Chronic villitis of unknown etiology and massive chronic intervillositis have similar immune cell composition

Article in Placenta · March 2015
DOI: 10.1016/j.placenta.2015.03.008 · Source: PubMed

4 authors, including:

Carlos Labarrere
California Medical Innovations Institute, San …
142 PUBLICATIONS 2,642 CITATIONS
SEE PROFILE

James Hardin
University of South Carolina
279 PUBLICATIONS 5,238 CITATIONS
SEE PROFILE

David M Haas
Indiana University-Purdue University Indiana…
108 PUBLICATIONS 1,238 CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project
Cigarette Warning Wearout Project View project
Chronic villitis of unknown etiology and massive chronic intervillositis have similar immune cell composition

C.A. Labarrere a, d, *, J.W. Hardin b, D.M. Haas c, G.S. Kassab d

a CBL Partners for Life, Indianapolis, IN, USA
b Obstetrics and Gynecology, Indiana University School of Medicine Wishard-Eskensazi Hospital, Indianapolis, IN, USA
c Epidemiology and Biostatistics, Columbia, SC, USA
d California Medical Innovations Institute, San Diego, CA, USA

ARTICLE INFO

Article history:
Accepted 21 March 2015

Keywords:
ICAM-1
Pregnancy
Villitis
Intervillitis
Spiral arteries
Failure of physiologic transformation
Atherosis

ABSTRACT

Introduction: Chronic villitis of unknown etiology (CVUE) and massive chronic intervillositis (MCI) are placental lesions associated with infiltration of mononuclear cells in the chorionic villi and the intervillosous spaces, respectively. It is not well known whether immune cells in CVUE and MCI have similar phenotypic characteristics.

Methods: A cross-sectional study of third trimester placentas was conducted to identify immune cell subpopulations in CVUE and MCI (n = 17/group). CVUE was diagnosed with H&E staining and antibody to CD3 in serial sections; and MCI, by the presence of massive infiltration of mononuclear cells in the inter villous spaces. Immune cells, ICAM-1 expression and nuclear factor kB (NF-κB) activation were determined immunohistochemically.

Results: CVUE and MCI showed similar infiltrates, mainly CD68+ and CD3+ cells. Most cells (>80%) were CD45RB+, and one third were CD45RO+ in both lesions. There were slightly more CD8+ than CD4+ cells in both CVUE and MCI. More than 90% of cells in CVUE and MCI were ICAM-1+ with NFκB nuclear localization. Syncytiotrophoblast ICAM-1 expression was significantly (p < 0.001) higher in MCI (mean of 81.0; range of 71.6–86.0) than in CVUE (52.4; 36.4–59.4) or normal placentas (0.2; 0.0–0.6). Both, failure of physiologic transformation of spiral arteries and placental atherosclerosis-like lesions of atherosclerosis were significantly more frequent in MCI than in CVUE or normal placentas (p = 0.044 and p = 0.007, respectively).

Discussion: These finding suggest that MCI and CVUE have very similar infiltration of immune cells although MCI has more severe placental lesions.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Chronic villitis of unknown etiology (CVUE) is a common and major placental lesion affecting 6.6%–33.8% of third-trimester placentas [1] (7.6–10% in the United States [2,3], 13.6% in the United Kingdom [4], 14.3% in New Zealand [5] and up to 33.8% in Argentina [6]). CVUE should be distinguished from villitis of infectious etiology [7], caused by specific maternal infections, like rubella [2,8], varicella [9], Chagas disease [10,11], toxoplasmosis [10,12], syphilis [13], cytomegalovirus [14], herpes simplex virus [15], influenza A/H1N1 [16], enterovirus [17], Mycobacterium tuberculosis [18] and malaria [19]. A small percentage of cases have a recognizable infectious cause; however, most villitis are of unknown etiology (CVUE) [4]. CVUE’s clinical significance depends on the etiology and severity of the fetal infection [20] and may lead to intrauterine fetal death, miscarriage, malformations, associated with elevation of serum alpha-fetoprotein, and intrauterine growth restriction (IUGR) [3,21–24]. CVUE is a major cause of IUGR and recurrent reproductive failure [7,20–23]. Morphologically, infectious villitis may show nonspecific characteristics and can pose as CVUE [25]. Both lesions have chronic inflammatory cells, mainly macrophages and lymphocytes, in the placental villous stroma [20,25].

