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D2-40/podoplanin expression in chronic villitis of the placenta

Expressão de podoplanina/D2-40 em vilosite crônica placentária

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ABSTRACT

D2-40/podoplanin (D2-40/PDPN) is a multifunctional protein that can be expressed in lymphatic endothelium and immune cells. D2-40/PDPN expression in chronic villitis (CV) has not been studied. In 22 cases of CV, we analyzed both D2-40/PDPN expression as well as its co-expression with immune cells markers, and the relationship with stromal cells. In the non-inflamed villi, the D2-40/PDPN positive plexiform pattern has a lymphatic-like conductive network. In the inflamed villi, the D2-40/PDPN expression, predominantly restricted to stromal cells forming a cellular network, is likely related to a phase of the inflammatory response, such as reorganization of the damaged tissue.

Key words: placenta; immunohistochemistry; stromal cells.

INTRODUCTION

D2-40, a monoclonal antibody to an Mr 40,000 O-linked sialoglycoprotein, specifically recognizes human podoplanin (PDPN), which is expressed on lymphatic endothelium, kidney podocytes, and pulmonary type I alveolar cells. Regarding immune cells, D2-40/PDPN has been shown to be expressed on inflammatory macrophages[1] and Th17 cells[2]. Th17 cells belong to the group of CD4+ helper T cells and interleukin (IL)-17 supports T cell activation increasing the induction of co-stimulatory molecules[3, 4]. In placentas, D2-40/PDPN expression has rarely been studied and was found: a) forming a villous plexiform network pattern, and b) on stromal cells[5, 6]. The former was interpreted as a lymphatic-like conductive network whereas D2-40/PDPN expression on stromal cells was thought to be related to cytoskeletal reorganization.

Chronic villitis (CV), an inflammatory lesion of placental villi, can be of infectious origin or of unknown etiology (CVUE) and has been associated with abortion, intrauterine fetal death, malformations and intrauterine growth restriction[7]. Macrophages and T lymphocytes are the predominant cells of CV, regardless of its etiology[8].

As D2-40/PDPN plays multiple roles, some of them are related to inflammatory response, the aim of this study was to investigate the expression of this protein in a series of CVUE in order to enhance our understanding of this lesion. CVUE has been reported to be the most common type of villitis, affecting between 5% and 15% of all third-trimester placentas[9].

METHODS

This study was approved by the Institutional Ethics Committee. Twenty two cases, which had been diagnosed as CV without an identifiable etiologic agent, were reviewed. Three term placentas without villitis were included as control.

Immunohistochemical studies were performed on one paraffin block from each case. All cases were stained with anti-CD3 (T cells) and D2-40 antibodies. Any CD3-positive cell was used as the criterion for villitis. D2-40/PDPN expression on villous stroma was analyzed in areas with and without villitis. The extension of D2-40/PDPN expression was evaluated semi-quantitatively as follows: - (absent), +/focal (present in 1%-50% of villi), ++/diffuse (> 50% of villi). The patterns of D2-40/PDPN expression on villous stroma were classified as: a) plexiform network, and b) cellular network.

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In the selected cases (five cases), additional studies were performed as follows: a) serial sections were stained for D2-40, alpha-smooth muscle actin (alpha-SMA) and vimentin antibodies to verify whether within the inflamed villi the amount of D2-40/PDPN positive stromal cells corresponded to that of alpha-SMA and/or vimentin positive cells; b) double-labeling immunohistochemical staining using D2-40, CD45 (leukocytes) and CD68 (macrophages) antibodies to verify the co-expression of D2-40/CD45 and D2-40/CD68. All antibodies were from Dakopatts S/A, Denmark and details of immunohistochemical staining are shown in **Table 1**. For all antibodies, we used sections of non-neoplastic palatine tonsil as positive control. Primary antibodies were left out as a negative control.

