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ORIGINAL ARTICLE

Expression of Toll-Like Receptors in Chronic Histiocytic Intervillositis of the Placenta

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Chronic histiocytic intervillositis of the placenta (CHI) shows monocytic/histiocytic infiltration of the intervillous space. Placental malaria has a CHI-like histopathology and induces an aberrant expression of Toll-like receptors (TLR) 3, 7–9. We hypothesized that, similar to placental malaria, CHI could be associated with increased TLR expression. TLR1-10 and other inflammation-associated factors were analyzed by real-time PCR and immunohistochemistry. A total of 31 formalin-fixed and paraffin-embedded placenta samples were evaluated: CHI ($n = 9$), and for control purposes, villitis of unknown etiology (VUE, $n = 8$) and placentas without inflammation ($n = 14$). CHI shows increased expression of monocytic TLR1, a receptor which is involved in bacterial lipopolysaccharide (LPS)-induced inflammation. This could indicate a TLR1-mediated immune mechanism in the placenta (e.g. triggered by transient, clinically inapparent maternal bacteraemia) which leads to massive monocytic/histiocytic accumulation in the intervillous space. The increased expression of TLR1 with no increased expression of TLR3 and TLR7-9 is different from that in malaria.

Keywords: histiocyte, monocyte, placenta, chronic histiocytic intervillositis, massive perivillous histiocytosis, Toll-like receptor, TLR

INTRODUCTION

A nonbacterial placental inflammatory lesion is chronic histiocytic intervillositis (CHI)/chronic intervillositis of unknown etiology (CIUE) [1–3]. The disease may occur in all trimesters and massive accumulation of monocytes/histiocytes is found in the intervillous space but no significant infiltration and destruction of villi. Few eosinophils and lymphocytes are present and intervillous fibrin depositions are variable features [1–3]. CHI is often associated with high rates of perinatal mortality or growth restriction and a tendency towards disease recurrence [1–3].

The pathobiology of this lesion is still not fully understood. Acute atherosclerosis-like lesions in decidual vessels with deposits of IgM and complement have been described which might be related to postinfectious immunological triggers [1]. The infectious counterpart is placental malaria which can show CHI-like lesions in ~5–10% of cases [4, 5]. It has been shown that Toll-like receptors (TLR) are increased in blood cells of newborns after maternal malaria infection [6]. Because CHI and malaria-associated

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Table 1. Characteristics of pregnancies with CHI, VUE and control placentas without atypical inflammation.

Diagnosis	CHI (<i>n</i> = 9)	VUE (<i>n</i> = 8)	Control placentas (<i>n</i> = 14)
Age of mothers (years)	33 (24–41)	30.5 (18–34)	29 (26–43)
Gravida	1–6	1–2	1–6
Para	0–4	1–2	1–3
Gestation age (weeks)	20 (8–33)	37.5 (30–41)	31 (20–36)
Intrauterine fetal growth restriction	3/9	6/8	2/14
Fetal death	5/9	0/8	2/14

Maternal ages were similar. The gestational age was significantly lower in CHI than in VUE ($p = 0.0011$); it has to be taken into account that, in general, VUE usually manifest in the late phases of pregnancies while CHI can occur at any time [2]. The gestational ages were similar between CHI versus controls and VUE versus controls (differences not significant). Median values (ranges) are summarized.

intervillositis have some morphological similarities [4, 5], we hypothesized that both diseases might share similar inflammatory pathways, in particular expression of TLR. These receptors are expressed on different leukocytes, such as monocytic cells, and are involved in immune system activation after pathogen contact but are also related to autoimmune diseases [7–10]. We therefore evaluated the expression profile of TLR1-10 and TLR/inflammation-associated factors in CHI.

MATERIAL AND METHODS

Study Groups

Study group I (screening of TLR profile) comprised four cases with massive CHI (these CHI cases have been characterized previously [11, 12]) and four placentas without inflammation. Gestational ages were similar in CHI (19, 25, 30, and 33 weeks) and control placentas without any sign of CHI, villitis or chorioamnionitis (20, 25, 30, and 33 weeks).

Study group II (re-evaluation of TLR1) comprised another five previously undescribed CHI cases (median gestational age 12 weeks, range 8–26), eight cases with villitis of unknown etiology (VUE; median 36 weeks, range 31–41) and ten control placentas without inflammation (median 31 weeks, range 21–35). Similar to CHI, VUE is a noninfections placenta disease [2].

