Decidual Macrophages and Their Roles at the Maternal-Fetal Interface

Brandy L. Houser

Stem Cell and Regenerative Biology Department, Harvard University, Boston, Massachusetts

The semi-allogeneic fetus, whose genome consists of maternally and paternally inherited alleles, must coexist with an active maternal immune system during its 9 months in utero. Macrophages are the second most abundant immune cell at the maternal-fetal interface, although populations and functions for these populations remain ill defined. We have previously reported two distinct subsets of CD14+ decidual macrophages found to be present in first trimester decidual tissue, 20 percent CD11cHI and 68 percent CD11cLO. Interestingly, CD11cHI decidual macrophages express genes associated with lipid metabolism, inflammation, and antigen presentation function and specifically upregulate CD1 molecules. Conversely, CD11cLO decidual macrophages express genes associated with extracellular matrix formation, muscle regulation, and tissue growth. The large abundance of CD11cHI decidual macrophages and their ability to process antigens more efficiently than CD11cLO macrophages suggests that CD11cHI macrophages may be important antigen processing and presenting cells at the maternal-fetal interface, while CD11cLO macrophages may perform necessary homeostatic functions during placental construction. Thus, macrophage heterogeneity may be an important and necessary division of labor that leads to both an induction of maternal immune cell tolerance to fetal antigens as well as basic homeostatic functions in human pregnancy.

Keywords: pregnancy, reproduction, macrophages, antigen presenting cells, NK cells, lipids

To whom all correspondence should be addressed: Brandy L. Houser, Harvard University, Stem Cell and Regenerative Biology Department, 101 Huntington Avenue, Boston, MA 02115; Tele: 617-999-0213; Fax: 617-954-9361; Email: bhouser@partners.org.

†Abbreviations: APC, antigen presenting cell; CD, cluster of differentiation; DC, dendritic cell; dMϕ, decidual macrophage; dNK, decidual natural killer cell; EVT, extravillous trophoblast; FACS, fluorescence-activated cell sorting; HLA, human leukocyte antigen; IFN-γ, interferon gamma; Ig, immunoglobulin; IL, interleukin; ITAM, immunoreceptor tyrosine-based activating motif; ITIM, immunoreceptor tyrosine-based inhibitory motif, KIR, killer Ig-like receptor; LPS, Lipopolysaccharides; MHC, major histocompatibility complex; MR, mannose receptor; PE, phycoerythrin; SA, scavenger receptor; TCR, T cell receptor; TLR, toll like receptor.
INTRODUCTION

Viviparity, or live birth, has evolved independently in several species. Eutheria, from the Greek for “well developed beast,” are a clade of viviparous mammals in which the fetus is nourished during gestation by a placenta [1]. The placenta consists of two basic elements: an inner vascular network and an outer epithelium [2]. The outer epithelium, comprised of trophoblast cells, provides the main structural and functional components of the placenta and allows for oxygen and nutrient exchange between mother and child. The inner vasculature and stroma are derived from embryonic mesoderm.

Placenta formation begins when fetal-derived trophoblast cells from the recently implanted blastocyst invade the uterine lining. Simultaneously, cells of the endometrium also begin to prepare for this invasion, in a process known as decidualization [3]. The mammalian chorioallantoic placenta is essential for the growth and development of the fetus and distinguishes Eutherian mammals from other organisms. There are three main types of Eutherian placentation: epitheliochorial, endotheliochorial, and hemochorial [4]. These distinctions are made based upon contact between trophoblast cells and the uterine lining. In epitheliochorial placentation, trophoblast cells can reach and sometimes fuse with the surface epithelium of the uterus, while in endotheliochorial placentation, trophoblasts can reach the maternal blood vessels [5]. Humans undergo hemochorial placentation, wherein fetal membranes are in direct contact with maternal tissue and blood (Figure 1). This intimate contact between the fetal-placental unit and mother was established in the last common crown group of Eutheria and gives credence that a successful pregnancy requires appropriate allore cognition and tolerance at the maternal-fetal interface [6].

THE HUMAN MATERNAL-FETAL INTERFACE

The maternal-fetal interface is a dynamic site that encompasses multiple cellular interactions in an environment rich in cytokines and hormones [7]. During the first trimester (weeks 1-12 post-fertilization), interstitial and endovascular infiltration of trophoblast cells elicit both the recruitment of maternal immune cells and the production of pro-inflammatory cytokines [8]. It is commonly thought that immune responses by the mother help to protect from trophoblast over-invasion while allowing for the acceptance of the semi-allogeneic fetal-placental unit.

Immunohistochemical staining against the leukocyte common antigen CD45 has shown that 40 percent of cells in the decidua during the first trimester are leukocytes [8]. An estimated 50 to 60 percent of decidual leukocytes are CD56\(^{bright}\)CD3\(^{-}\)NK cells [9]. The remaining leukocytic infiltrate is comprised of roughly 10 percent T cells, 1 to 2 percent dendritic cells (DCs\(^+\)), and 20 to 25 percent M\(\phi\)s [10]. The decidual macrophage (dM\(\phi\)) compartment consists of at least two distinct subsets based upon differential expression of the complement receptor CD11c and are now termed CD11c\(^{hi}\) and CD11c\(^{lo}\) [11]. Decidual leukocytes at the maternal-fetal interface play important roles in both allore cognition of fetal antigens and in the development of the fetal-placental unit.

