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Intercellular adhesion molecule-1 expression in massive chronic intervillositis: Implications for the invasion of maternal cells into fetal tissues

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ABSTRACT

Introduction: Massive chronic intervillositis (MCI), also known as chronic intervillositis of unknown etiology, is a placental lesion associated with massive infiltration of mononuclear cells in the intervillous space, poor perinatal outcome, and high rate of recurrence. Our previous demonstration of increased syncytiotrophoblast (st) intercellular adhesion molecule-1 (ICAM-1) expression in villitis lesions and the finding of extensive monocyte/macrophagic cells in the maternal intervillous space in MCI, led us to further investigate stICAM-1 in MCI.

Materials and methods: A cross-sectional study of placentas from the third trimester of pregnancy (34–41 weeks gestation) was conducted to determine stICAM-1 in MCI ($n = 7$). MCI stICAM-1 expression was compared to stICAM-1 in villitis ($n = 7$) and in normal villi from placentas with ($n = 7$) and without ($n = 7$) villitis. Maternal cells within villi in MCI were identified in placentas mismatched for maternal/fetal human leukocyte antigen (HLA)-DRw52. Villitis was diagnosed with hematoxylin and eosin staining and antibody to CD3 in serial sections, and ICAM-1 in syncytiotrophoblasts was confirmed with antibodies to ICAM-1 and cytokeratin.

Results: Placentas with MCI had higher stICAM-1 (79.8%) than placentas with villitis (27.1%), normal villi from placentas with villitis (11.5%), and normal villi from placentas without villitis (0.3%). Maternal cells were identified within villi of placentas ($n = 5$) mismatched (mothers positive, fetuses negative) for HLA-DRw52.

Conclusions: Placentas with MCI have more stICAM-1 than placentas with or without villitis lacking MCI. The finding that MCI and villitis have prominent stICAM-1 and maternal cells in the villi suggests that MCI and villitis could have a similar pathophysiologic mechanism.

1. Introduction

Massive chronic intervillositis (MCI) is a placental lesion recognized for the first time in 1987 by our group [1] and characterized by an infiltrate of mononuclear cells in the intervillous spaces of the human placenta, frequently in the absence of villous inflammation. Originally, the lesion was named MCI because of the massive infiltration of the intervillous space and the type of cells that were present in the infiltrate, macrophages and lymphocytes. Since our original publication, many groups have used different terms for this entity: chronic intervillositis of unknown etiology [2]; chronic intervillositis [3–5]; chronic histiocytic intervillositis of unknown etiology [6,7]; chronic histiocytic intervillositis [8–10]; massive perivillous histiocytosis [11]; and intervillitis [12,13]. Although we believe that the original name, MCI, is the best term to define this placental lesion, a good alternative may be chronic intervillositis of unknown etiology (CIUE).

The pathogenesis of MCI remains undetermined, but it may be related to the mechanism that causes chronic villitis of unknown etiology (CVUE). MCI, a disease in which the inflammatory infiltrate is located almost exclusively between the villi, may be an extreme

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variant of CVUE [1]. MCI is usually associated with variable amounts of fibrin deposition, although the presence of fibrin is not necessary to make the diagnosis [2,7,8,14]. Atherosis-like lesions in decidual vessels have also been found [1,15].

Parant et al. concluded that there is a relationship between the histological lesions in patients with MCI and clinical outcome, with the most striking characteristics of patients with MCI being poor perinatal outcome and high rate of recurrence (100.0%) [2]. Contro et al. also reported a high recurrence rate (80%) [5]. Interestingly, they further reported that the live birth rate without treatment was 58.9%, and the birth rate dropped to 30.8% with treatment. This difference, however, was not statistically significant [5]. Nevertheless, Contro et al. concluded that intervention with drug therapy is of no demonstrable benefit and may even be harmful [5].

MCI has been linked to cases of neonatal alloimmune thrombocytopenia [16] and abnormal pregnancies that result in intrauterine growth restriction/retardation, fetal death, and recurrent pregnancy loss [1,3,9,17–20]. Moreover, MCI can be observed in patients who abuse heroin or have preeclampsia, hypertension, diabetes, lupus, Sjogren's syndrome [21], or malaria [12,13,17–19].

The principal immunopathologic characteristic of MCI is the presence of CD45- and CD68-reactive monocytes/macrophages that fill the intervillous spaces of the placenta [7,8]. CD3-reactive T-lymphocytes are rarely identified [8,9], being equally CD4+ and CD8+ cells [9]. Capuani et al. recently found that MCI is associated with an increase in Treg lymphocytes in the decidua basalis and the intervillous space and suggested that their finding would support the hypothesis of an immunopathological disorder for MCI [6].

As proposed for placental villitis, MCI could also be the expression of a maternal immune reaction against fetal tissues, although infections or other nonimmune causes of inflammation cannot be excluded at the present time. Intervillous monocyte recruitment and the development of villous inflammation could be facilitated by adhesion molecule expression, like intercellular adhesion molecule-1 (ICAM-1), among others, in the villous syncytiotrophoblast. Marchaudon et al. [7] and Heller [10] recently concluded that a diagnosis of MCI must be considered in cases of severe obstetric complications. Marchaudon et al. [7] further hypothesized that the elevated alkaline phosphatases observed during pregnancy in their study demonstrate the presence of syncytiotrophoblastic lesions (before fibrin deposits cover them) due to histiocytosis in the intervillous space.