No samples from initial CHI/VUE and the corresponding recurrent lesions in the following pregnancy were available for analysis. CHI, VUE and control cases were unrelated to each other. Clinical characteristics are summarized in Table 1. Formalin-fixed and paraffin-embedded (FFPE) placental samples were analyzed. The retrospective analysis was approved by the local Ethics Committee at Hannover Medical School.

Gene Expression Analysis

Transcript expression analysis of 45 target genes and endogenous control genes was performed with samples from study group I with a 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) as described [12]. TLR1 and endogenous control gene polymerase (RNA) II (DNA directed) polypeptide A, 220kDa (POL2RA) were re-evaluated with a TaqMan (R) assay (Applied Biosystems) in study group II. Statistical evaluation was performed with Prism 5.0 (GraphPad Software, San Diego, CA, USA) by applying the Mann–Whitney test.

Immunohistochemistry

Immunostaining was performed on FFPE sections with anti-TLR1 antibody (1:200, AbD, Düsseldorf, Germany) and a ZytoChemPlus HRP Polymer Kit (Zytomed Systems, Berlin, Germany).

RESULTS

In screening cohort I, we found higher levels of TLR1 and monocytic colony stimulating factor 1 receptor (CSF1R) in CHI (both $p = 0.029$), while TLR2-9 were not significantly deregulated (Fig. 1, Supplementary table 1). In one CHI, TLR1 and TLR2 were both increased and in another CHI, TLR1 and TLR4 were co-expressed at elevated levels (Fig. 1). In general, TLR1-9 were expressed at low levels (mean relative expression levels <1) and TLR10 was not detectable. TLR signaling-associated factors were not deregulated at the transcript level, including Toll interleukin-1 receptor domain containing adapter protein (TIRAP), myeloid differentiation primary response 88 (MYD88) and interleukin-1 receptor-associated kinase 1 (IRAK1). TNF receptor-associated factor 3 (TRAF3) had a tendency for higher levels in CHI but the difference was not significant ($p = 0.057$). TRAF-associated CD40 was not deregulated.

Increased expression of TLR1 in a sub-fraction of CHI was verified in a second cohort, and corresponding to the low transcript levels, weak TLR1 protein expression was detectable in monocytes but not in the few intermingled lymphocytes or eosinophils (Fig. 1). Increased levels of TLR1 were not specific for CHI but were also found in a sub-fraction of placentas affected by VUE, another noninfectious placenta lesion (Fig. 1).

DISCUSSION

In this analysis, deregulation of the TLR expression profile was evaluated as a possible factor in CHI pathobiology. TLR signaling can be activated by bacterial lipopolysaccharides (LPS) and can lead to gene transcription via nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) [7–10]. TLR1 and TLR2 can form heterodimers which allows recognition of a broader panel of LPS [7–10] but in most CHI, increased expression of TLR1 was not paralleled by increased expression of TLR2. TLR1 was increased in most CHI above control levels while TLR1/TLR2 co-increase was observed only in one CHI under investigation (Fig. 1). TLR1 and TLR2 mediate cell signaling via TIRAP and MYD88 which are located at the intracellular TLR domains [7–10]. These factors induce signal transduction by activating IRAK1 and other TLR-associated factors which finally leads to activation of gene transcription for monocyte-activating cytokines by NFKB1 [7–10]. CSF1R is the central receptor for monocytic differentiation and proliferation [13]. The CSF1R-related cytokine is colony stimulating factor 2 (granulocyte-macrophage) (GM-CSF/CSF2) [13] and CSF1R as well as CSF2 are expressed in normal placentas [14]. CSF2 is increased in response to bacterial LPS and endogenous tumor necrosis factor alpha (TNF alpha) [13]. Our previous analyses revealed that in CHI only low levels of TNF alpha were detectable [12]. In this current analysis, TNF receptor-associated TRAF3 was upregulated in CHI while the TRAF3-associated TNF receptor superfamily member 5 (CD40) was expressed at similar levels in CHI and controls. These results indicate that increased expression of CSF1R is a result of monocytic accumulation while TNF-associated activation is unlikely the main cause of CHI.