IMMUNOBIOLOGY OF REPRODUCTIVE FAILURE IN HUMANS

Statistically, human pregnancy is remarkably inefficient. It has been estimated that approximately 50 to 60 percent of all human concepti die prior to birth [12]. The majority of these deaths occur before implantation; however, between 15 and 20 percent of otherwise successful embryo implants will result in an early spontaneous abortion [13,14]. Although pregnancy loss has been attributed to vague complications such as genetic, endocrinological, and anatomical abnormalities, the majority of miscarriages remain unexplained.

Haemolytic disease of the newborn was the first recognized immunological complication of human pregnancy [15]. This disease develops because the mother is
immunized by antigens on fetal erythrocytes from an earlier pregnancy. This leads to antibody-mediated haemolysis of the fetus in a subsequent pregnancy. These antigens are termed “Rhesus factor” (Rh) because early studies utilized red blood cells from Rhesus macaques [16]. These data contributed to Sir Peter Medawar’s development of the concept of maternal tolerance to the fetus, and he proposed three potential explanations regarding why the maternal immune system does not reject the fetus: physical separation of mother and fetus, antigenic immaturity of fetal tissues, and immunological inertness of the mother [17,18]. However, none of these three proposed concepts account for maternal tolerance to fetal antigens.

There are now several well-characterized immunological factors known to aid in fetal tolerance, including complement inhibitory receptors [19,20], absence of major MHC expression, expression of non-polymorphic non-classical presenting molecules [21], and cytokine balance. Although the fetal-placental unit is often equated to a vascularized allograft, classical allogeneic rejection of invading fetal cells is avoided because of the absence of MHC class II. However, there are several disorders, including tubal pregnancy, placenta accreta, and preeclampsia, that occur in part due to immune misregulation [5].

Preeclampsia occurs in as many as 10 percent of all human pregnancies [22] and is
the primary pathogenesis of inadequate invasion of extra-villous trophoblasts (EVT) and insufficient remodeling of the maternal-spiral arteries [23-25]. This ultimately leads to a lack of maternal blood flow into the intervillous space and manifests in the mother as proteinuria, edema, and hypertension. Although other factors have been shown to contribute to preeclampsia, it is generally thought of as an immunological manifestation of the misregulation of trophoblast invasion by maternal leukocytes [26]. Further evidence of immunological involvement in preeclampsia has been shown in cases where a primipara who had preeclampsia has a reduced risk with a change in partner [27]. In addition, in the case of oocyte donation in which the fetus is entirely non-self, the risk of preeclampsia is elevated to 30 percent [28].

Macrophages have been shown to play important roles in pregnancy maladies, including preeclampsia. In normal human pregnancies, dMφs are located in the surrounding stroma and near the spiral arteries; however, in preeclamptic pregnancies, the dMφs are mostly located within and around the spiral arteries and appear to physically inhibit trophoblast remodeling [29,30]. Moreover, it has been reported through ex vivo studies that dMφs can limit EVT invasion of spiral arteries through apoptosis mediated secretion of TNF-α [30,31]. In summary, Mφs at the human maternal-fetal interface play important roles during normal placental development, and the misregulation of these cells can result in pregnancy complications.

MACROPHAGE HETEROGENEITY

Peripheral blood monocytes give rise to Mφs, tissue resident phagocytic cells whose phenotype is specific to the tissue type. These are involved in tissue homeostasis via apoptotic cell clearance and the production of important cytokines, chemokines, and growth factors [32]. They are foremost prodigious phagocytic cells that clear more than 2X10¹¹ erythrocytes per day, recycling iron as a necessary homeostatic process [33]. Moreover, Mφs are the dedicated janitors of the body, clearing cellular debris from effete cells during tissue remodeling processes without eliciting an immune response. Phagocytosis of cellular components, along with varying environmental cues, may lead to Mφ plasticity [34]. Such plasticity generates different types of Mφs possessing distinct phenotypes and functions.

Emulating the Th1/Th2 nomenclature, polarized Mφs have been broadly categorized as either pro-inflammatory (M1) or anti-inflammatory (M2) [35]. It is well established that classically activated M1 Mφs are potent inducers of IL-1, TNF-α, and IL-12 following stimulation by a microbial antigen or pro-inflammatory cytokines [34]. Originally described as Mφs activated by IL-4, alternatively activated Mφs or M2 Mφs have been described as anti-inflammatory mediators [36,37]. Polarization of the M2 phenotype can be induced not only with IL-4, but also with immune complexes, IL-10, glucocorticoid, or sex steroid hormones [38]. The M1/M2 paradigm has been comprehensively studied by transcriptional approaches, which defined skewed gene expression based on the stimulus used to generate each type of macrophage [34,39]. Recently, the M1/M2 paradigm has come under scrutiny, as tissue resident Mφs are neither M1 nor M2 [11,40]. Thus, since Mφs are uniquely plastic cells, it has been proposed to define Mφs based on functions that are involved in maintaining homeostasis, such as host defense, wound healing, and immune regulation [33].