Massive chronic intervillositis (MCI) is a rare lesion (<1% of pregnancies) described in 1987 by Labarrere and Mullen [26]. The lesion has massive mononuclear cell inflammatory infiltrates predominantly monocytes/macrophages and lymphocytes in the placental inter villous spaces in the absence of significant
inflammatory compromise of the chorionic villi. Similar to CVUE, MCI is thought to have an immunopathological background in terms of “graft” rejection [27,28]. The infectious counterpart of primary MCI is MCI secondary to placental malaria or human herpesvirus 5/cytomegalovirus (HHV5/CMV) [29,30]. MCI can occur in all trimesters while CVUE tends to manifest in later stages of gestation [27,28]. Both diseases have an elevated risk of recurrence in subsequent pregnancies and, particularly MCI, a poor obstetric history with recurrent pregnancy loss, IUGR, and fetal death [26,28,31–35].

There are two different proposed hypotheses regarding pathogenesis of CVUE: 1) a fetal response to an infectious agent within the chorionic villi [7], and 2) a maternal immune response to fetal antigens in chorionic villi [6]. It has also been proposed that MCI is a placental lesion related to a maternal immune response to fetal tissues and could be an extreme variant of CVUE [26]. An important premise in these hypotheses is the understanding of the different immune cells involved in both lesions. Accordingly, the objective of this study is to determine the phenotype of the immune cells implicated in CVUE and MCI.

2. Material and methods

2.1. Study design

A cross-sectional study was performed to evaluate the phenotypic characteristics of immune cells in third trimester placentas (34–42 weeks gestation) with CVUE and MCI obtained by 2012 from the Hospital Italiano de Buenos Aires, Argentina. Placentas with CVUE (n = 17), MCI (n = 17) or without CVUE or MCI (n = 17) were studied. All cases and controls were live births. To minimize confounding effects of different pathologies of pregnancy, placentas with CVUE, MCI and controls were matched for clinical diagnosis (i.e., preterm, preclampsia and/or IUGR). Pregnancies with no medical/obstetric complications and birth weight appropriate for gestation of the percentage of immune cells in placentas [36].

2.2. Immunohistochemistry

Placentas were processed for light microscopy, single and double immunohistochemistry [36]. Primary antibodies used were: mouse anti-human CD68R (LCA, 2B1, Dako), CD3 (C11, 1B5, Dako), CD45RO/B (H129, Dako), CD3 (C11, 1B5, Dako), ICAM-1 (C5, sc-8439, Santa Cruz Biotechnology, Santa Cruz, CA), CD68 (KP1, Dako), CD3 (FT2.38, Dako), CD4 (AB12, Dako), CD8 (CL4/14, Dako), CD20 (L26, Dako), CD15 (Carb-3, Dako) and rabbit anti-human CD3 (A0452, Dako), HLA-DR (ab137832, Abcam, Cambridge, MA), NF-κB p-65 (phosphorylated) (ab68299) and cytokertain (A0575, Dako). Immunoperoxidase studies were performed as described [36].

2.3. Criteria for histopathologic diagnosis

MCI was diagnosed by massive infiltrates of mononuclear cells in the placental intervillous spaces irrespective of the presence or absence of CVUE. CVUE was defined by mononuclear cell infiltrates in the villous stroma, often with destruction/necrosis of the villous parenchyma commonly found in a patchy pattern, usually involving no more than 10 villi per focus [38] in H&E stained sections [39]; and immunohistochemistry with monoclonal antibody to CD3 and concomitant H&E staining of an immediate serial section [40]. The percentage of villi with CVUE was calculated in each placenta. Syncytiotrophoblast (sT CAM-1 was considered positive either when there were only spotty or circumferential villous reactivity [36]. SICAM-1 in CVUE was confirmed using double-antibody immunohistochemistry with antibodies to CD3 and ICAM-1. Immune cell activation was measured by ICAM-1 expression and nuclear localization of nuclear factor kappa B (NF-κB). SICAM-1 was demonstrated using a double-antibody technique with antibodies to ICAM-1 and cytokertain. Quantitation of the percentage of immune cells in placentas with MCI was performed by counting cells in the intervillous spaces of 5 different fields (640x) in the placental parenchyma located between the center of the placenta and the basal plate, but excluding the basal plate itself to avoid inclusion of lesions of anchoring villiis. The percentage of cells with a particular phenotype was calculated as the number of cells with that phenotype/total number of cells × 100. These calculations were performed in placentas with MCI and in areas of CVUE.

2.4. Statistical analysis

All continuous measures were summarized using median and range, and all categorical measures were summarized as frequency (% of categories). Among the three placenta groups, demographic measures were compared using Fisher’s exact and Kruskal–Wallis tests. Pairwise comparisons of percentage of cells with different phenotypes among placenta groups with MCI and CVUE were carried out using the Wilcoxon Rank Sum test. A probability value <0.05 was used to establish statistical significance.

