Placenta xxx (2014) 1–8

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Dynamic changes in maternal decidual leukocyte populations from first to second trimester gestation

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ARTICLE INFO

Article history:
Accepted 25 September 2014

Keywords:
dNK
Macrophages
Dendritic cells
T cells
Placenta
Decidua

ABSTRACT

Introduction: Decidual leukocytes are critical to the development of the fetomaternal interface, regulating tolerance to the semi-allogeneic fetus and vascular transformation of the uterine spiral arteries. Despite the continuation of these processes beyond the first trimester of pregnancy, the second trimester has largely been unstudied, with investigation focusing on early gestation and term tissues. We sought to characterize changes in decidual leukocyte populations from first to second trimester.

Methods: Multicolor flow cytometry was performed on isolated decidual leukocytes from elective terminations of pregnancy between 6 and 20 weeks of gestation for study of first (6–12 weeks) and second trimesters (13–20 weeks). Specific subpopulations were identified by comparison to isotype and fluorescent-minus-one (FMO) controls.

Results: Decidual natural killer cells ($CD56^+CD16^-CD3^-$) did not change in number, although a population of dNK with decreased CD56 brightness was observed in second trimester decidua. $CD14^+$HLA-DR^+ macrophage numbers declined from first to second trimester ($p = 0.031$), yet a $CD163^+CD206^+$ subset designating alternatively activated M2-like macrophages increased during the same period ($p = 0.015$). Intermediate $CD205^+$ dendritic cells demonstrated significant decline ($p = 0.022$), but immature $CD209^+$ and mature $CD83^+$ dendritic cells did not differ between trimesters. Total $CD3^+$ and $CD3^+CD4^+$ T lymphocytes increased ($p = 0.0079$, $p = 0.0028$); $CD3^+CD8^+$ T cells trended towards increase but did not differ significantly.

Conclusion: Several changes in leukocyte subsets are observed in the second trimester that promote a tolerogenic and angiogenic decidual microenvironment through mid-gestation.

1. Introduction

Pregnancy-specific leukocyte populations found within the decidua are critical to the development of the uteroplacental interface, mediating maternal tolerance to the fetal allograft and uterine spiral artery remodeling. Aberrant leukocyte activation or alterations in the proportions of specific leukocyte subtypes may disrupt these crucial processes and increase the risk of developing serious complications of pregnancy [1]. Decidual leukocytes are present in normal cycling endometrium and peak in number during the late secretory phase of the menstrual cycle [2]. Following fertilization, leukocyte numbers continue to rise with progressive endometrial decidualization, reaching a population of 30–40% of decidual cells in early pregnancy [3].

$CD56^{bright}CD16^-$ decidual natural killer (dNK) cells comprise up to 70% of decidual leukocytes in early pregnancy, representing a distinct NK cell type whose lack of cytolytic activity is a result of selective interaction of EVT-expressed MHC Class I molecules HLA-G, HLA-C, and HLA-E with inhibitory NK cell surface receptors [4]. In both murine and human pregnancy, numerous studies have established a critical role for dNK in regulation of trophoblast invasion and vascular remodeling events [5–8].

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http://dx.doi.org/10.1016/j.placenta.2014.09.018
0143-4004/

Macrophages represent the second largest population of immune cells in early gestation at approximately 30% of total decidual leukocytes, and are present in an atypical alternatively-activated phenotype characterized by tissue repair and immunogenic tolerance through secretion of anti-inflammatory, growth and matrix remodeling factors [9–12]. dNK and macrophage mediate trophoblast-independent stages of decidual spiral artery remodeling, and are observed infiltrating the vessel wall prior to trophoblast invasion in both decidua basalis and placenta-decidua co-cultures [7,8].

In addition to regulation of maternal T cell responses to the fetal allograft, the importance of dendritic cells (DC) to proper implantation and formation of the decidua is well established in mice [13,14]. DC can exert pro-angiogenic effects via secretion of angiogenic factors which stimulate the vascular endothelium, including CXCL8 and CCL2, or cytokines which increase integrin expression on endothelial cells and expression of pro-angiogenic cytokines by other cell types (e.g. IL-6, TGFβ, TNFα) [15]. T cells represent 10% of decidual leukocytes in the first trimester and govern maternal tolerance to the fetus as directed by macrophages and DC. Several populations of T cells have previously been reported in the decidua and interact to prevent rejection of the fetus and trophoblast by the maternal immune system [16].

While it is well accepted that uterine vascular transformation continues into the second trimester [17], there is a paucity of information concerning leukocyte populations during this period, as studies typically focus on readily available decidual tissue from early first trimester gestation or term pregnancy. Recent ultrasound studies of the fetomaternal interface have documented changes in uterine blood flow through the second and third trimesters, suggesting that vascular transformation may continue into later pregnancy [18,19]. Furthermore, remodeling of the deeper decidual and myometrial sections of spiral arteries, the primary location of defective vascular transformation in cases of placental insufficiency, occurs at this time [17,20]. Thus, characterization of the abundance and phenotype of second trimester decidual leukocyte subpopulations and comparison to first trimester populations is crucial to defining the roles of specific leukocyte subtypes during the mid-gestation period and understanding how vascular transformation at the decidual–myometrial junction may be compromised in pathologic pregnancies.