Interestingly, Mφ plasticity plays important roles not only in homeostasis and infection but also in cancer. As early as the late 1970s, it was found that tumor growth was promoted by tumor-associated macrophages (TAMs), a predominant leukocyte population present in tumors with poor prognosis for therapeutic outcome [41-43]. TAMs are different from Gr1+ myeloid-derived suppressor cells (MDSCs) in mice, which are a heterogeneous population of cells of the myeloid lineage also associated with cancer [44]. However, because their defining marker, Gr1, is only found in mice and has no homologue in humans, the precise characterization...
| Macrophage type | Characteristics and Hallmarks | Function | Humans/Mice | Reference |
|-----------------|-------------------------------|----------|-------------|-----------|
| M1 Mφ           | TLR2/4, CD16/32/64, CD80/86, TNF-α, IL-1, IL-6, CCL2 | Microbicidal activity, clearance of pathogen, pro-inflammatory | Humans | [34,38] |
| M2 Mφ           | SRA/B, MR, CD163, CD23, IL-10, CXCR1, CXCR2, CCL22/24/17 | Anti-inflammatory, immune regulators, tissue repair | Humans | [35-38] |
| TR- Host defense | Induced by Th1 or NK cell production of IFN-γ or other TLR-stimulated APC TNF-α production | Classical activation, microbicidal activity | Humans/Mice | [33] |
| TR- Wound healing | Induced by Th2 or granulocyte production of IL-4 | Wound healing | Humans/Mice | [33] |
| TR- Immune regulation | Induced by TReg IL-10 production or IC, prostaglandins, GPCR ligands, glucocorticoids, apoptotic cells | Anti-inflammatory activity | Humans/Mice | [33] |
| Tumor associated macrophage (TAM) | Found in cancers, produce angiogenic and lymphoangiogenic factors including VEGF production | Neoplastic assisted growth development | Humans/Mice | [43,45,47] |
| Myeloid Derived Suppressor Cell (MDSC) | A heterogeneous population of myeloid lineage cells, CD11b+Gr1+ or CD11b+CD14-CD33+, potent suppressors of T cells | Accumulate in lymphoid organs and in tumors in pathological conditions, notably cancer | Mice/Humans (to some degree) | [44] |
| CD11c^{hi} dMφ | CD206^{low}, CD209^{low}, lipid metabolism, inflammatory markers, antigen presentation function, mobile | Antigen presentation and immune regulation | Humans | [11] |
| CD11c^{lo} dMφ | CD206^{hi}, CD209^{hi}, extracellular communication, large phagolysosomes | Clearance of cellular debris and effete cells during tissue remodeling processes | Humans | [11] |
and role of MDSCs in human infections and cancer remains ill defined. TAMs, however, have been found to aggregate in the hypoxic regions of tumors, thereby promoting hypoxia-driven programs including angiogenesis, TNF-α and TGF-β production, and CXCL8 secretion [45]. Other chemokines including CXCL1 and related molecules and CCL2 have been associated with TAM accumulation [42]. Although TAMs have been most closely associated to the M2 phenotype, they are known drivers of chronic inflammatory processes that promote epithelial hyperproliferation, tissue remodeling, and angiogenesis. Ultimately, this is followed by dysplasia and invasive carcinoma [46]. Moreover, their production of TNF-α and IL-1β strongly indicates their regulation of cellular metastasis [47].

TAMs, as well as MΦs in general, also have been shown to have a role in the connection between sex steroids and inflammation in the promotion of cancer [48]. One study focused on gender disparities in hepatocellular carcinoma, a cancer to which males are more susceptible [49]. This study found that liver MΦs in males produce higher levels of IL-6 during carcinoma development following induction via the hepatitis virus. Furthermore, carcinoma development was inhibited in males that were IL-6 deficient. Interestingly, females produce higher levels of estrogen steroid hormones, which were found to inhibit IL-6 production in liver MΦs and thereby protect them from the development of cancer. This study emphasizes the link between sex-steroids, inflammation, and MΦ regulation in the development of some cancers.

Hormones in tissue play important roles in the development of tissue-resident MΦs. For example, glucocorticoids, which are released by adrenal cells in response to stress, have been shown to inhibit MΦ-mediated host defenses via the production of pro-inflammatory cytokines, leading to increased susceptibility to pathogen infections [50]. However, other MΦ functions, such as phagocytosis, are not impaired in the presence of glucocorticoids [51], suggesting that glucocorticoids can directly impact tissue-resident MΦ immune regulation. Progesterone, an important hormone during the menstrual cycle and pregnancy, has been shown to inhibit MΦ production of TNF-α in a pre-transcriptional manner [52] as well as IL-12 induced nitric oxide (NO) production in response to TLR4-mediated agonists [53]. Thus, it is conceivable that pregnancy-specific hormones may directly contribute to the rise and frequency of dMΦ populations and their responses to fetal-derived or pathogen-derived antigens.

