Do the twin’s placenta differ (morphological aspects) – preliminary study

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**Abstract**

Placenta forms a barrier between two organisms, namely the mother and the fetus. It also could form a barrier in far more complicated circumstances, see eg. twin pregnancy. The microenvironment changes during pregnancy might determine post-natal immune system function. The aim of the present study was to evaluate in twin placentas the expression of MyD88 and NOD2 factors involved in immune response pathways. The studies were done on paired twin placenta specimens selected from 50 cases of twins. The expression of MyD88 and NOD2 were determined by immunohistochemical studies and semiquantitative morphometric analysis. The average expression levels of MyD88 and NOD2 in syncytiotrophoblasts, decidual cells and cytotrophoblast were found to be different in some twin pairs. The levels of expression were even up to 50% different between twins within one pair. These results of this studies suggest that each twin within a pair might differ on the epigenetic level.

**Key words:** placenta, twins, immunology

**Introduction**

As it is written in some reference books placenta is problematic for the pathologist [1]. It comes from the fact that it forms a barrier between two organisms, namely the mother and the fetus. It also could form a barrier in far more complicated circumstances, see eg. twin pregnancy. Placenta structure is composed by: the villous parenchyma with maternal decidual tissues, the umbilical cord, and membranes. Additionally, it has a complex derivation. It originates from maternal tissues, fetal elements and extraembryonic tissues (for review see [1]). As it was already proved the amniotic epithelium and stroma, and umbilical cord are of embryonic derivation. While the chorionic epithelium and stroma, chorionic villous stroma and endothelium, and all trophoblast subtypes are extraembryonic derivatives [2]. Nowadays, it is believed that placenta, yolk sac comprise independent hematopoetic microenvironment [2, 3]. In the umbilical cord within the Wharton’s jelly there are numerous mast cells and only few macrophages. There is no vasa vasorum or lymphatic channels [1]. This makes placenta a unique structure. It is even more interesting field for studies, when we could focus on placenta of twins. For instance, in the common believe, there is a zealous expectation that monozygotic twins should be “identical”. But many of nowadays publications revealed that there genetic and epigenetic proves the diversity of twins. The nice review was recently published by Obel et al. [4]. Those authors on the idea that genetic differences have been proposed to play a strong role in risk of death from infectious diseases. Their performed a huge study base on 44,005 included all same-sex twin pairs born in 1870-2001 (appropriate data were available for 18,359 deaths). Then using such database, there were calculated concordance rates for same-sex monozygotic and dizygotic twin pairs. The probandwise concordance rates for monozygotic twin pairs were consistently higher than for dizygotic twin pairs. Moreover, the concordance rates were generally low, although a genetic influence on the risk of death from infectious diseases could be demonstrated, the absolute effect of the genetic component on mortality was small [4]. The same was found by others in studies on otitis media in twins. In the later studies discuss the issue, that even if there could strong evidences of genetic predisposition to the disease (as published in some other studies), it becomes less evident with time [5]. Such observations could be explained by the strong role of post-natal environment.
that modulates immunology of the patients [5, 6]. While there are also publications focusing on cell transfer between mother and fetus, and even between the twins. Using cells trafficking it was proved that such phenomenon occurs even between fetuses with separate placentas and separate chorions [7, 8]. The theories of twin discordance were summarized in Figure 1.

The aim of the present study was to evaluate the expression of selected factors of immune response pathways in twin placentas.

**Material and methods**

The studies were done on 50 twin pairs. For the further studies selected samples of placenta, as well as umbilical cord and amniotic membranes were taken. The whole material was fixed in 10% buffered formalin and processed according to standard protocol. Finally, paraffin blocks were prepared. After preliminary evaluation of hematoxillin and eosin slides, the material from placenta was selected for immunohistochemical studies. We used primary antibodies against MyD88 (R&D Systems cat. no. 316603) and NOD2 (Sigma Aldrich cat. no. PRS 2511) and for detection EnVision system (DAKO). Antigen expression evaluation in cytotrophoblast, syncytiotrophoblast and decidual cells was carried out using Remmele-Stegner scale. For those studies, the standardized protocols described elsewhere were used.

**Results**

The morphological analysis revealed cytoplasmic expression of the determined parameters (MyD88 and NOD2) in cytotrophoblast, syncytiotrophoblast and decidual cells. The average expression level of MyD88 in syncytiotrophoblast was 9 on a scale Remmele-Stegner and 8 for NOD2 (Figure 2).
The expression level of MyD88 was different by 30 to 40% in four twins. While for NOD2, the level of expression was different by 30% in one case. The average expression level of MyD88 in decidual cells was 9 and 5 for NOD2 (Figure 3). MyD88 expression level was different by 30 or even up to 50% in two twins. In three twins NOD2 expression levels were different by 30%. The average expression level of MyD88 in cytotrophoblast was 5 and 2 for NOD2 (Figure 4).

In two twins, the levels of MyD88 expression were different by 15 to 40%. The expression levels of NOD2 were different up to 10-25% in three twins. Comparing the expression levels between NOD2 and MyD88, the largest differences were observed in cytotrophoblast and the lowest in syncytiotrophoblast.

