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DC-SIGN+ CD163+ Macrophages Expressing Hyaluronan Receptor LYVE-1 Are Located within Chorion Villi of the Placenta

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Abstract

The purpose of this study was to investigate with immunohistochemical methods antigen presenting cells and their relationship to blood and lymphatic vessels in human term placenta.

Fetal placental antigen presenting cells, historically also known as Hofbauer cells, were located in the chorionic villi below the syncytiotrophoblast and in the vicinity of fetal capillaries. DC-SIGN/CD209 expression was observed on CD163+, CD68+, CD45+, HLA-A,B,C+, DC-LAMP/CD208−, CD86−, Langerin/CD207−, FXIIIa−, CD1a− cells consistent with the macrophage nature of these cells.

These fetal DC-SIGN+ cells lack HLA-DR, -DP, -DQ expression. Moreover, we show for the first time that they co-express the hyaluronan receptor LYVE-1. In contrast, no LYVE-1+ vessel structures, i.e. lymphatic vessels, were detected.

Human term decidua hosted a variety of CD45+ cells, further phenotyped as CD163+, DC-SIGN+, CD68+, HLA-DR+, HLA-A,B,C+. Mature dendritic cells were never observed in human term placenta.

In summary, human term placenta is an immunoprivileged organ without lymphatic drainage and with numerous DC-SIGN+ macrophages within the chorionic villi. We hypothesize that these cells may fulfil a function in innate responses against pathogens as well as be involved in the homeostasis of hyaluronan metabolism in the rapidly differentiating placenta.

Keywords: Macrophages; Dendritic cells; Fetomaternal interface; Immunology; LYVE-1; DC-SIGN; CD163; HLA-DR, -DP, -DQ; Antigen presentation; Tissue remodelling

1. Introduction

The human placenta is considered to be an immunological privileged site that must play a vital role in protecting the allogeneic fetus from the maternal immune system for nine months. On the other hand the immune cells must be able to respond to different types of foreign antigens, including pathogenic organisms [1].

The characterization of antigen presenting cells in the decidua has been the subject of intensive research. Human early pregnancy decidua harbours the following three antigen presenting cell populations: CD14+ macrophages, CD83− immature dendritic cells and CD83+ mature dendritic cells [2,3].

Within chorion villi numerous macrophages were described. The distribution and characterization of fetal macrophages in first trimester placenta were investigated [4,5]. Only few reports suggested a dendritic cell phenotype for these cells [6]. Soilleux et al. observed DC-SIGN expression on HLA-DR+, -DP+, -DQ+ CD68+ CD14+ CD4+ S100+ CD83− villous cells consistent with Hofbauer cells, which have a phenotype most similar to that of immature dendritic cells.

Macrophages have a close relationship to lymphatic vessels and migrate through lymphatic vessels to the lymph node. Therefore, lymphatic vessels are an essential part of the

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immune system. Little is known about the lymphatic network in human term placenta [7]. Recently, two research groups used LYVE-1 [8,9], which is a hyaluronan receptor and a powerful marker for lymphatic vessels, for the investigation of lymphatic vessels in human placenta. However, several recent studies [10] have shown that LYVE-1 is not exclusive to the lymphatic vessels. Apart from the lymphatic endothelium, LYVE-1 is also expressed by activated tissue macrophages.

This prompted us to investigate the occurrence, distribution, and phenotypical characteristics of antigen presenting cells in human term placenta. Moreover, we examined – using lymphatic endothelial cell markers – human term placental specimens in order to support the general view that this tissue completely lacks lymphatic vessel.

2. Materials and methods

Tissue was obtained from eight healthy women undergoing a Caesarean section. The sections were at term (38–40 gestation week) and optional. All investigations were in accordance with the guidelines of the local ethical committee.

Cryosections were incubated with human IgG (Beriglobin P^{TM}, Aventis Behring; Marburg, Germany; final concentration 0.6 mg/ml) for blocking Fc-receptors. Afterwards, we applied the diluted unconjugated primary antibodies (Table 1a) and proceeded as described [11].

For negative controls, the primary antibodies were replaced by isotype-matched immunoglobulins of irrelevant specificity and at equal concentrations. Staining placental tissue with various antibodies was additionally controlled by applying the same antibodies simultaneously on human skin or tonsil specimens, i.e. tissues with well know immunolabeling profiles for the antibodies used.