Environmental cues can lead to MΦ plasticity and give rise to different populations of MΦs that occur at various frequencies. MΦs at the maternal-fetal interface experience a shift in hormonal production both locally and systemically, encounter potential pathogens, clear effete cells, and notably interface and respond to non-self invading trophoblast cells [54]. The variety of environmental cues during placental development can lead to uniquely diverse, in both phenotype and function, dMΦ populations in order to maximize different necessary processes (Table 1). In fact, we have now described two distinct dMΦ populations at the maternal-fetal interface [11].

DECIDUAL MACROPHAGES AS ANTIGEN PRESENTING CELLS

The large abundance of MΦs and near absence of DCs [55] suggests that dMΦs may be the most important antigen processing and presenting cells at the maternal-fetal interface. dMΦs, as a professional APC, may be important in regulating both adaptive T cell responses as well as innate NK cell responses at the maternal-fetal interface during early human pregnancy.

MΦs are equipped with a recognition system for a host of different pathogen-associated molecular patterns (PAMPs) and are specialized in the initial capture and processing of these potential antigens. They are also important in the development of an adaptive immune response [56]. Although MΦs are known for their powerful phagocytic and endocytotic capacities, antigen presentation, by default, is left to the DC.
This may be in part because their high levels of lysosomal proteases completely degrade engulfed antigens, a property not shared with their DC counterparts [57-59]. It has now been shown, however, that through Mφ activation, early phagosomes actually have limited proteolysis and can effectively generate epitopes and present antigens, while late phagolysosomes maintain highly degradative capacity [60].

Tissue-resident APCs must be simultaneously capable of initiating a T cell response to invading pathogens while avoiding the risk of prematurely priming T cells to seemingly innocuous events such as apoptosis and cellular turnover. During pregnancy, where the fetus is only partially derived from its mother, this delicate balance is necessary for fetal survival. It is known that the maternal adaptive immune system can recognize paternal antigens, including anecdotal evidence from women who had multiple miscarriages but upon a switch in partner they were successfully able to carry a child to term [61]. In addition, mixed lymphocyte reactions have shown that there is maternal immune cell suppression of fetal or paternal antigens during pregnancy [62]. Another study also demonstrated that female mice can accept an allogeneic tumor of paternal origin during the course of their pregnancy, but will reject the same tumor if pregnant with a “third-party” father [63].

Although most studies emphasize the role of suppression or regulation of T cell responses, very few have focused on the role of the APC. An elegant study done in mice demonstrated that DCs lose their ability to migrate to draining lymph nodes following decidualization and are consequently retained within the uterus [64]. Both mouse and human endometrium are largely lacking in lymphatic vessels; however, during human decidualization, there is heightened lymphangiogenesis that is not seen in mice [65]. This suggests that, in humans, if APCs are capable of migrating to draining lymph nodes, they could be equipped with potent signals to alert effector T cells, emphasizing the importance of a non-migratory APC, such as the Mφ at the maternal-fetal interface.

We have now demonstrated that there are two distinct subsets of dMϕs found in the early human placenta that can be separated based upon CD11c expression and are termed CD11c^{hi} and CD11c^{lo} [11]. Interestingly, CD11c^{hi} dMϕs were more efficient at protein antigen processing and express genes consistent with APC function, including elevated levels of lipid-antigen presenting molecules such as CD1a, CD1c, and CD1d compared to CD11c^{lo} dMϕs.

Although CD11c, a complement receptor, is often exploited as a single marker to track murine DCs, all human monocytes express CD11c and may retain protein expression following tissue extravasation. Furthermore, expression is maintained during DC or Mφ differentiation processes [66] and therefore cannot be utilized in the same way to differentiate between human DCs and Mϕs [67]. The similarities between murine CD11c^{+} decidual DCs and human CD11c^{hi} dMϕs are apparent based upon antigen processing and presentation capacity ([11] and LG, BLH, JLS manuscript in preparation). However, murine CD11c^{+} decidual DCs are capable of migrating into the draining lymphnodes during pregnancy and are phenotypically distinct from F4/80^{+} Mϕs within the murine decidua tissue [68]. Moreover, both CD11c^{hi} and CD11c^{lo} dMϕs are phenotypically and functionally macrophages and are equivalently capable of phagocytosis [11]. CD11c^{hi} dMϕs are found in abundance compared to CD14^{+} HLA-DR^{+} DCs [69], which comprise less than 1 percent of the immune cell compartment at the maternal-fetal interface [55]. These differences in abundance suggest that although there may be overlapping functions, they are likely to be performing specialized and distinct functions. However, due to the low numbers of human decidual DCs, experimentation, and therefore a greater understanding of their function, is extremely difficult.

**LIPID ANTIGEN PRESENTATION**

Lipids are important for normal homeostasis, including wound healing, growth
and hormone production. Blood lipid concentrations are elevated during pregnancy, presumably for necessary fat storage and fetal supply of fatty acids [70]. They are also important for the development of a variety of hormones, which play integral roles during pregnancy. It is therefore important that phagocytic immune cells of the placenta are capable of recognizing lipids derived from cellular debris of effete decidual and trophoblast cells versus lipids derived from bacterial pathogens that threaten the health of the mother and fetus.

