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Research Article

Localization of Myeloid Cells in the Basal Plate of Term Placentas from Uncomplicated Gestations

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ABSTRACT

Decidual macrophages are in close contact with trophoblast cells during placental development, and an appropriate cross talk between these cellular compartments is crucial for a successful pregnancy. During the phases of gestation, macrophages undergo dynamic changes to adjust to the different stages of fetal development, involved in immune tolerance, trophoblast invasion, tissue and vascular remodeling, embryo growth, and initiation of parturition.We aimed to identify and localizemyeloid cells in the basal plate of term placentas from uncomplicated gestations. We found that antigen presenting cells, which express MHC class II, are localized throughout the basal plate without apparent selective localization adjacent to spiral arterioles. We also showed that most MHC-II$^+$ cells express CD14, characterizing them as monocyte/macrophage-like cells. We speculate that the MHC II$^+$/CD14$^+$ macrophages are key players of the immunological responses typical of the basal plate interface of term human placentas.

INTRODUCTION

Pregnancy uniquely challenges the maternal immune system. A healthy pregnancydepends on remarkable immunological changes that allow acceptance of the fetal allograft, as well as multiple metabolic and hormonal adaptations. The female reproductive tract must balance protection against pathogenic infections and inflammatory processeswith the necessary acceptance offoreign antigens of paternalorigin to support implantation andpregnancy related development of the conceptus.Immune function in a successful pregnancy is neitheran overtinflammatory nor immunosuppressive condition but instead, is a complex seriesoftightly controlled immune modulations. The characteristics of this modulation changes systemically, and most notably, *in utero* at the maternal-fetal interface during the course of pregnancy [1-3], especially through antigen presenting cells (APCs; [4]). APCs present antigenic peptides to T lymphocytes through MHCclass II surface molecules [5].The secretory response of T lymphocytes will differ depending on the antigens presented, yielding both co-stimulatory and co-inhibitory cytokines, chemokines, and growth factors. A pivotal player in maternal tolerance is the human chorioallantoic placenta, which physically interfaces with the mother's decidual uterine surface and with the fetus

contained within the chorioamnion membranous sac. Placental villi evolve to transfer nutrients to the fetus, dispose fetal wastes, and secrete hormones into the maternal blood that sustain pregnancy. The site of blastocyst implantation in the uterus also undergoes remarkable changes, including decidualization, invasion of immune cells, and prominent penetration of Extravillous Trophoblast (EVT) from the tips of chorionic villi. The EVT penetrates the decidual extracellular matrix,and modify the maternal spiral arterioles into dilated funnels, with vascular smooth muscle depleted from their walls and replaced by hypocellular fibrinoid [7]. By the end of the first trimester, this area of intimate maternal-fetal contact to the uterine decidua is called the Basal Plate (BP), to distinguish from the fetal-facing chorionic plate of the placenta.The BP surface in the delivered placenta offers an accessible source of tissue for the study of cellular relationships and immune expression [9,10]. We previously identified a subset of $CD14^+$ MHC^+ myeloid cells with immune regulatory signature in human placenta. Now, we further advance knowledge of these cells, characterizing their location in the critical maternal-fetal interface.

MATERIALS AND METHODS

Placenta acquisition and tissue preparation

This study was approved by the Institutional Review Board of the Washington University School of Medicine in St. Louis, MO, and written informed consent was obtained from all participating women. Placentas were obtained from normal singleton gestations at 37 - 40 weeks, after uncomplicated antenatal courses. Deliveries were by cesarean section under conduction anesthesia without labor, or vaginal delivery after spontaneous onset of labor whose duration was < 6 h. Placentas were retrieved in the delivery room, kept at 4°C until sampling, with an average time between delivery and sampling of 60 min (range,30 to 120 min). The BP was submitted to systematic random sampling, according to recommended procedures for placental sample collection [8]. Five to 10 *en face* sections of Basal Plate (BP) tissue were dissected with dimensions ~1.5 cm^2 surface area and < 0.5 cm thickness, fixed in 10% formalin for 24 h, embedded in paraffin,and cut into 5 µm-thick sections for Immunohistochemistry (IHC) or Immunofluorescence (IF).