2. Materials & methods

2.1. Tissue collection

Decidual tissue was obtained following informed consent from women undergoing elective termination at the Morgentaler and Mount Sinai Second Trimester Interruption of Pregnancy (STIPS) clinics during the first (6–12 weeks, mean gestational age = 9 weeks; $n = 14$) and second (13–20 weeks, mean gestational age = 16 weeks; $n = 15$) trimesters of gestation. Tissues were collected via vacuum aspiration. In patients beyond 16 weeks of gestation, tissues were obtained following cervical insertion of laminaria 24 h earlier. The study and sample collection were approved by the Morgentaler and Mount Sinai Hospital Research Ethics Boards. Patient information is found in Table 1.

2.2. Isolation of decidual leukocytes

Decidual tissue was washed immediately after collection in cold, sterile HBSS with Ca^{2+}/Mg^{2+}. Tissue was then minced finely and flushed with $HBSS^{+/+}$ 3 times to ensure maximal release of leukocytes. The resulting suspension was filtered through 100 μm and 70 μm sieves and centrifuged twice at 700× g for 10 min at 4 °C to collect cells. Isolated cells were resuspended in RPMI-1640 + 10% FBS and incubated at 37 °C for 20 min to eliminate fibroblasts through differential attachment. Following incubation, remaining cells were passed through a 40 μm filter and incubated in erythrocyte lysis buffer Buffer EL (Qiagen, Toronto, ON, Canada) for 20 min at 4 °C. The final cell suspension was incubated in serum-free protein block (Dako, Burlington, ON, Canada) for 1 h on ice and diluted to a final concentration of 10^6 cells/mL.

Table 1
Clinical characteristics of maternal subjects.

	6–12 weeks ($n = 14$)	13–20 weeks ($n = 15$)
Maternal age (years ± SD)	24.2 ± 4.8	31.7 ± 10.0
Gravidity (median (range))	2.5 (1–7)	2 (1–5)
Parity		
Nulliparous	5	5
Multiparous	5	5
Unknown	4	5

Of 29 total subjects, data was not available for 9 patients (4 from 1st trimester, 5 from 2nd trimester).

2.3. Cell labeling and FACS analysis

Isolated cells were incubated with fluorochrome-conjugated mouse monoclonal antibodies in 200 μl staining volume for 45 min at 4 °C in the dark. Cells were washed in PBS and resuspended in 200 μl stabilizing-fixative buffer (BD Biosciences, Mississauga, ON, Canada) to prevent dissociation of tandem dyes and analyzed using a FACSAria flow cytometer (BD Biosciences, Mississauga, ON, Canada) and FlowJo software (Tree Star, Ashland, OR, USA). Antibody information is listed in Table 2.

2.4. Gating strategy

Non-leukocyte events were excluded from analysis using threshold gates set on CD45 fluorescence. Cell debris and aggregates were excluded by gating on forward vs. side scatter, then by height vs. width of forward and side scatter plots prior to $CD45^+$ population discrimination (Supplementary Fig. 1). Positive leukocyte subpopulations were identified by comparison of fully-stained samples to FMO (fluorescence-minus-one) controls (Supplementary Fig. 2).

Table 2
Antibodies and combinations used for leukocyte population discrimination.

Antigen	Clone	Isotype	Fluorochrome	Dilution	BD Cat.#
CD45	2D1	Ms: IgG_1,κ	APC-Cy7	1:50	557833
CD3	UCHT1	Ms: IgG_1,κ	Alexa Fluor 488	1:25	557694
CD4	RPA-T4	Ms: IgG_1,κ	APC	1:20	555349
CD8	HITa	Ms: IgG_1,κ	PE	1:40	555635
CD25	M-A251	Ms: IgG_1,κ	PE-Cy5	1:25	555433
CD45	–	–	–	–	–
CD56	B159	Ms: IgG_1,κ	PE-Cy7	1:200	557797
CD16	3G8	Ms: IgG_1,κ	PE	1:40	555407
CD45	–	–	–	–	–
CD14	M5E2	Ms: IgG_{2a},κ	PE-Cy7	1:30	557742
CD163	GHI/61	Ms: IgG_1,κ	PE	1:20	556018
CD206	19.2	Ms: IgG_1,κ	APC	1:15	550889
HLA-DR	G46-6	Ms: IgG_{2a},κ	PE-Cy5	1:40	555813
CD45	–	–	–	–	–
CD14	–	–	–	–	–
CD83	HB15e	Ms: IgG_1,κ	APC	1:20	551073
CD205	MG38	Ms: IgG_{2b},κ	PE	1:40	558069
CD209	DCN46	Ms: IgG_{2b},κ	PerCP-Cy5.5	1:40	558263

Please cite this article in press as: Kwan M, et al., Dynamic changes in maternal decidual leukocyte populations from first to second trimester gestation, Placenta (2014), http://dx.doi.org/10.1016/j.placenta.2014.09.018