Lipids are not water soluble and are therefore always associated with membranes or lipid-binding proteins, thus making the immunogenicity of lipids different from that of peptides. Lipids are transported throughout the body in complex with apolipoproteins, internalized via the LDL receptor, and delivered to the endocytic compartment, where the lipid-protein complex is dismantled and distributed according to cellular need [71]. Other receptors, such as scavenger receptor A (SR-A), lectin-type oxidized LDL receptor 1 (LOX1 or OLR1), CD36, and other C-type lectins, can also bind modified forms of LDL, including that expressed by apoptotic cells [72]. This suggests several potential routes of entry for a range of environmental lipid antigens.

Several lipid antigens that have been characterized are either of bacterial or somatic origin. Generally lipid antigens are found as either glycolipids or lipo-peptides and are differentially distributed among endocytic compartments [72]. Because different types of lipids are sorted into different endocytic compartments, it has been proposed that CD1 trafficking evolved to sample the most appropriate lipid-containing compartment [73]. CD1 molecules are genetically non-polymorphic cell-surface glycoproteins that present glycolipids and lipo-peptides [73]. CD1 genes have a similar intron/exon structure to MHC class I genes and encode integral membrane proteins consisting of three α helices and an associated β-2 microglobulin domain [74,75]. The α3 domain is the most similar between all of the CD1 molecules, but is not completely homologous, to the MHC class I α3 domain [76]. Loaded CD1 molecules that reach the surface will be capable of presenting to T or invariant TCR NK (iNKT) cells. TCRs recognizing group I CD1 loaded with microbial antigens have highly diverse TCR α and β chains, with a level of heterogeneity similar to that of peptide-recognizing TCRs [77].

Based upon protein sequence, CD1 isoforms can be classified into three groups: group 1, which is comprised of CD1a, CD1b, and CD1c; group 2, which is comprised of CD1d; and group 3, which is comprised of CD1e [72]. Humans express all CD1 isoforms, but these are generally restricted to DCs and other professional APCs. It has been shown that CD1a, expressed by Langerhans cells, is able to efficiently present antigens to CD1a-restricted T cells [78]. Dermal DCs and interdigitating DCs in lymph nodes express CD1b [79,80]. CD1c is largely expressed on B cell subsets, including lymph node mantle zones and germinal centers, in marginal zone B cells of spleen and on a subpopulation of B cells in adult and fetal peripheral blood [81-83].

Moreover, human CD1a, CD1b, CD1c, and CD1d are all expressed by DCs, but appear at different stages of the monocyte-DC differentiation process [84]. Different expression patterns of CD1 on the cell surface, early and late endosomal compartments, lead to different rates of internalization into endosomes [85], suggesting that each CD1 isoform may have a distinct role in the immune response [73,86]. The different patterns of CD1 expression are not completely understood. In vitro studies using monocyte-derived DCs have demonstrated that differing amounts of IgG in tissues can direct CD1 expression profiles, an effect shown to be mediated by FcγRIIa on myeloid cells [87]. Also, Leslie and colleagues demonstrated that lysophosphatidic acid and cardiolipin, lipids in normal human serum, are modulators of CD1 expression via peroxisome proliferator-activated receptor (PPAR) nuclear hormone receptors [88].

Placental lipids remain ill defined, and their potential role in dMϕ expression of
CD1 has yet to be characterized. However, CD1 expression and lipid trafficking may play currently unknown roles at the human maternal-fetal interface. Recent observations from our lab found that CD1a and CD1c molecules on the surface of CD11c+ dMφs are functionally capable of presentation to clonal T cell lines (LG, BLH, and JLS manuscript in preparation). These data, along with the observation that there are CD1 autoreactive decidual T cell clones, lends further credence to the possibility that placental lipids and CD1 presentation may contribute to maternal-fetal immunotolerance. These observations may help to better understand lipids in pregnancy and in other inflammatory processes.

**DECIDUAL MACROPHAGES AND NK CELL CROSS TALK**

NK cells were originally characterized based on their innate cytolytic capacities, which, unlike cytotoxic T cells, can directly induce death of tumor cells or virus infected cells [89]. NK cells are also integral cytokine producers in both physiological and pathological conditions. Although NK cell cytotoxic responses directly impact infected cells, it is now thought that NK cell cytolytic and cytokine responses can also regulate antigen specific adaptive immunity via APC priming and cross presentation [90].