Immunohistochemistry and Immunofluorescence

De-paraffinized sections of BP tissue were stained with Hematoxylin and Eosin (H & E) or used for Immunohistochemistry (IHC), employing anti-MHC class II (1:500, IgG1, mouse monoclonal anti-MHC class II, 3.8B1, PMID:9151699), with secondary antibodies and antigen detection done using the R.T.U. Vectastain Universal Elite kit with ImmPACT DAB substrate (Vector labs, Burlingame, CA), followed by hematoxylin counterstaining. MHC class II quantification was performed using Image J software (NIH), considering 3 slides per placenta, 10placentas, 10 fields randomly photographed and grid placed for point counting. Sections for Immunofluorescence (IF) were stained by monoclonal anti-mouse (1:200 MHC) class II (IgG1- 38B1) and anti-rabbit (1:200) anti-CD14 (Sigma Chemical Co., St. Louis, MO, #141103LVI) and anti-cytokeratin-7 (1:1000).

RESULTS AND DISCUSSION

The chorionic plate (Figure 1A,B) is surfaced by umbilical cord derived fetal vessels which course over the surface to penetrate the chorioallantoic placental villous trees. The basal plate site of attachment to the decidua in the delivered placenta is characterized by a series of rounded, indented, demarcations called cotyledons, which each consist of a main stem and branches of a chorionic villus. The latter is surfaced by a trophoblast bilayer whose basement membrane delimits the villous core connective tissue through which fetal vessels pass. Tissue sampling in the BP was designedto include cotyledonary regions randomly. The histology of the area is shown in Figures 1C-F. There are spiral arterioles penetrating the BP (Figure 1C-F), carrying maternal blood to perfuse the cotyledon's intervillous space to bathe villi with nutrient-rich maternal blood. The spiral arteriole walls are characteristically thin, without the abundant smooth muscle typical of the spiral arterioles of the cycling non-pregnant uterus. EVTs are embedded in the vessel walls (Figure 1C-F), in the extracellular matrix, and sometimes lining the lumen of the modified spiral arterioles. Many of the EVT cells are in close proximity with not only decidual cells but the multiple phenotypes of immune cells distributed among the extracellular matrix. Glandular structures (Figure 1C,D) are present and secretory products from the glands continue to be secreted into the decidua throughout gestation.

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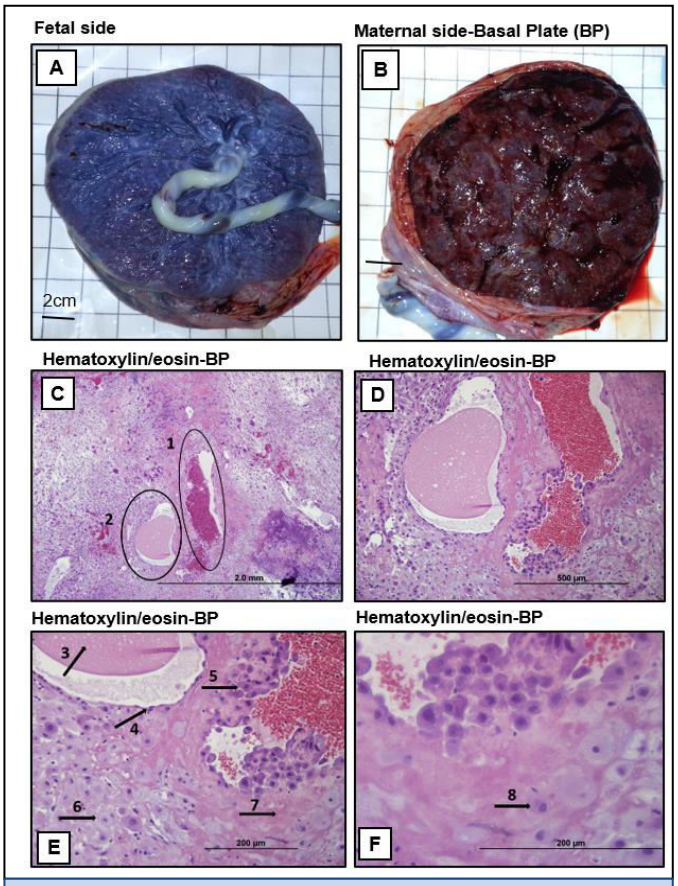

