma coming from embryos with severe changes of acute chorioamnionitis in fetal membranes (original magnification x200).

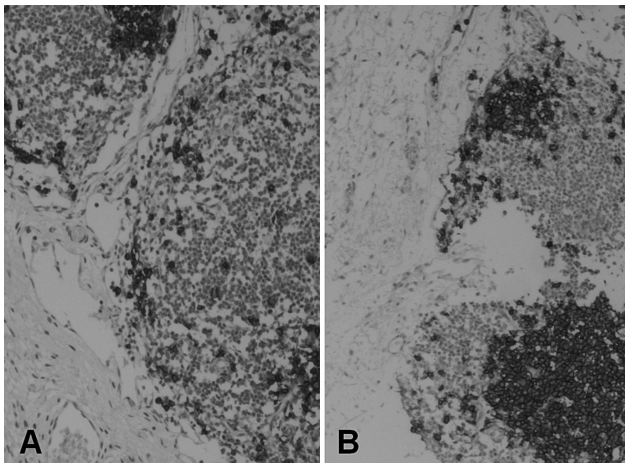

Figure 2. — Representative micrographs immunostaining for CD3 (A) and CD20 (B) in intermediate cells of the lung parenchyma coming from embryos with severe changes of acute chorioamnionitis in fetal membranes (original magnification x200).

CD20 and CD3 was increased and associated with the severity of inflammation degree in all studied groups.

Discussion

Mucosae-associated lymphoid tissue (MALT) can be surely classified to the most fundamental obstacles, which resists effectively to the factors that originate from the environment [9].

From a physiological vision, these system's mechanisms are well understood, after much study. Nevertheless, its maturation in humans still constitutes a mystery. The explanation can be attributed to the fact that mucosal tissues of an infant, is difficult to be isolated and studied, for ethical reasons. It is also difficult to foresee the whole organization and possible functions of MALT of a newborn child, in comparison with a fetus or an adult [10].

Edward Klein is considered to be the first to describe lymphoid tissue correlating with bronchial mucosa in 1875. Furthermore, he recognized the resemblance between this tissue and MALT in other positions such as Peyer's Patches in small bowel mucosa [11]. A century later, Bienenstock *et al.* transacted a study of lymphoid tissue in rabbit lungs, focusing at the level of morphology and functions of this tissue [12].

Tscherning *et al.* proved in their study of a series of 145 fetal and neonatal lungs that the presence of BALT in 100% of cases is associated with chorioamnionitis [13]. Barman *et al.* showed in their study that BALT did not develop in prenatal periods and they demonstrated that BALT in the bronchial lamina propria of normal and diseased lungs is associated with potent antigens [14].

In accordance with this, other studies demonstrated that the development of BALT constitutes a response to antigen [15]. In the case of chorioamnionitis. this can be explained by the mix of amniotic fluid with lung fluid by fetal breathing causing lung exposure [16], and three pulmonary outcomes of concern for preterm infants – respiratory distress syndrome (RDS), pneumonia/sepsis, and bronchopulmonary dysplasia (BPD) can be caused [17].

Emery and Dinsdale proved in their study that in the context of unexplained childhood deaths, according to the measurement of the antigenic state, many of those children showed a higher number of lymphoreticular aggregates [18]. They also proved that the presence of lymphoreticular aggregates used as measure of the concentration of biologically derived antigens, first emerged a week after birth and progressively increased in number, entirely presented by the age of five years [19].

Sminia *et al.* recognised the interaction between antigens and BALT, resulting in local and systemic immune reactions finding a similar role of BALT with Peyer's patches [20]. Furthermore, Meuwissen *et al.* demonstrated that BALT was mentioned to be more conspicuous around bronchiole and smaller bronchi, correlating the hyperplasia of BALT with the degree of the inflammation of the respiratory system [21]. Other studies with fetal sheep exposed to intra-amniotic LPS showed persistently activated leucocytes in the airways, CD3 positive lymphocytes to lung tissue, increased expression of toll-like receptors 2 and 4, decreased caveolin-1 expression, and changes in multiple other signalling pathways [22, 23].

Getahun *et al.* showed the results of a study involving the effects of chorioamnionitis on early childhood asthma. They conducted a retrospective cohort study in children born alive in Kaiser Permanent Southern California (KPSC) health maintenance organization. They included 510,216 live and still births, excluding 8,738 cases of stillbirths, spontaneous and induced abortions, pregnancies delivered at fewer than 23 weeks gestation, children with birth defect, and neonatal mortality. They defined as exposure variable the clinically diagnosed chorioamnionitis and as the outcome variable the physician-diagnosed asthma in children younger than eight years. The results demonstrated that in contrast with children born around 37 and 38 weeks of gestation and not exhibited to chorioamnionitis in utero, the probability of asthma manifestations in children born preterm and exposed to chorioamnionitis in utero were much larger. This conclusion can be explained by the fact that chorioamnionitis releases microorganisms, toxic substances, and inflammation mediators in the growing bronchi. Thus, inflammation, injury, apoptosis, and airway remodelling is created and that results probably in bronchopulmonary dysplasia, suggesting the fetal origin of asthma [24].

According to the provided data of the literature, there is strong evidence that acute chorioamnionitis is associated with fetal lung development. The results of the present study suggest that the inflammation of the fetal membranes clearly affects the expression's pattern of intermediate fil-

aments proteins in the lung parenchyma and also induces the development of BALT in fetal period. Thus, the association between chorioamnionitis and preterm gestation may result in increased risk of childhood asthma. Elucidation of the effects of inflammation on the fetal lungs and other organs will allow more refined approaches to the care of preterm infants exposed to inflammation in utero. Intrauterine inflammation induces the expression of enzymes responsible for prostaglandin production in fetal lung tissue. Inhibition of prostaglandin production prevents, at least in part, the effects of inflammation on fetal lungs [25]. Preterm infants are at risk of acute respiratory distress as a result of lung immaturity; evidence of exposure to infection and/or inflammation before birth is associated with a reduced risk of neonatal RDS [26].

Based on the results of this study, the authors conclude that there is positive correlation between exposure of the fetus to a severe inflammatory response and lung development, and the possibility of causing chronic lung disease of prematurity. However, additional research is needed to confirm the findings in order to lead to a better understanding of the pathoetiologic mechanism of the disease and its prevention.

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