ICAM-1 is a member of the immunoglobulin superfamily that can be induced in numerous cell types, including endothelial cells [22]. Endothelial cell surface ICAM-1 appears to contribute to the adhesion and transmigration of leukocytes (neutrophils, monocytes, lymphocytes and natural killer cells) that express leukocyte function-associated antigen-1 (LFA-1) ligand molecules [23,24]. The syncytiotrophoblast, which forms the boundary between the maternal circulation and the villous stroma, behaves as an endothelial surface that enables the regulation of maternal–fetal exchange [25]. Aberrant expression of ICAM-1 has been described in cultured syncytiotrophoblasts pretreated with inflammatory cytokines, which also have the property of increasing the adhesion of monocytes to the syncytiotrophoblast [25]. In addition, it has also been shown that monocytes adhering by LFA-1 to the placental syncytiotrophoblast induce tumor necrosis factor (TNF)-α-dependent apoptosis accompanied by focal disruption of the trophoblast culture [26]. Based on these findings, it has been suggested that focal damage of the placental barrier could be a route for maternal leukocyte infiltration into the villi and a possible mechanism of villitis onset [26].

The demonstration by our laboratory [27] and by Juliano et al. [28] of increased ICAM-1 expression in villitis lesions and the finding of extensive numbers of monocyte/macrophagic cells in the intervillous spaces of the placenta in MCI, led us to investigate whether (a) mononuclear cells in the maternal intervillous space were associated with the expression of adhesion molecules in the villous syncytiotrophoblast, and (b) whether ICAM-1 expression on the syncytiotrophoblast (stICAM-1) in MCI could favor the transmission of maternal cells into the villous fetal compartment.

2. Material and methods

2.1. Study design

A cross-sectional study was performed to evaluate the expression of stICAM-1 in villi from placentas with MCI from the third trimester of pregnancy (34–41 weeks gestation) obtained by 2010 from the Hospital Italiano of Buenos Aires, Argentina. Placentas with MCI ($n = 7$) and control tissues from placentas with CVUE ($n = 7$) or without evidence of inflammation ($n = 7$) were included in the study. All cases and controls were live births. To minimize the possible effects of different pathologies of pregnancy upon placental immunopathology, control placentas without MCI (placentas with and without villitis) were matched for the clinical diagnosis (i.e., preterm, preeclampsia and/or intrauterine growth restriction) attributed to placentas with MCI to eliminate confounding. Pregnancies were considered normal when there was no evidence of medical and/or obstetric complications and the birth weight was appropriate for the gestational age at term (≥37 weeks of gestation). Preeclampsia was defined as hypertension (systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg on at least two occasions, 4 h to 1 week apart), edema, and proteinuria (≥300 mg in a 24-h urine collection or one dipstick measurement of ≥2+). Infants with intrauterine growth restriction were defined as those infants whose weight was under the 10th percentile for their respective gestational age. Infants whose weight was over the 10th percentile were considered adequate for their gestational age. Pregnancies were considered preterm when the gestational age was less than 37 weeks. Placentas with chorioamnionitis (histologic evidence of neutrophil infiltration of placental amnion and chorion) or those from mothers with obesity (BMI of 30 or greater), known to be associated with a proinflammatory state [29], were not included in the study. Written informed consent was obtained from all participants, and the Institutional Review Board at Hospital Italiano of Buenos Aires approved the study protocol.

2.2. Placental specimens and immunohistochemistry

Placentas were fixed in 10% formalin, and one block per placenta was obtained from the central region. Each block used in the study measured 0.5 cm in width and 0.3 cm in thickness and comprised the full thickness of the placenta from maternal to fetal surfaces. Blocks were embedded in paraffin, and sections from each block were obtained and stained with hematoxylin and eosin to evaluate the histological characteristics of the placentas. Serial sections (5 μm each) were obtained and stained for use with light microscopy and single and double immunohistochemical techniques. The following primary antibodies were used: mouse anti-human ICAM-1 (G5, sc-8439, Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-human leukocyte antigen (HLA)-DRw52 (7.3.19.1, Accurate Chemical and Scientific Corporation, Westbury, NY), mouse anti-human CD68 (KP1, DakoCytomation, Carpinteria, CA), rabbit anti-human CD3 (A0452, DakoCytomation, Carpinteria, CA), and rabbit anti-human cytokeratin (A0575, DakoCytomation, Carpinteria, CA).

2.3. Immunoperoxidase technique

Slides obtained from paraffin blocks were dried for 30 min at 60 °C. They were deparaffinized, hydrated, and rinsed with TRIS buffer saline (DAKO). All antibodies were antigen retrieved using DAKO Target Retrieval solution (pH 6.0), cooled, and rinsed with TRIS buffer saline. Slides were processed in the DAKO Autostainer and blocked using an avidin/biotin blocking system (DAKO). Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. A protein block (DAKO) was also used. Primary antibodies were applied for 60 min at room temperature. Slides were developed using DAKO's EnVision+ Dual Link, HRP kit. Double antibody immunohistochemistry was performed using the Envision double stain system.

2.4. Criteria for histopathologic diagnosis

MCI was diagnosed by the identification of massive infiltrates of monocyte/macrophage-like cells in the placental intervillous spaces. Chronic villitis was defined as the presence of a mononuclear cell (lymphocytes and/or histiocytes) infiltrate 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 [15] in placental sections using conventional hematoxylin and eosin staining [30]; and immunohistochemical techniques with monoclonal antibody to CD3 and concomitant hematoxylin and eosin staining of an immediate serial section, as described [27]. ICAM-1 in the syncytiotrophoblast of the chorionic villi was considered spatially if there were only spotty or circumferential reactivity. ICAM-1 reactivity on the syncytiotrophoblast of chorionic villi in areas of villitis was confirmed using a double-antibody immunohistochemical technique with antibodies to CD3 and ICAM-1. The expression of ICAM-1 on syncytiotrophoblastic cells