Figure 1: Gross (A & B) and basal plate histology (C - F) from a placenta delivered after an uncomplicated pregnancy.The gross appearance distinguishes the surface exposed to the fetus (A) and that attached to maternal decidua, the BP (B). The region of the BP sampled is noted within the outlined area. A 5mm *en face* section of basal plate tissue was stained with hematoxylin and eosin and examined by light microscopy. The C and D panel shows a 100X magnification and the E and F panel is a 400X image. Indicated are spiral artery (1); gland (in the Decidua) (2); mucin glycogen (3); epithelial cells (look flat due to pressure from secretion) (4); trophoblast (5); decidual cell (6); fibrin (comes from degenerating vessels; red blood cells) (7) and macrophage (8).

Multiple studies have explored the assessment of immune cells and the differences in numbers of selective cellsat the maternal-fetal interface are implicatedin deregulation of immune balance to lead to sub-optimal outcomes, such as growth restriction or preeclampsia [11-14]. Our group has characterized two distinct subsets of myeloid cells from the placental BP of term, uncomplicated pregnancies, with different pro-inflammatory and regulatory capacities despite similar phenotypes. Whereas one subset predominantly produces the immune-modulating cytokine IL-10, the other subset had increased capacity to secrete pro-inflammatory mediators, such as IL-1β and IL-6 [15]. We studied the localization of MHC class II^+ myeloid cellsand found that theywere distributed throughout the basal plate (Figure 2) without apparent selective localization adjacent to spiral arterioles and in close contact to trophoblasts (Figure 2 E,F). Around 7% of the tissue volume was occupied by MHC class II cells based on morphometric analysis (Supplementary Figure). Many of the MHC class II^+ cells co-localized with $CD14^+$ cells, indicating they were from the monocytic/macrophagic lineage (Figure 3).

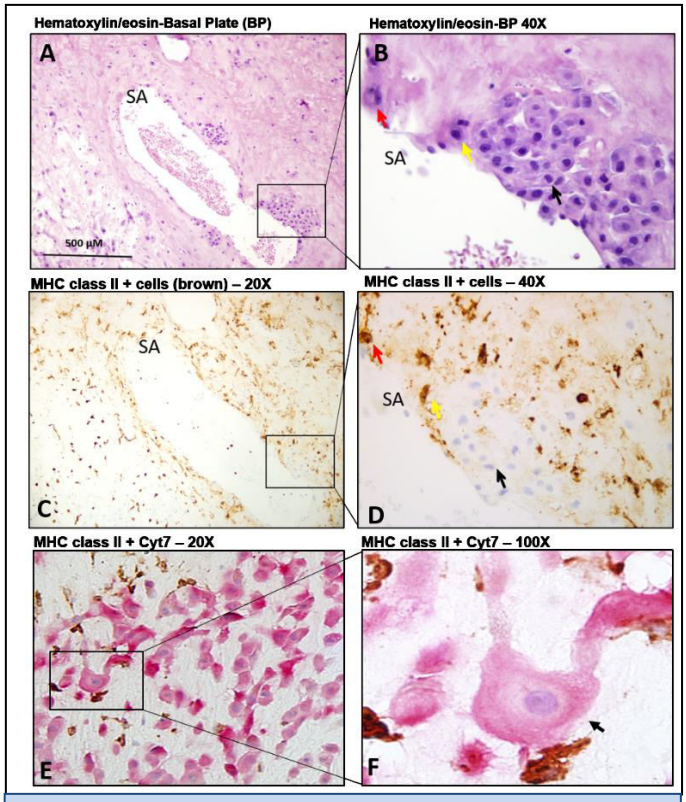

Figure 2: Immunohistochemistry of Antigen Presenting Cells (APC) in human placental basal plate. H/E staining, MHC class II positive cells and cytokeratin 7 positive cells. Sequential sections of formalin-fixed, paraffin-embedded basal plate tissue from a term placenta of an uncomplicated pregnancy were stained with hematoxylin and eosin (H/E; A&B), primary antibody to MHC class II followed by DAB detection (brown; C &D) and primary antibody to cykeratin7 followed by DAB detection (E & F). Right panel is the higher magnification (40X in B and D; 100X in F) of boxed area, identifying spiral artery (SA), extravillous trophoblasts (black arrow), decidual cells (yellow arrow) and immune cells (red arrow).