3. Results

The observations shown and described in this section are representative for all the placentae examined.

The distribution patterns of immunohistochemical staining in the placental tissues investigated are summarized in Table 1b.

3.1. Chorionic villi – antigen presenting cell antibodies

Chorion villi represented 80% of the placental tissue. Within chorion villi, DC-SIGN+ cells were the main cell population besides endothelial cells and fibroblasts. They always showed the same topographical position within the chorion villi suggesting a fixed distribution pattern. Numerous DC-SIGN+ cells surrounded the CD31+ microvasculature of chorion villi. Additionally, they formed a collar-like ring directly

Table 1a
Dendritic and endothelial cell antibodies

Antibody	Clone	Isotype	Source	Location	Dilution/final concentration
Antigen presenting cell antibodies					
DC-SIGN	DCN46	mouse IgG2b	BD Biosciences	San Diego, CA, USA	1:60/0.52 μg/ml
CD1a	HI149	mouse IgG1	BD Biosciences	San Diego, CA, USA	1:10/10 μg/ml
CD86	BU63	mouse IgG1	Dako A/S	Glostrup, Denmark	1:20/7.5 μg/ml
DC-LAMP	104.G4	mouse IgG1	Beckman Coulter Immunotech	Marseille, France	1:40/5 μg/ml
Langerin	DCGM4	mouse IgG1	Beckman Coulter Immunotech	Marseille, France	1:40/5 μg/ml
CD68	KP1	mouse IgG1	Dako A/S	Glostrup, Denmark	1:25/4 μg/ml
CD45	4B2/HB-196	mouse IgG2a	ATTC	Manassas, VA, USA	hybridoma cell supernatant
CD45	VIT 200	mouse IgG2a	Prof. W. Knapp	Vienna, Austria	1:100/10 μg/ml
HLA-A,B,C	W6/32	mouse IgG2a	ATTC	Manassas, VA, USA	hybridoma cell supernatant
HLA-DR	G 46-6	mouse IgG2a	BD Biosciences	San Diego, CA, USA	1:50/10 μg/ml
HLA-DQ	SPV-L3	mouse IgG2a	AbD Serotec	Oxford, UK	1:10/5 μg/ml
HLA-DP	BRA-FB6	mouse IgG2b	Biogenesis	Kingston, USA	1:100/3.7 μg/ml
CD163	FC6-FAT	mouse IgG1	Acris Antibodies	Hiddenhausen, Germany	1:100/10 μg/ml
FXIIIa	polyclonal	rabbit serum	Thermo Scientific	Fremont, CA, USA	1:100/10 μg/ml
Lymphatic endothelial cell antibodies					
LYVE-1	polyclonal	rabbit serum	COACROM	Vienna, Austria	1:100/2 μg/ml
Podoplanin	18H5	mouse IgG1	RDI	Flanders, NJ, USA	1:20
Prox-1	polyclonal	rabbit serum	COACROM	Vienna, Austria	1:20/10 μg/ml
FITC-conjugated antibodies					
DC-SIGN	DCN 46	mouse IgG2b	BD Biosciences	San Diego, CA, USA	1:10
CD31	WM 59	mouse IgG1	BD Biosciences	San Diego, CA, USA	1:5
CD34	581	mouse IgG1	BD Biosciences	San Diego, CA, USA	1:2
CD68	KP 1	mouse IgG1	Dako	Glostrup, Denmark	1:10/10 μg/ml
DC-SIGNR	120604	mouse IgG2b	R&D Systems	Tustin, CA, USA	1:5/10 μg/ml
HLA-A,B,C	G46-2.6	mouse IgG1	BD Biosciences	San Diego, CA, USA	1:5
HLA-DR	L243	mouse IgG2a	BD Biosciences	San Diego, CA, USA	1:5
Negative control antibodies					
IgG1	DAK-GO1	mouse IgG1	Dako	Glostrup, Denmark	1:10/100 μg/ml
IgG2b	DAK-GO9	mouse IgG2b	Dako	Glostrup, Denmark	1:20/100 μg/ml
IgG2b-FITC	MPC-11	mouse IgG2b	Beckman Coulter	Fullerton, CA, USA	1:5
IgG2a	RPC 5	mouse IgG2a	Ancell Corp.	Bayport, USA	1:100/1000 μg/ml