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ICAM-1 expression on immune cells in chronic villitis

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ABSTRACT

Introduction: ICAM-1 expression on the villous syncytiotrophoblast (ST) is believed to participate in migration of maternal cells into the inflamed villi regardless of villitis etiology. However, its expression on immune cells in chronic villitis (CV) has yet to be analyzed. ICAM-1 induces cell–cell adhesion allowing intercellular communication, T cell-mediated defense mechanism, and inflammatory response.
Material and methods: 21 cases of CV (all without an identifiable etiologic agent) and 3 control placentas were analyzed using ICAM-1, and for immune cells CD45, CD3 and CD68. These cells were subdivided according to their location in inflamed villi: a) within the inflamed villi and b) outside forming perivillous aggregates.
Results: Large amounts of CD45, CD3 and CD68 were found within the inflamed villi and forming perivillous aggregates attached to areas of trophoblastic loss. Inflamed villi usually showed ICAM-1+ ST. The majority of immune cells surrounding areas of trophoblastic rupture presented marked expression of ICAM-1. In contrast, a small number of immune cells within the inflamed villi exhibited ICAM-1 expression. Only some (<5%) inflamed villi without trophoblastic rupture and with ICAM-1+ ST presented adherence of immune cells.
Discussion: In inflamed villi of chronic villitis, the level of ICAM-1 expression on immune cells depends on their location: high in number of cells in the perivillous region and low within the villi. The strongest expression of ICAM-1 on immune cells attached to areas of trophoblastic rupture suggests that the loss of trophoblast can lead to an amplification of the inflammatory response.

1. Introduction

Chronic villitis, an inflammatory lesion of placental villi, has been associated with the transmission of infection between mother and fetus (e.g. rubella, toxoplasmosis, etc) as well as with the maternal immune aggression of fetal tissues [1–3]. In inflamed villi, most of the immune cells are of maternal origin, suggesting that mononuclear cells have the capacity to migrate across the trophoblastic barrier into villous stroma [4–7].

Intercellular adhesion molecule-1 (ICAM-1), a transmembrane glycoprotein, is expressed constitutively on the cell surface of a variety of cell types, such as immune cells, epithelial cells, endothelial cells, fibroblasts, etc. Among immune cells, ICAM-1 is expressed on monocytes/macrophages, B lymphocytes, plasma cells, and on memory and activated T lymphocytes. In placental cells, ICAM-1 has been detected at low levels on the villous trophoblast [8]. However, ICAM-1 expression can be up-regulated by a number of factors, including proinflammatory cytokines and virus infection. In placentas, overexpression of ICAM-1 has been reported on syncytiotrophoblast (ST) in chronic villitis, massive chronic intervillositis and infections by Plasmodium falciparum, human cytomegalovirus and HIV-1 [9–14].

ICAM-1 plays important roles in the adhesion phenomena involved in the transendothelial migration of leukocytes and in the immune system. The ST of chorionic villi presents an endothelial-like function and in chronic villitis, ICAM-1 expression on the ST is believed to participate in migration of maternal cells into the placental villi [12,13]. In cultured ST, it has been described that monocytes adhered by ICAM-1 receptor LFA-1 (leukocyte function-associated antigen-1) induce TNF-alpha-dependent apoptosis

Abbreviations: ST, syncytiotrophoblast; CV, chronic villitis; ICAM-1, Intercellular adhesion molecule-1; LFA-1, leukocyte function-associated antigen-1.

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accompanied by focal disruption of the trophoblast [15]. This focal damage of the placental barrier has been thought to be a route for maternal leukocyte infiltration into the villi and a possible mechanism of villitis onset [15]. In fact, in a previous study, our group showed that in the inflamed villi, regardless of the villitis etiology, there was trophoblast overexpression of ICAM-1 close to areas of rupture, which frequently presented leukocyte adherence [13].

In relation to the immune system, ICAM-1 induces cell–cell adhesion allowing intercellular communication, T cell-mediated defense mechanism, and inflammatory response. In chronic villitis, to date, the studies on ICAM-1 have only focused on its expression on ST [12,13]. However, the inflammatory cascade initiated in the villitis onset and maintained during its evolution can potentially induce enhancement of ICAM-1 expression on other cell types that participate in the inflammatory process. In order to broaden our understanding of the mechanisms involved in the development of chronic villitis, we investigated ICAM-1 expression on immune cells in inflamed villi.

2. Material and methods

2.1. Cases

This study was approved by the Institutional Ethics Committee. The surgical pathology archives of the Hospital of the University of Campinas (UNICAMP), São Paulo-Brazil were reviewed between 2008 and 2012 and contained 21 cases which had been diagnosed as chronic villitis without an identifiable etiologic agent. All cases were from term/near term singleton pregnancies and had hematoxylin and eosin (H&E) slides and/or paraffin blocks. Chronic inflammatory infiltrate was observed in the stroma of >one chorionic villi in all cases. Three term placentas without villitis were included as control.

2.2. Immunohistochemistry

One paraffin block from each case was chosen for immunohistochemical studies. The following primary antibodies were used: anti-CD45 (IgG2a, clone 2B11 + PD7/26, dilution 1/100), anti-CD68 (IgG2a, clone KP1, 1/1000), anti-CD3 (IgM, clone C3D-1, 1/1000), anti CD34 (IgG1, clone QBEnd, 1/50) anti-ICAM-1 (IgG2a, SC-8439, 1/4000). All antibodies were from Dakopatts S/A, Denmark, except for anti-ICAM-1, which was from Santa Cruz Biotechnology Inc, USA. Briefly, the immunohistochemical staining was performed as follows: the 5-μm sections were deparaffinized, hydrated and endogenous peroxidase activity was quenched by immersion of the slides in 10% hydrogen peroxide. The antigen retrieval (AR) was achieved by boiling, in a steamer, in citrate buffer (pH: 6.0) or Tris–EDTA buffer (pH: 8.9) according to the primary antibody used. After cooling, the sections were incubated at 4 °C with the primary antibody overnight and then with the EnVision polymer for 1 h at 37 °C. Subsequently, sections were stained for 5 min at 37 °C with 3,3′-diaminobenzidine tetrahydrochloride (DAB) and counter-stained with hematoxylin.