Placental CHI differs in the TLR profile from malaria-associated receptor profiles. After maternal malaria infection, monocytes/histiocytes in the placentas have not been evaluated but, in blood cells of new-born children, TLR3 and TLR7-9 are

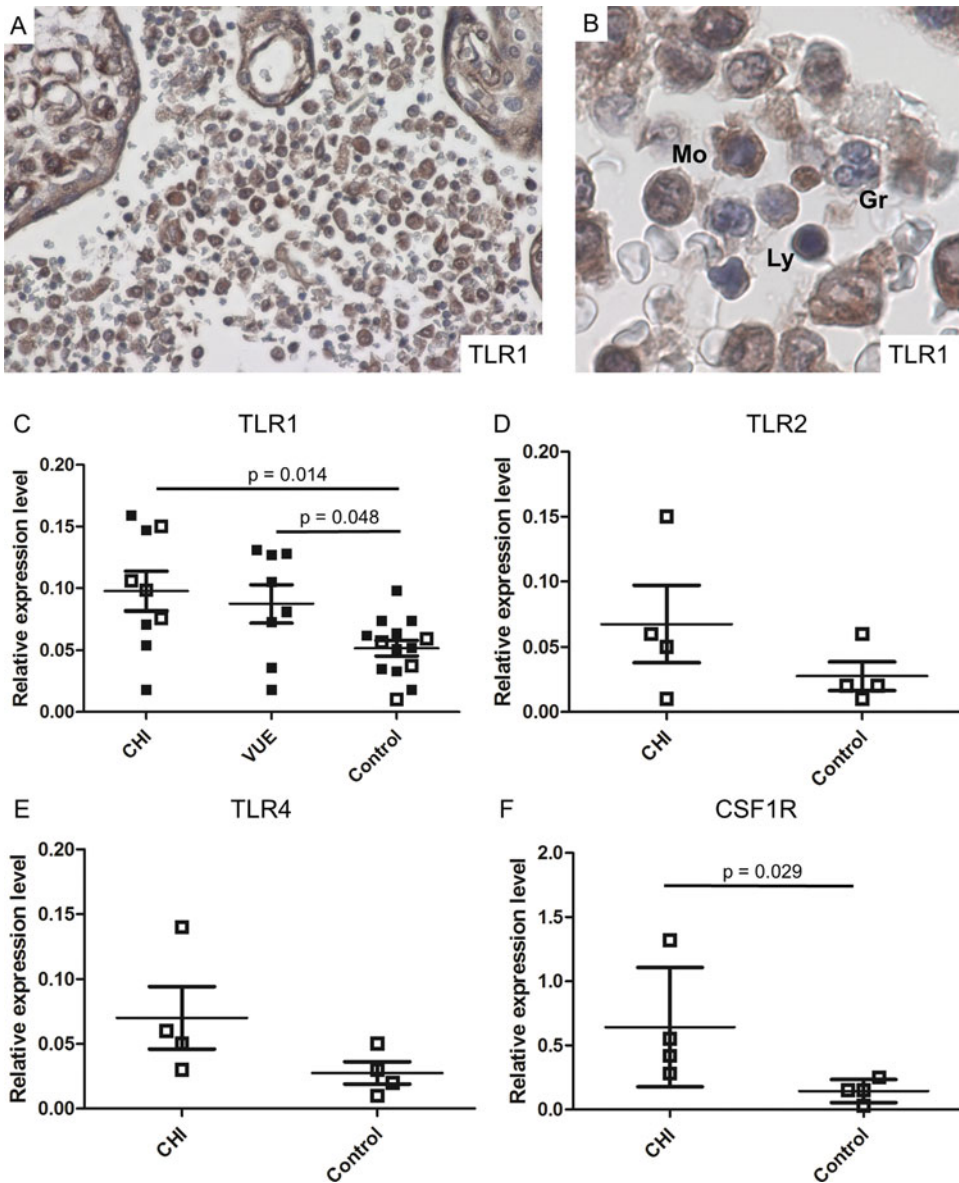


Figure 1. Aberrant expression of inflammation-associated receptors in CHI. (A) Immunohistochemistry shows expression of TLR1 in intervillosal monocytes in CHI (Original magnification 400 \times ; microscopic images were produced with a BZ-9000 slide scanner, Keyence, Neu-Isenburg, Germany). (B) Higher magnification (1000 \times) reveals weak TLR1 protein expression in monocytes (Mo) but not in intermingled lymphocytes (Ly) or granulocytes (Gr). (C) TLR1 transcript levels are increased in CHI and VUE. Samples of the screening cohort I (4 CHI versus 4 controls) are depicted as black squares with white centers and the re-evaluation cohort II (5 CHI versus 8 VUE versus 10 controls) is depicted as black squares. Note that the p values refer to cohorts I + II. The p value of CHI versus controls from cohort I is 0.029. Mean and standard deviation are represented by bars. (D) TLR1-related TLR2 is increased in only one CHI; in this CHI case both TLR1 and TLR2 were simultaneously increased. (E) TLR4 was increased in only one case which also had elevated TLR1 expression levels but low levels of TLR2. (F) CSF1R, which is known to be expressed in monocytes [13], is increased in CHI.

increased and not TLR1 or TLR2 [6]. Accumulation of monocytes in the intervillous space is associated with expression of intercellular adhesion molecule-1 (ICAM1) in syncytiotrophoblast cells and monocytes, both in CHI and placental malaria [15, 16]. It has to be taken into account that detection of ICAM1 is a common finding in several inflammatory placenta lesions, including VUE [17]. In general, ICAM1 expression is related to TLR2 and TLR4 signaling [18]. Although we found no significant upregulation, the expression of these two receptors was detectable in CHI, which could lead to ICAM1 expression via increased receptor activation rather than increased expression of TLR2 and TLR4 receptor molecules. Presumably, very low amounts and/or transient presence of circulating LPS in the maternal blood could be the immune trigger (e.g. clinically inapparent transient bacteremia before CHI manifestation) but it is unlikely that, at the time of CHI manifestation, a significant amount of bacterial pathogens are present. Otherwise, regular activation of full anti-bacterial reaction, including neutrophils, would be expected. In autoimmune diseases with lymphohistiocytic reaction (psoriasis of the skin and Sjögren