Confirmation of the co-localization of MHC class II immunogenicity and CD14 positivity is shown by IF staining using different fluorescent probes for each antigen (Figure 4), and the cells colocalizing were closely associated, if not touching, adjacent cells that were neither CD14 nor MHC class II positive (Figure 3,4).

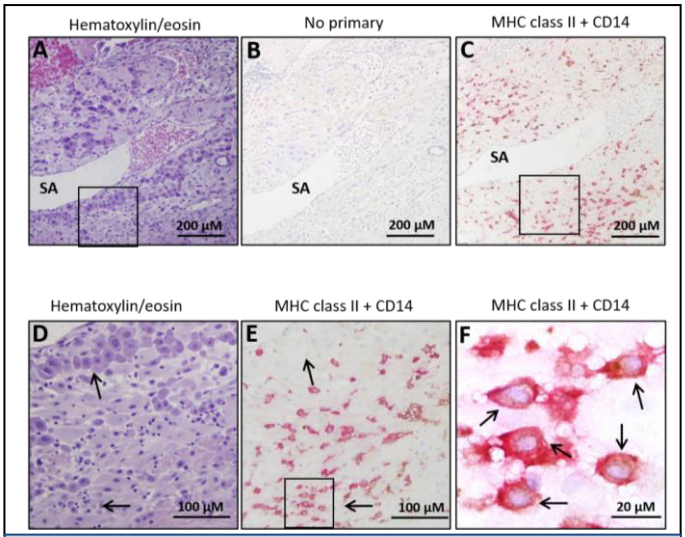

Figure 3: Immunohistochemistry of antigen presenting cells (APC) in human placental basal plate. H/E staining anddouble staining CD14 and MHC class II - showing co-localization. Sequential sections of formalin-fixed, paraffin-embedded basal plate tissue from a term placenta of an uncomplicated pregnancy were stained with hematoxylin and eosin (H/E; A & D), no primary antibody-control (B), or primary antibody to MHC class II followed by DAB detection (brown) and CD14 followed by AP detection (Red;C,E, & F). Panels D and F show boxed regions marked in A and C at higher magnification. H/E staining shows the morphology of the basal plate, with a spiral artery (SA) and different cell types pointed in magnification (D), trophoblasts in upper arrow and immune cells in lower arrow. CD14 and MHC class II positive cells are abundant in the maternal basal plate, and mostly co-localize, as noted by arrows in panel F.

According to Zhang [16,17], macrophages represent up to 25% of the total decidual leukocytes and the predominant subset of APCs at the maternal–fetal interface. Our data show these macrophages are in close proximity to the EVT andare adjacent to spiral arteries. Macrophages are involved in several processes required for a successful pregnancy, including immune tolerance, trophoblast invasion, tissue and vascular remodeling, embryo growth, and initiation of parturition [18]. Allthese distinct roles are possible because of macrophage plasticity and heterogeneity. Macrophages polarization can skew towards a continuum within a large spectrum between the classically activated M1and the alternatively activated M2 subsets, which simplistically elicit Th1 and Th2 responses, respectively [19-21]. M2 macrophages, however, have been further classified as M2a, M2b, M2c and M2d based on their regulatory proprieties and the mediators involved in their polarization and function [22].