Discussion

Twin pregnancies are more prone to complications than single pregnancies, with an increased rate of gestational hypertension, gestational diabetes, postpartum hemorrhage, miscarriage, preterm birth, intrauterine growth restriction (IUGR), malformations and compli-
cations such as twin-twin transfusion syndrome (TTTS) [9]. But according to genetic backgrounds, assuming that monozygotic twins have virtually the same genetic heritage, they may serve as an elegant study material to understand the influence of pregnancy microenvironment and/or post-partum environment influence on the development of diseases and susceptibility for pathologies. This is especially interesting for monozygotic twins. In such cases although they share the same genes, but some of them might be active in one twin and not in the other. This raises the idea of twins as being genetically identical but not epigenetically. For the studies of the influence of pregnancy microenvironment on immune response to pathogens in the extra-uterine life, probably twin placentas would be the model. Unfortunately it could be difficult to conduct such studies as it was published by some authors, that even in academic centers only about 10-20% of placentas are examined [1].

After conception and placentation almost in whole pregnancy, we can observe an ongoing remodeling of the fetal-maternal contact area. This is controlled by complex signaling networks that regulate tightly co-ordinated events such as: trophoblast invasion, villous and extra-villous trophoblast differentiation and vascular tree remodeling [10]. Some of those processes are under control of leukocytes, which are an important component of endometrial stroma and placenta. According to some studies in the first trimester of pregnancy approximately 30-40% of endometrial (decidual) stromal cells are leukocytes [11]. The main leukocytic populations include: macrophages, dendritic cells, natural killer T cells as well as regulatory T cells. All aforementioned cells probably take part in control of regulation of trophoblast invasion, spiral artery remodeling and immune tolerance [11].

Recently, some studies were published on the role of toll-like receptors (TLRs) which are involved in innate immunity [12, 13]. TLRs are present on immune cells, but also on non-immune cells such as trophoblast and decidual cells. They expression in those sides were found to be changing during pregnancy [13]. The presence of TLRs on maternal-fetal interphase is attributed (among others reasons) to initiate innate immune response towards infections [12]. To understand the mechanisms of receptors activation the studies on activation pathways have been carried. One of such example is present work with emphasis on MyD88 and NOD2. The first attempt done by us revealed the different expression of those markers in twin placentas. The varied expression of MyD88 and NOD2 in the material may indicate the different mechanisms of signal transduction which activates the transcription factor NF-κB and consequently inflammation process (Figure 5).

**Fig. 5. Model of the molecular interactions between NOD2 and MyD88 signalling pathway**

Bacterial cell wall is composed of PGN (peptidoglycan). PGN as well as LPS (lipopolysaccharide) is a potential activator of TLR receptors and may alternatively be broken down into MDP (muramyl dipeptide). MDP binding to the LRR region (leucine-rich repeat) of the NOD2 protein, affects the recruitment of RIP2 kinase. RIP2 activates IKK complex. This process triggers the release of nuclear factor NF-κB (nuclear factor-κB) by phosphorylation, degradation and ubiquitination IKKB. On the other hand, TLR-activated receptors activates MyD88, which recruits IRAK4 protein (interleukin-1 receptor (IL-1R) and IRAK1. Then the TRAF6 protein (tumor necrosis-factor-receptor-associated factor 6) binds IRAK1, which directly affects the creation of complex three proteins TAK1 (transforming-growth-factor-activated kinase 1), TAB1 (TAK1-binding protein 1) and TAB2. This complex ubiquitinates TRAF6 which activates TAK1 kinase and ultimately allows phosphorylation of the IKK complex and activate the NF-κB. MyD88 can also activate NF-κB factor by an alternative pathway through RICK (receptor-interacting serine/threonine kinase). TRAF6 protein can also activate MAP kinases such as p38, JNK and ERK. Finally together with the NF-κB lead to the production of inflammatory cytokines.

It might reflect a possibility of different outcomes of immune system function of a given patient in the future. The first steps in discordance probably occur during intrauterine live according to changes of microenvironment. The TLRs are not the only one element of this complex system. Their expression and functional effects could be correlated with expression of such factors as: nitric oxide synthase 2, interferon-γ, IL-1b, IL-1RA, IL-12 TNF-α, IL-10, and even vitamin D receptor and vitamin
D-binding protein or E-cadherin turnover [14, 15]. In those processes Wnt signaling pathway is involved [16]. And even HLA haplotype sharing may occur [17]. Nevertheless, as it was published by several authors, further epigenetic differences arise during lifetime [18-21]. Then, even in monozygotic twins we can observe differences in susceptibility to diseases and wide range of anthropomorphic features and neoplasms development [18, 22, 23]. Some authors refer that processes as being genetic/epigenetic and pre- as such as post-natal environmental post-zygotic mechanisms [6]. The mechanisms of epigenetic changes are probably associated with complex traits, e.g. epigenetic asymmetry in the zygote or in utero cells sharing [7, 19, 24, 25]. But to understand all processes further extensive studies are needed.

Acknowledgment
The authors are in depth to Ms. D. Vogt for her expert technical support.

References