syndrome of the salivary glands), increased expression of TLR1/TLR2 has been found in epithelial cells [19]. Because TLR1 and TLR2 are involved in LPS-induced inflammation, it could be possible that CHI imitate a TLR1-mediated LPS-like reaction without involvement of neutrophils but monocyte attraction, e.g. via chemokine (C-C motif) ligand 2 (CCL2) [12], and intraplacental monocytic activation via CSF1R.

CHI has a tendency of disease recurrence in the subsequent pregnancy [1-3]. In these recurrent cases, persistent or recurrent bacterial/LPS stimulation of TLR1 could be possible. Although previous miscarriages were frequent in our CHI cohort, we have no case with proven CHI in at least two pregnancies (e.g. because not all placentas were sent in for examination). Similar to other diseases [7], TLR inhibitors could be used for preventing aberrant TLR signaling in subsequent pregnancies with suspected disease recurrence. However, TLR1 expression is increased only in a subfraction of cases, which indicates that this receptor type is not the major driver of aberrant leukocyte accumulation in the placenta. In our previous evaluation of inflammatory signaling factors in CHI, we have already noted the discrepancy of massive histiocytic accumulation and the lack of striking upregulation of proinflammatory factors [12]. These minor to moderate changes in the expression of receptors and signaling factors could be the basis of the typical lack of placenta tissue infiltration despite massive ICAM1-associated intervillous leukocyte accumulation in CHI. In contrast to CHI, in VUE maternal monocytes infiltrate into the placental villi but both lesions show similar TLR1 expression levels. This could indicate that CHI and VUE share the same initial TLR1-associated trigger but, after initiation of the aberrant immune reaction, two pathways with different cytokine profiles [12] lead to different disease phenotypes.

In summary, increased expression of TLR1 in CHI could indicate an LPS-like immune mechanism in the placenta which leads to massive monocytic/histiocytic accumulation in the intervillous space.

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Author Contribution

Kais Hussein: design of the study, molecular analysis, statistical analysis. Angelika Stucki-Koch: molecular analysis, immunohistochemistry, statistical analysis.

Henning Feist, Angelika Stucki-Koch, Hans Kreipe, Kais Hussein: interpretation of data, manuscript preparation.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Supplementary Material

A supplemental table for this article may be found online at www.tandfonline.com/ipdp.

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