3. Results

A total of 51 placentas (17 with MCI, 17 with villitis, and 17 without villitis or MCI) were included in the study. Clinical and demographic data of the study population and placental characteristics of the different groups are summarized in Table 1. Forty-seven percent (8/17) placentas with MCI had lesions of CVUE, although the percentage of villi with CVUE in these placentas was significantly lower than in placentas with CVUE without MCI (0.9% vs. 19.6%, p = 0.001). SICAM-1 expression was significantly higher in MCI than in CVUE or in placentas without MCI or CVUE (Table 1). Failure of physiologic transformation of spiral arteries and atherosclerosis-like lesions of atherosis were more frequently identified in placentas with MCI than in other groups (Table 1; p = 0.013).

All placentas with MCI (17/17, 100%) showed a predominant infiltration of CD68§ and CD3§ immune cells in the intervillous spaces, as shown in Table 2 and Fig. 1. CD8§ T-cells were slightly more frequently identified than CD4§ T-cells. Most cells in MCI were CD45R§+, a third of the immune cells were CD45RO+ and very few of them were CD45RA+ (Table 2 and Fig. 1). Fewer cells CD20+ or CD15+ were also identified. More than 90% of the immune cells in the intervillous spaces of placentas with MCI were ICAM-1+, ICAM-1 was always identified in cytokertain-reactive syncytiotrophoblasts as well as in the immune cells of MCI (Fig. 1). Immune cells in MCI frequently showed enhanced expression of cytoplasmic Nfkb with concomitant Nfkb nuclear localization (Fig. 1). Areas of CVUE were strongly positive with monoclonal antibody to CD3 and consistently showed stICAM-1 expression (Fig. 2). All placentas with CVUE (17/17, 100%) showed a predominant infiltration of CD68§ and CD3§ immune cells in villitis lesions, as shown in Table 2 and Fig. 2. CD8§ T-cells were more frequently identified than CD4§ T-cells. Most cells in CVUE were CD45R§+, a third of those immune cells were CD45RO+ and very few of them were CD45RA+ (Table 2 and Fig. 2). Fewer CD20+ or CD15+ were also identified. More than 90% of the immune cells in lesions of CVUE were ICAM-1+. ICAM-1 was always identified in cytokertain-reactive syncytiotrophoblasts as well as in immune cells of villitis areas (Fig. 2), and immune cells in CVUE always showed frequent enhancement of Nfkb cytoplasmic expression as well as Nfkb nuclear localization (Fig. 2).

We quantified different immune cells in placentas with MCI and CVUE, as well as in control placentas. Placentas without MCI or CVUE had very few and isolated immune cells (CD68§ and CD3§) in the intervillous spaces (data not shown). Both, placentas with MCI and CVUE, had predominantly CD68§ and CD3§ cells, most of them CD45R§+ (Table 2). Immune cells showed signs of activation in both MCI and CVUE, identified by ICAM-1 expression and nuclear localization of Nfkb (Fig. 1 and 2). When we compared the different cells in both MCI and CVUE, we detected almost identical phenotypic characteristics in both lesions (Table 2).
4. Discussion

Immunomorphological studies in infectious villitis and CVUE have been described [6,11,14,39,41–44]. Labarrere et al. [6] found that in CVUE the predominant inflammatory cells were CD68+ activated macrophages expressing class II major histocompatibility complex antigens and CD4+ helper T-cells. Few CD8+ T-cells and no B-cells were identified [6]. These findings are in contrast with those in villitis of Chagas’ disease, in which CD8+ T-cells outnumbered CD4+ T-cells [11]. Brito et al. [42] found no immunohistochemical differences between the predominant macrophage and CD8+ T-cell infiltrates in infectious villitis or CVUE. Whereas a number of studies provided phenotypic characterization of CVUE infiltrates [6,11,14,39,41–44], fewer identified cell phenotypes in MCI [29,31,32] and none has shown a comparison of immune cell infiltrates in both CVUE and MCI. This is the first study showing that immune cells in CVUE and MCI are nearly identical. The dominant immune cells in both lesions are CD68+ monocytes/macroages and CD3+ T-lymphocytes. CD4+ T-cells were slightly more frequently identified than CD4+ T-cells in both lesions. Immune cells in both CVUE and MCI showed signs of activation (ICAM-1 expression and nuclear localization of NFkB). These findings suggest that both lesions have similar common characteristics.