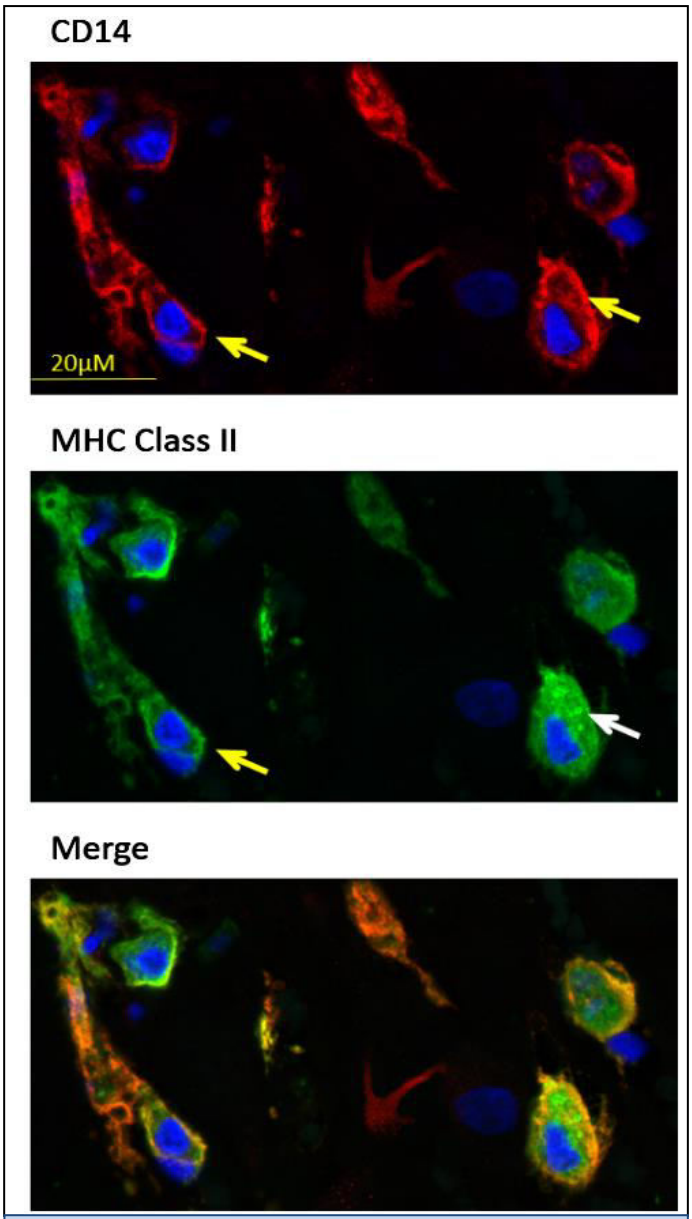

Figure 4: Immunofluorescence of Antigen Presenting Cells (APC) in human placental basal plate. Patterns of CD14 (red) and MHC class II (green) positive cells in placental basal plate tissue, as detected by immunofluorescence and confocal microscopy. Tissue was co-stained for DNA (blue), CD14 (red) and MHC class II (green). The lower panel presents merged image, with co-localization of positive CD14 and MHC class II cells. Arrows point to patterns within positive cells, denotinglocalization to the plasma membrane (yellow arrows)orpunctate, cytoplasmic staining(white arrows).

Decidual macrophages are therefore a heterogeneous population with multiple phenotypes that facilitate adaptive responses to the ever-changing environment. Interestingly, decidual macrophages do not completely fit the criteria for either the M1 orM2 subsets [23]. However, some studies have suggested that M2 macrophages, or M2 subgroups, are the

predominant phenotype in the decidua [24], a sub-set associated with the support for trophoblast invasion and placental growth [25]. In fact, macrophages polarization is driven by the local environment milieu, which changes throughout gestation and therefore, finely tunes the local immune response. M2-like macrophages are increased in number as pregnancy progresses, being responsible for the maintenance of tolerance to fetus, as well as contributing to the development and function of the placenta. Parturition, on the other hand, is characterized by an inflammatory process, with abundance of M1 macrophage in the decidua of laboring tissue [26].

Pregnancy is a dynamic and highly regulated immunologic process [27], with costimulatory interactions of decidual T cells with surrounding pregnancy-related MHC class II/CD14 positive cells and EVT cells in modulating maternal immune responses. More study of the macrophages now identified at the maternal-fetal interface is needed to understand how defects in reproductive immunology, such as a defect in supporting macrophage polarization, might lead to dysregulation of the immune system and pregnancy complications such as preeclampsia and preterm birth [28,29].