NK cell function is based upon fine-tuning of cell surface receptors that activate or inhibit their responses [91]. These receptors signal through corresponding secondary molecules that express immunoreceptor tyrosine activation motifs (ITAMs) or immunoreceptor tyrosine inhibitory motifs (ITIMs). Each NK cell has a particular repertoire of inhibitory and activating receptors on their surface [92]. NK cell activation can be induced by overexpression of activating ligands on cellular surfaces in the absence/reduced expression of inhibitory ligands. For example, NKG2D interacts with several ligands that can be upregulated in response to cellular duress, including DNA damage responses, and induces NK cell activation [93]. Alternatively, NK cells can respond to the absence of MHC class I surface expression (“missing self”) [94]. MHC class I can be down-regulated by virus infection or cellular transformation. NK cells can become activated because inhibitory ligands such as CD94/NKG2A that would normally recognize HLA-E or a variety of killer Ig-like receptors (KIRs) that recognize HLA-A, B, and C on the cell surface are missing, thereby tipping the balance between inhibitory and activating receptors, leading to NK cell activation [89,95]. These results suggest that NK cells in steady-state conditions with more inhibitory receptors are poised for recognition of missing self and therefore rapid clearance of MHC class I deficient cells, whereas NK cells with lower levels of inhibitory receptors are poised for mobilization in response to pathogen infections [89].

NK cells are the most abundant immune cell type at the maternal-fetal interface [9]. Decidual NK cells are all CD56bright CD16− and contain cytotoxic granules [96] but are unique compared to CD56bright peripheral NK cells [9]. In an autologous setting, healthy cells are spared from cytolysis due to a high expression of self-MHC [97]. However, at the maternal-fetal interface, trophoblast cells lack HLA-A and -B antigens, yet there is no NK cell cytolysis. This may be in part due to the fact that trophoblast cells express the minimally polymorphic HLA-C and other non-classical HLA molecules including HLA-E, -F, and -G [25] that are recognized by dNK cells. Although it is possible to envision that dNK cells contact trophoblast cells with an inhibitory synapse as opposed to an activating synapse, it has now been shown that dNK cells do in fact form an activating synapse with MHC I null cells but are unable to coordinate their microtubule organizing center (MTOC) with perforin-containing cytotoxic granules, thereby disabling them from killing their target [98]. Combined recognition of non-classical HLA molecules along with the inability to polarize granules may spare fetal trophoblast cells from dNK cell-mediated destruction.

The relatively large abundance of dNK cells may be a potent source of cytokines and
growth factors that are necessary for placental development. dNK cells are important regulators of trophoblast invasion during maternal vasculature reconstruction [99]. However, the induction of dNK cell activation as well as regulation of NK cell responses has yet to be characterized. One possible source of dNK cell regulations is the dMϕ population. dNK cells are in close proximity to CD14+ dMϕs or DC-SIGN+ dMϕs [100], implicating dMϕs as potential mediators.

Alternatively, it was found that in mice with MHC class I-negative tumors that CD11c+ DCs adoptively transferred promoted NK cell-dependent anti-tumor effects and that these effects were contact dependent [101]. These results suggest that APCs can regulate NK cell function in vivo. Furthermore, it has been shown that constant cross-talk between NK cells and immature DCs leads to DC maturation as well as an initial priming event in NK cells. This suggests that continuous conversations between innate immune cells can lead to enhanced innate and adaptive immune responses [102]. Interestingly, CD1 molecules presented on NK cell target lines that are HLA class I deficient are capable of inhibiting NK cell cytolytic responses [103,104]. Moreover, in the same system, it was demonstrated specifically that targets expressing CD1b pulsed with a known bacterial lipid antigen for CD1b, enhanced NK cell inhibitory effects. These results together suggest that dMϕs, or a specific population of dMϕs that are functionally close to DCs, might regulate the large abundance of dNK cells at the maternal-fetal interface. Moreover, this particular interaction may occur by non-traditional receptor-ligand interactions such as CD1 molecules. Thus, we propose that the innate plasticity of Mϕs may allow for environmental signals to give rise to distinct dMϕ populations that play specific roles in regulating both innate and adaptive immune responses at the human maternal-fetal interface.

**CONCLUSIONS**

Human pregnancy and hemochorial placentation challenges the conventional view of the regulation of immune recognition of foreign antigens. The understanding of how Mϕs, or populations of Mϕs, participate in the maintenance of fetal-placental tolerance could lead to a better understanding of how the innate immune system regulates both itself and the adaptive immune system in order to induce tolerance to what are non-self but non-pathogenic antigens. By characterizing these findings, it is possible that the mechanisms discovered could be exploited for the development of therapeutics and/or therapeutic strategies to alleviate human autoimmune and alloimmune disease/complications.

Macrophage heterogeneity plays important roles in the induction and cessation of inflammatory events, including those necessary at the human maternal-fetal interface. We propose that two distinct dMϕ populations, CD11cHI and CD11cLO, allow for integral processes to be done in concert by specialized macrophage populations. Specifically, CD11cHI dMϕs are likely to be important for the processing and presentation of lipid antigen to decidual T cells through specific CD1 molecules (Figure 2). This is consistent with a mechanism by which CD11cHI dMϕs are capable of separating pathogenic and non-pathogenic lipids in order to amount to an appropriate immune response at the maternal-fetal interface without interfering with ongoing tolerogenic mechanisms to fetal antigens. It would be interesting to identify the lipid components found at the human maternal-fetal interface and their ability to dictate CD1 expression on dMϕ cell surface. Furthermore, the lipids may vary in their ability to be loaded into different CD1 molecules and their level of antigenicity. Moreover, irrespective of lipid processing and presentation, CD1 expression by CD11cHI dMϕs may be important in regulating dNK cell cytokine responses and cellular expansion. This does not discount the fact that dNK cells, as well as other cells, and CD11cLO dMϕs may also interact in alternative ways to regulate or respond to dNK cells. However, CD11cLO dMϕs may have more phagocyte-specific function, which is important for organogenesis and placental construction.
There are many aspects to understanding how human dMΦ populations confer tolerance to fetal antigens. However, the key may be in immune cell interaction and regulation with a particular emphasis on placental lipid diversity (or specificity) and CD1 molecules. The distinct decidual macrophage populations that we described [11] may help to better understand how macrophage heterogeneity in pregnancy, and in other tissues, plays specific roles. Furthermore, the fact that only one population of dMΦs express CD1 molecules may indicate important antigen presentation function and lipid composition at the maternal-fetal interface, an area that remains to be extensively explored.