A relevant finding in this study is the expression of CD45 antigens in immune cells in both CVUE and MCI. Protein tyrosine phosphatase, receptor type C (also known as CD45 antigen) is an essential regulator of T- and B-cell antigen receptor signaling. Protein tyrosine phosphatases are signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. CD45RA is located on naive T cells and CD45RO on memory T cells. Activated and memory T-lymphocytes express the shortest CD45 isoform, CD45RO, and a third of cells in CVUE and MCI in our study expressed this antigen. Reciprocal expression of CD45RA and CD45RO in human CD4+ T-cells defines populations understood to be naive cells (CD45RA+CD45RO−) and memory cells (CD45RA−CD45RO+) [45]. Thus, differential expression of CD45RB within CD45RA-cells defines two subsets that have similar properties except for somewhat greater IFN-γ production and proliferative responses by CD45RO−RBright cells. Variability in CD45RB expression may represent a mechanism for fine-tuning the responsiveness of memory cells in vivo. CD45RB can play an important role in the maintenance of inflammation by T-cell activation [46].

CVUE inflammatory infiltrates are predominantly CD68+ macrophages and CD3+ T-lymphocytes [47]. Different studies have shown that the lymphocytic infiltrate in villitis is heterogeneous including CD3+ T-lymphocytes, CD4+ helper T-cells, CD8+ suppressor T-cells, and CD25/FOXP3+ regulatory T-cells, but few, if any, natural killer cells or B-cells [13,39,47–50]. Altemani [43] identified macrophages and T-lymphocytes, and most cells were HLA-DR-reactive as previously described by Labarrere’s group [39,51,52]. Greco et al. [14] found macrophages and T-lymphocytes expressing HLA-DR in villitis from mothers with syphilis, cytomegalovirus and CVUE. Tuberculosis placentas had CD68+ and CD3+ cells as well as myeloperoxidase-positive neutrophils in villitis and intervillositis [18]. Both infectious villitis and CVUE have mostly macrophages...
and CD8+ T-cells [42]. Kim et al. [49] showed numerous CD68+ cells, and that CD8+ T-cells outnumbered CD4+ T-cells in CVUE. Other studies showed the predominance of CD8+ T-cells over CD4+ T-cells in CVUE and infectious villitis. As suggested by Kim et al. [49], the discrepancy with our initial study reporting predominant CD4+ T-cells in CVUE [6], may be explained by different immunohistochemical techniques, different antibodies used, different populations studied or other confounding factors.

Maternal cells have been identified within chorionic villi in both CVUE and MCI [36,48,51], suggesting maternal cells cross the trophoblastic barrier with (CVUE) or without (MCI) disruption of the syncytiotrophoblast layer. Kim et al. [49] found numerous Hofbauer cells in CVUE and suggested that those cells may be responsible for antigen presentation to recipient maternal cells that can penetrate the villous stroma developing a host versus graft response. This idea is supported by their finding of maternal T-cells in the lesions, and previous studies [48,51] showing maternal cells in CVUE. A study by Myerson et al. [50] indicated that invasion of fetal villi by maternal T-cells is associated with focal syncytiotrophoblast destruction. Recently, Egal et al. [44] found large amounts of CD45+, CD3+ and CD68+ cells in CVUE and a high number of those cells in the perivillous region expressed ICAM-1, suggesting that trophoblast destruction can lead to amplification of the inflammatory response.

The primary cause of MCI remains unknown but the histologic pattern and clinical profile do not suggest infection [33].

**Fig. 1.** Placental massive chronic intervillositis (MCI). MCI is characterized by massive infiltrates of mononuclear cells in intervillous spaces of the placenta (a); 83.3% cells are CD68+ (b); 85.3% cells are CD45RB+ (c); 7.7% cells are CD8+ (d); 7.7% cells are CD8+ (e); 90.9% cells are ICAM-1+ (f); most CD68+ cells are ICAM-1+ (black arrow in f, g and h); numerous cells show NFkB nuclear localization (black arrow in i and j). Note syncytiotrophoblast ICAM-1 reactivity in MCI (red arrows in d, f-j). Original magnification X640.