TABLE 1 – Antibodies used in the study*

Antibody	Manufacturer	Clone	Dilution	Antigen retrieval
D2-40	Dako	D2401	1:200	Triz/EDTA
CD45	Dako	2B11+PD7/26	1:100	Citrate
CD68	Dako	clone KP1	1:1000	Citrate
CD3	Dako	F.7. 238	1:1000	Citrate
Vimentin	Dako	V9	1:100	Citrate
Alpha-SMA	Dako	IA4	1:200	-

**All the antibodies are monoclonal.*
EDTA: ethylenediaminetetraacetic acid; alpha-SMA: alpha-smooth muscle actin.

RESULTS

In all cases focal villitis was observed, affecting only terminal and stem villi and showing CD3 positive cells. Both control placentas and the non-inflamed villi of placentas with CV presented D2-40/PDPN expression forming plexiform network pattern around villous core fetal vessels in most of cases (**Table 2**). This pattern of D2-40/PDPN expression was also detected in the inflamed villi, but only in areas without inflammatory infiltrate and in a few cases (3%-13.6%). In the inflamed villi, D2-40/PDPN was strongly expressed in the villous stroma in 63.6% of cases (Table 2). In these villi, D2-40/PDPN positive cells formed a dense cellular network similar to that seen in the sections stained for vimentin. D2-40/PDPN expression was not observed on CD45 positive cells, although rare CD68 positive macrophages in the inflamed villi showed co-expression of D2-40/PDPN. In normal placentas, alpha-SMA was positive in stromal cells around fetal vessels of stem villi, vascular walls and pericytes in terminal villi. In the inflamed villi, alpha-SMA positive stromal cells were absent or very rare, even in those containing numerous D2-40/PDPN positive stromal cells (**Figure**).

TABLE 2 – D2-40 expression in 22 cases of chronic villitis subdivided according to its expression pattern in inflamed and non-inflamed villi

	Inflamed villi			Non-inflamed villi		
	Quantity of positive cells			Quantity of positive cells		
D2-40 expression patterns	- (%)	+ (%)	++ (%)	- (%)	+ (%)	++ (%)
Plexiform network	19/22 (86.4)	2/22 (9.1)	1/22 (4.4)	2/22 (9.1)	8/22 (36.4)	10/22 (45.5)
Cellular network	8/22 (36.4)	7/22 (31.8)	7/22 (31.8)	20/22 (90.9)	2/22 (9.1)	0/22 (0)

-: absent; +: ≤ 50% of positive cells; ++: > 50% of positive cells.