CONCLUSION

In the present study, we showed the localization of APCs, MHC class II^+ cells, in placenta of uncomplicated gestations. We also found that most MHC class II^+ cells also express CD14, which shows that most of APCs in placenta are of the monocytic/macrophagic linage.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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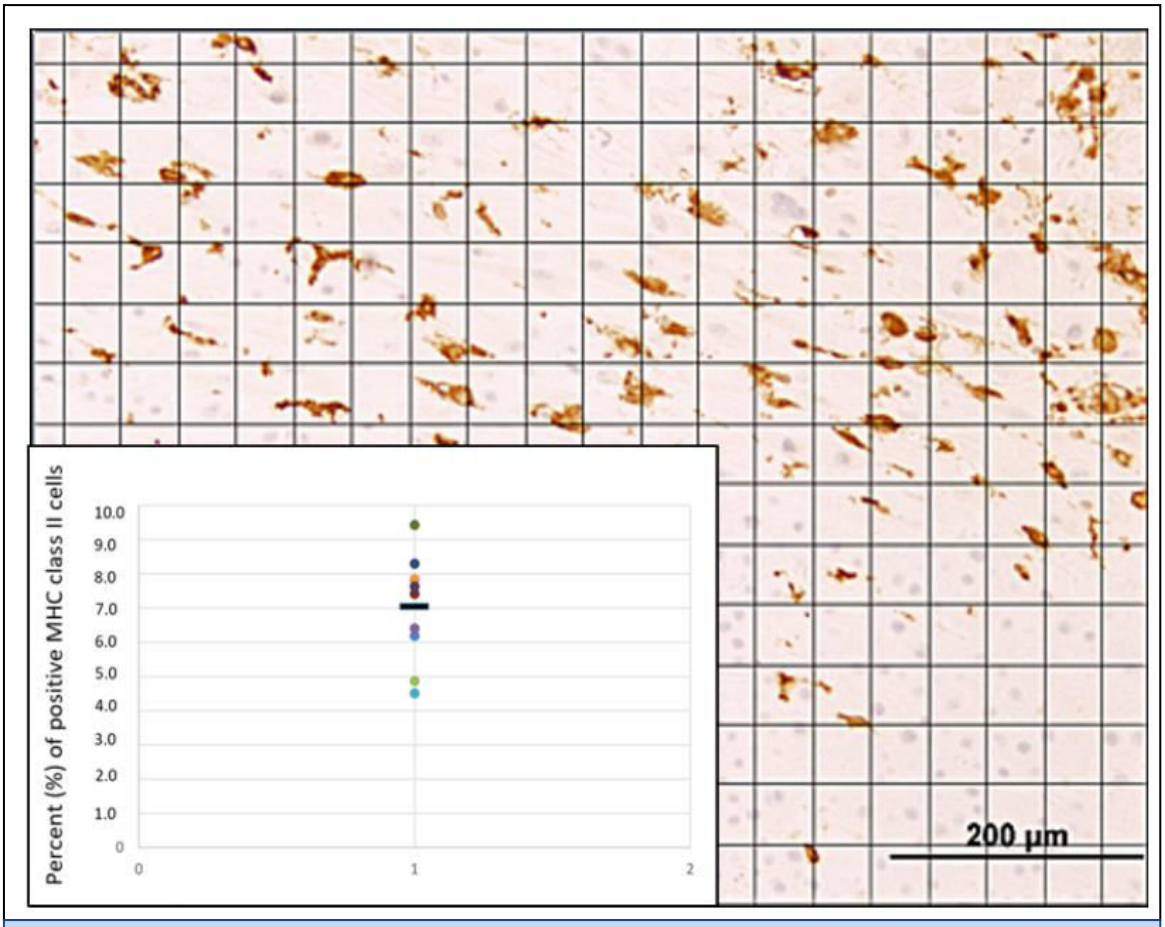
SUPPLEMENTARY FIGURE



Quantification of MHC class II cells in the placenta basal plate of term, uncomplicated pregnancies: MHC class II quantification was performed using ImageJ software (NIH), considering 3 slides per placenta, 10 placentas, 10 fields randomly photographed, and grid placed for point counting (one example represented in the figure). Immunohistochemistry with primary antibody to MHC class II followed by DAB detection (brown). The boxed area presents results on quantification. Each colored dot represents mean values for individual placentas. The mean percent of MHC class II positive cells is marked and represents 7.0% of the considered tissue.