**Fig. 2.** Placental chronic villitis of unknown etiology (CVUE). CVUE is characterized by placental fibrinoid necrosis and villous infiltrates of mononuclear cells (a); 83.8% cells are CD68+ (b); 85.3% cells are CD45RB+ (c); 13.4% cells are CD3+ (d); 7.9% cells are CD8+ (e); 90.7% cells are ICAM-1+ (f); most CD68+ cells are ICAM-1+ (black arrow in f, g and h); syncytiotrophoblast in CVUE is ICAM-1+ (red arrow in f, g, h and j) and HLA-DR+ (brown reactivity, red arrow in h); numerous cells show NFkB nuclear localization (i, and black arrow in j). Original magnification X640.
may be considered an allograft bearing foreign, paternally derived, transplantation antigens grafted into the maternal uterus. Solid organ transplants show different signs of rejection, among them cell damage with infiltration of recipient's immune cells (macrophages and T-cells) and atherosclerosis-like vasculopathies that develop very quickly within the first year of transplantation [53–55]. We proposed that CVUE, MCI and atherosclerosis may be expression of placental rejection [22,26,56]. CVUE and MCI have numerous recipient maternal cells that not only surround the chorionic villi but cross the syncytiotrophoblast barrier into the fetal compartment [36,48,51]. The transmission of maternal cells into the fetal compartment could be explained by induction of syncytiotrophoblast focal damage, characteristic of malaria parasitic infection [57], mediated by TNF-α [58]secreted by activated maternal monocytes bound to trophoblast. StICAM-1 expression can be upregulated in vitro by HIV-1 and malaria infections [59,60].

The most significant finding associated with active malarial infection is MCI [61]. The presence of macrophages and T-lymphocytes in malarial MCI can explain that, in human placenta, malaria elicits TNF-α, IL-2, and IFN-γ release [62]. TNF-α and IFN-γ may be associated with subsequent expression of stICAM-1. As suggested for malaria infection, the syncytiotrophoblast is capable of responding immunologically to the interaction with maternal immune cells in the intervillous space [63,64]. Maternal immune cells could not only induce up-regulation of ICAM-1 expression, but also induce secretion of immune factors that could facilitate recruitment of maternal immune cells to the intervillous space and migration towards the syncytiotrophoblast. However, up-regulation of such molecules might be secondary to a still unknown (autoimmune) trigger, which is responsible for the unusual mononuclear infiltrate in MCI [31].

The predominantly massive infiltrates in placental intervillous spaces associated with numerous maternal cells in the fetal compartment in MCI with or without CVUE [36], suggests that MCI may be a maximum expression of CVUE in which immune cells simply cross the syncytiotrophoblastic barrier without disrupting it. How maternal cells get into the fetal compartment remains unknown. Two alternatives emerge as possible explanations: 1) cytokines and chemokines attract maternal cells into the chorionic villi, and 2) adhesion molecule expression in villous syncytiotrophoblast allows maternal cells to penetrate into the fetal compartment. Although a striking difference in cytokine or chemokine expression was not found in MCI [31], a systemic derangement of CXC chemokines in maternal and fetal circulation distinguishes CVUE [49]. The worse outcomes in MCI compared with CVUE can be explained on the basis of our findings in the present study. The cells involved in both lesions are practically the same, as found in other solid organ transplants, suggesting that the recipient of a transplant (e.g., a heart or a placenta) responds in a similar fashion. The development of different lesions can relate to levels of cytokines, chemokines or adhesion molecules. Although CVUE has less stICAM-1 and more cytokines and chemokines, MCI has no differences in cytokines or chemokines but has a massive expression of ICAM-1 in syncytiotrophoblast and immune cells in the placental intervillous spaces. A possible scenario may be that CVUE contains villous damage, fetal vessel obliteration in the lesions and lower expression of adhesion molecules that can limit the invasion of maternal cells into the fetal compartment. Given MCI’s massive stICAM-1 expression, it can facilitate maternal cell invasion into the villi without disrupting the trophoblastic barrier and without causing villous damage as seen in CVUE. These findings enhance our understanding of important placental lesions that may lead to new ways to treat or prevent development of those lesions and ultimately improve pregnancy outcomes.

Conflict of interest

None.

Acknowledgments

We sincerely appreciate the help and support from Drs. Mario Wernicke and Eduardo Mullen from the Department of Pathology of the Hospital Italiano de Buenos Aires, who provided the tissues for the present study. We are deeply indebted to them, especially Dr. Mullen who originally described the entity with the leading author of the present article. We sincerely appreciate the help of Mr. Adam Essex who helped with the artwork and configuring the references of this manuscript.

References

[15] Syridou G, Spanakis N, Konstantinidou A, Piperaki E-T, Kafetzis D, Patsouris E, Mr. Adam Essex who helped with the artwork and con
[16] References of this manuscript.

Please cite this article in press as: Labarrere CA, et al., Chronic villitis of unknown etiology and massive chronic intervillitis have similar immune cell composition, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.03.008


[40] Labarrere CA, Jaeger BR. Biomarkers of heart transplant rejection: the good, the bad, and the ugly! Transplantation 1990;50:812.