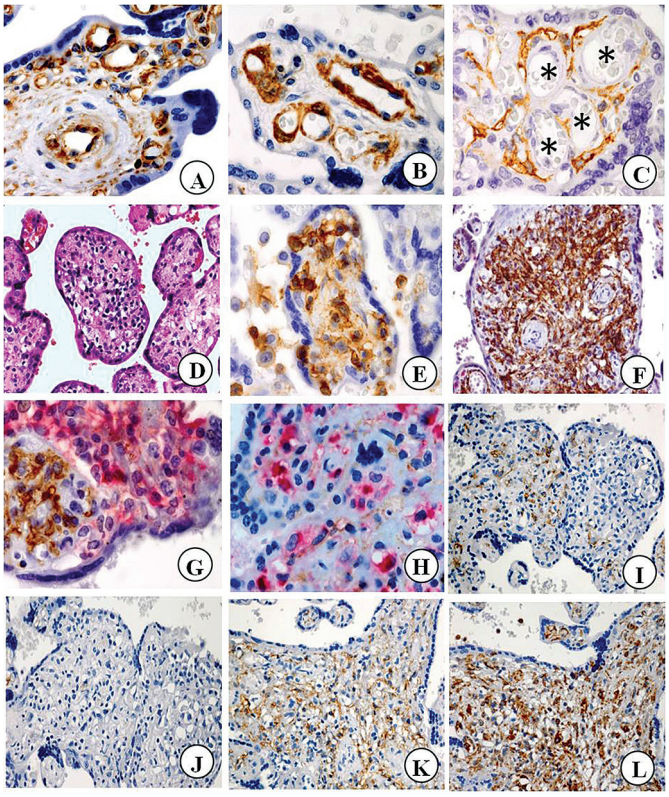

FIGURE – *Immunohistochemical findings in normal and inflamed villi*

Images A to C – normal villi of control placentas: immunostaining of vimentin (A), alpha-SMA (B) and D2-40 (C) in villous tissue sections. Note vimentin expression in vascular walls and extravascular stromal cells; alpha-SMA expression in vascular walls; D2-40+ cells delineating a lymphatic-like conductive network around fetal blood vessels (*) (original magnification 1000×). Images D to L – inflamed villi of placentas with chronic villitis. D: immune cells within villous stroma (HE staining, original magnification 400×); E: CD3+ T lymphocytes within villous stroma (original magnification 1000×); F: D2-40+ cells form a dense cellular network (original magnification 400×); G and H: double-labeling immunohistochemical staining using D2-40 (brown), CD45 (red in G) and CD68 (red in H) antibodies: co-expressions of D2-40/CD45 and D2-40/CD68 are not observed (original magnification 1000×); I and J: serial sections stained with D2-40 (I) and alpha-SMA (J): D2-40+ stromal cells are seen in (I) whereas alpha-SMA+ cells are absent in (J) (original magnification 400×); K and L: serial sections stained with D2-40 (K) and vimentin (L): D2-40 and vimentin staining similar amounts of stromal cells (original magnification 400×).

Alpha-SMA: alpha-smooth muscle actin; HE: hematoxylin and eosin.

DISCUSSION

There are a few studies on placental lymphatic development and lymphatic circulation was not found[6]. However, recently Wang *et al*. (2011)[5], based on immunohistochemical expression of D2-40/PDPN in villous stroma, proposed that a lymphatic-like conductive network with ability to maintain homeostasis may exist in the human placenta.

In the current study, our findings in the non-inflamed villi showing that D2-40/PDPN positive cells frequently delineated tubular structures strengthen this hypothesis. However, in the inflamed villi, we detected a modification of D2-40/PDPN expression, i.e., D2-40/PDPN positive cells formed a dense network but without the plexiform pattern. As no significant co-expression of D2-40/PDPN with CD45 or CD68 was seen in our series, we supposed that the D2-40/PDPN positive cellular network could represent the stromal cells modified by the microenvironmental stimuli in the inflamed villi. D2-40/PDPN has been found to be expressed in fibroblasts from both chronically inflamed tissues and myofibroblasts[10, 11]. Regarding the latter, alpha-SMA expression (a marker of myofibroblastic differentiation) was not observed in the inflamed villi, even in those with numerous D2-40/PDPN positive cells. In contrast, these villi with numerous D2-40/PDPN positive cells presented a staining pattern similar to that observed for vimentin, suggesting that these cells could be fibroblastic stromal cells. As D2-40/PDPN has been implicated in cytoskeletal reorganization[12], we believe that in the inflamed villi of CV, microenvironmental stimuli may lead to cytoskeletal modification of the stromal cells.

In summary, in the non-inflamed villi, D2-40/PDPN plexiform pattern suggests a lymphatic-like conductive network, whereas in the inflamed villi D2-40/PDPN expression is predominantly restricted to stromal cells forming a cellular network. As this alteration occurs only in some inflamed villi, it is possible that its induction may be related to a phase of the inflammatory response, such as reorganization of the damaged tissue.

RESUMO

Podoplanina/D2-40 (PDPN/D2-40) é uma proteína multifuncional que pode ser expressa no endotélio linfático e nas células imunes. Na vilosite crônica (VC), a expressão de PDPN/D2-40 ainda não foi estudada. Em 22 casos de VC, analisamos tanto a expressão de PDPN/D2-40 como sua coexpressão com marcadores de células imunes, além da relação com células estromais. Nas vilosidades não inflamadas, o padrão plexiforme PDPN/D2-40 positivo tem aspecto de rede condutora linfática. Nas vilosidades inflamadas, a expressão de PDPN/D2-40, com predominância restrita às células estromais, formando rede densa está, possivelmente, relacionada com uma fase da resposta inflamatória, como a reorganização do tecido danificado.

Unitermos: *placenta; imuno-histoquímica; células estromais.*

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