In selected cases (5 cases) double-labeling immunohistochemical staining was performed (EnVision doublestain, code K1395, DAKO, SA, Denmark). The antibodies CD45, CD68, CD3 and CD 34 were applied after antigen retrieval using citrate buffer (pH: 6.0) and incubated 30 min at 25 °C; detection was achieved using the EnVision anti-mouse and anti-rabbit polymer HRP and DAB to visualize the binding of the first antibody. The sections were then incubated with an antibody against ICAM-1 at 25 °C for 30 min. EnVision polymer linked to alkaline phosphatase and fast red as a substrate chromogen system were used to complete the second immunostaining.

2.3. Analysis of positive cells

Serial tissue sections were used and each of the different antibodies (anti-CD45, anti-CD68, anti CD3, anti ICAM-1) was applied on a different section of the same block. Any CD3-positive cell was used as the criterion for villitis. Immune cells in villitis areas were subdivided according to their location: a) within the inflamed villi and b) outside forming perivillous aggregates. The relative numbers of stained leukocytes were considered in relation to all inflammatory cells seen in each location, i.e. a) all immune within the inflamed villi and b) all extravillous immune cells forming perivillous aggregates. Regarding ICAM-1 expression on ST, the number of positive villi was evaluated in areas with and without villitis in relation to all inflamed and normal villi, respectively. The immunoreactivity for ICAM-1 on leukocytes in both compartments (inside and outside the villi) was assessed using a three-tiered scale: a) 0 = no reactive cells, b) 0.1–50% of cells, c) >50%.

3. Results

In all cases, villitis was focal and involved only terminal and stem villi. A variable number of inflamed villi presented foci of trophoblastic necrosis. The inflammatory infiltrate within the villi was composed of mononuclear cells and these also formed aggregates in the intervillous space around the areas of trophoblastic rupture (Fig. 1a).

The immunohistochemical findings are in Table 1. All cases of villitis showed CD45 positive cells within the inflamed villi as well as in the perivillous region surrounding the areas of trophoblastic rupture (Fig. 1b). In the majority of cases, the inflamed villi exhibited numerous CD68 and CD3 positive cells (macrophages/monocytes and T cells, respectively) within and outside the villi; the outside (extravillous) cells surrounded the foci of trophoblastic damage (Fig. 1c, d). The majority of cases (57.8%) presented a similar proportion of CD3+ and CD68+ cells inside and outside the villi (Table 2). In 26.3% of cases, CD3+ lymphocytes and CD68+ macrophages/monocytes were the predominant immune cells inside and outside the villi, respectively (Table 2).

Regarding ICAM-1 expression on ST, the molecule was expressed on the ST of inflamed villi in variable extension in all cases of villitis (Fig. 2): in 76% (16 cases) in >50% of inflamed villi and 23.8% in <50%. In contrast, few non-inflamed villi showed ICAM-1 on ST (Fig. 2c). In inflamed villi, ICAM-1 expression on the ST was frequently associated with areas of trophoblastic rupture. In these areas, there usually was adherence of several perivillous immune cells (Fig. 2a, b; 3a). In inflamed villi without trophoblastic rupture and with ICAM-1 expression on the ST, we found in few villi (<5%) adherence of immune cells but the number of cells was usually small (Fig. 3b). Non-inflamed villi with ICAM-1 expression on the ST did not show such findings (Fig. 2c).

In relation to ICAM-1 positive immune cells, the number varied according to their location in the inflamed villi. The majority of immune cells (>50%) surrounding the areas of trophoblastic rupture presented marked expression of ICAM-1 in all cases but one (Fig. 2a, b). In contrast, a small number of immune cells within the inflamed villi showed ICAM-1 expression (Fig. 2a, b). This expression was usually less marked than the one presented by immune cells in the perivillous region. Double-labeling immunohistochemical staining was performed to verify the quantity of CD45+, CD68+ cells and CD3+ cells that was ICAM-1 positive in the two compartments (inside and outside the villi). In all cases the majority of CD45+, CD68+ and CD3+ cells outside the villi (>50% of cells) was ICAM-1 positive whereas <50% of these cells expressed ICAM-1 inside the villi (Fig. 4a, b, c). As endothelial cells of villous fetal vessels were ICAM-1 positive as well, double-labeling immunohistochemical staining for CD34 (pan-endothelial marker) and ICAM-1 was performed. Inflamed villi frequently presented absence of CD34+ fetal vessels (Fig. 4d) and slight increase of ICAM-1 expression on stromal cells, which formed a thin network (see villous stroma in Fig. 4a,b,d).

In control placentas, ICAM-1 expression was only detected on ST of rare terminal villi and on endothelial cells of fetal vessels (Fig. 2d).

4. Discussion

This study further expands our previous investigations on trophoblastic ICAM-1 overexpression in human villitis showing that: a) in inflamed villi, immune cells expressed ICAM-1 and such expression was particularly marked on those adhered to foci of trophoblastic rupture, and b) inflamed villi with ICAM-1 overexpression on ST but without trophoblastic rupture rarely present adherence of immune cells.

In relation to immune cells, ICAM-1 functions as a co-activation signal for activation of T cells [16]. ICAM-1 is an accessory molecule, which helps stabilize the interaction between the T cell receptor (TCR) and antigen in association with MHC class I (all