REFERENCES

1. Archibald JD. Fossil Evidence for a Late Cretaceous Origin of “Hoofed” Mammals. Science. 1996;272(5265):1150-3.
2. Cross JC. Genetic insights into trophoblast differentiation and placental morphogenesis. Semin Cell Dev Biol. 2000;11(2):105-13.
3. Hunt JS. Stranger in a strange land. Immunol Rev. 2006;213:36-47.
4. Vogel P. The current molecular phylogeny of Eutherian mammals challenges previous interpretations of placental evolution. Placenta. 2005;26(8-9):591-6.
5. Moffett A, Loke C. Immunology of placentaion in eutherian mammals. Nat Rev Immunol. 2006;6(8):584-94.
6. Wildman DE, Chen C, Erez O, Grossman LI, Goodman M, Romero R. Evolution of the mammalian placenta revealed by phylogenetic analysis. Proc Natl Acad Sci USA. 2006;103(9):3203-8.
7. Tayade C, Black GP, Fang Y, Croy BA. Differential gene expression in endometrium, endometrial lymphocytes, and trophoblasts during successful and abortive embryo implantation. J Immunol. 2006;176(1):148-56.
8. von Rango U. Fetal tolerance in human pregnancy—a crucial balance between acceptance and limitation of trophoblast invasion. Immunol Lett. 2008;115(1):21-32.
9. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. J Exp Med. 2003;198(8):1201-12.
10. Trundley A, Gardner L, Northfield J, Chang C, Moffett A. Methods for isolation of cells from the human fetal-maternal interface. Methods Mol Med. 2006;122:109-22.
11. Houser BL, Tilburgs T, Hill J, Nicotra ML, Strominger JL. Two unique human decidual macrophage populations. J Immunol. 2011;186(4):2633-42.
12. Sapin V, Blanchon L, Serre AF, Lemery D, Dastugue B, Ward SJ. Use of transgenic mice model for understanding the placentaion: to-
wards clinical applications in human obstetrical pathologies? Transgenic Res. 2001;10(5):377-98.
13. Arck PC, Rucke M, Rose M, Szekeres-Bartho J, Douglas AJ, Pritsch M, et al. Early risk factors for miscarriage: a prospective cohort study in pregnant women. Reprod Biol Online. 2008;17(1):101-13.
14. Gracia CR, Sammel MD, Chittams J, Hummel AC, Shaunik A, Barnhart KT. Risk factors for spontaneous abortion in early symptomatic first-trimester pregnancies. Obstet Gynecol. 2005;106(5 Pt 1):993-9.
15. Heard DH, Hinde IT, Mynors LS. An experimental study of haemolytic disease of the newborn due to isoimmunization of pregnancy. J Hyg (Lond). 1949;47(2):119-31.
16. Landsteiner K, Wiener AS. Studies on an Agglutinin (Rh) in Human Blood Reacting with Anti-Rhesus Sera and with Human Isoantibodies. J Exp Med. 1941;74(4):309-20.
17. Billingham RE, Brent L, Medawar PB. Activated acquired tolerance of foreign cells. Nature. 1953;172(4379):603-6.
18. Billington WD. The immunological problem of pregnancy: 50 years with the hope of progress. A tribute to Peter Medawar. J Reprod Immunol. 2003;60(1):1-11.
19. Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator cr3 in fetomaternal tolerance. Science. 2000;287(5452):498-501.
20. Girardi G, Yarilin D, Thurman JM, Holers VM, Salmon JE. Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. J Exp Med. 2006;203(9):2165-75.
21. Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J. 2005;19(7):681-93.
22. Hjartardottir S, Leifsson BG, Geirsson RT, Hunt JS, Petroff MG, McIntire RH, Ober C. Activation of murine monocytes, macrophages, and dendritic cells. Lab Invest. 2001;81(8):1143-52.
23. Pijnenborg R, Vercruysse L, Verbiest L, Van Assche FA. Interaction of interstitial trophoblast with placental bed capillaries and venules of normotensive and pre-eclamptic pregnancies. Placenta. 1998;19(8):569-75.
24. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science. 2010;327(5966):656-61.
25. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):656-61.
26. Rutherford AJ, Tomlinson AJ, et al. The influence of donated gametes on the incidence of hypertensive disorders of pregnancy. Hum Reprod. 1999;14(9):2268-73.
27. Kim YM, Chaiworapongs A, Gomez R, Bujoyd E, Yoon BH, Rotmensh S, et al. Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. Am J Obstet Gynecol. 2002;187(5):1137-42.
28. Reister F, Frank HG, Kingdom JC, Heyl W, Kaufmann P, Rath W, et al. Macrophage-induced apoptosis limits endovascular trophoblast invasion in the uterine wall of preeclamptic women. Lab Invest. 2001;81(8):1143-52.
29. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol. 2006;177(10):5303-11.
30. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol. 2009;27:451-83.
31. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. J Exp Med. 1992;176(1):287-92.
32. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003;3(1):23-35.
33. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677-86.
34. Ghassabeh GH, De Baetselier P, Blys L, Noel W, Van Ginneken J, Meerschaut S, et al. Identification of a common gene signature for type II cytokine-associated myeloid cells elicited in vivo in different pathologic conditions. Blood. 2006;107(2):573-83.
35. Daley JM, Brancato SK, Thomay AA, Reichen JS, Albina JE. The phenotype of murine wound macrophages. J Leukoc Biol. 2010;87(1):59-67.
36. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):867-70.
37. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539-45.
38. Schoppmann SF, Bimer P, Stockl J, Kalt R, Ulrich R, Cauig C, et al. Tumor-associated
macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Am J Pathol. 2002;161(3):947-56.

44. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9(3):162-74.

45. Allavena P, Sica A, Garlanda C, Mantovani A. The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. Immunol Rev. 2008;222:155-61.

46. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol. 2005;5(8):641-54.

47. Giavazzi R, Garofalo A, Bani MR, Abbate M, Giaccone G. Inhibition of CD11c+ macrophages in breast cancer by vaccination with tumor lysates. Cancer Res. 1990;50(15):4771-5.

48. Mantovani A. Cancer: an infernal triangle. Nature. 2007;448(7153):547-8.

49. Naugler WE, Sakurai T, Kim S, Maeda S, Lee NJ, Kim YK, et al. Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. Cancer Res. 1990;50(15):4771-5.

50. Sternberg EM. Neural regulation of innate immunity. Annu Rev Neurosci. 2002;25:487-516.

51. Liu Y, Cousin JM, Hughes J, Van Damme J, Seckl JR, Haslett C, et al. Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. J Immunol. 1999;162(6):3639-46.

52. Miller L, Hunt JS. Sex steroid hormones and macrophage function. Life Sci. 1996;59(1):1-4.

53. Jones LA, Anthony JP, Henriquez FL, Lyons RE, Nickdel MB, Carter KC, et al. Toll-like receptor-4 mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors. Immunology. 2008;125(1):59-69.

54. Romero R, Mor G. Macrophages and apoptotic cell clearance during pregnancy. Am J Reprod Immunol. 2004;51(4):275-82.

55. Gardner L, Moffett A. Dendritic cells in the human decidua. Biol Reprod. 2003;69(4):1438-46.

56. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. Science. 2002;296(5566):298-300.

57. Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. Science. 2005;307(5715):1630-4.

58. Steinman RM, Swanson J. The endocytic activity of dendritic cells. J Exp Med. 1995;182(2):283-8.

59. Lennon-Dumenil AM, Bakker AH, Maehr R, Fiebig E, Overkleeft HS, Rosenblatt M, et al. Analysis of protease activity in live antigen-presenting cells shows regulation of the phagosomal proteolytic contents during dendritic cell activation. J Exp Med. 2002;196(4):529-40.

60. Yates RM, Hermetter A, Taylor GA, Russell DG. Macrophage activation downregulates the degradative capacity of the phagosome. Traffic. 2007;8(3):241-50.

61. Pearson H. Reproductive immunity: Immunity's pregnant pause. Nature. 2002;420(6913):265-6.

62. Aluvihare VR, Kalikokourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5(3):266-71.

63. Tafuri A, Alferink J, Moller P, Hammerling GJ, Arnold B. T cell awareness of paternal alloantigens during pregnancy. Science. 1995;270(5236):630-3.

64. Collins MK, Tay CS, Erlebacher A. Dendritic cell entrapment within the pregnant uterus inhibits immune surveillance of the maternal/fetal interface in mice. J Clin Invest. 2009;119(7):2062-73.

65. Red-Horse K, Rivera J, Schanz A, Zhou Y, Winn V, Kapidzic M, et al. Cytotrophoblast induction of arterial apoptosis and lymphangiogenesis in an in vivo model of human placentation. J Clin Invest. 2006;116(10):2643-52.

66. Bradford BM, Sester DP, Hume DA, Mabbott NA. Defining the anatomical localisation of subsets of the murine mononuclear phagocyte system using integrin alpha X (Itgax, CD11c) and colony stimulating factor 1 receptor (Csf1r, CD115) expression fails to discriminate dendritic cells from macrophages. Immunobiology. 2011;216(11):1228-37.

67. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, et al. Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. Diabetes. 2010;59(7):1648-56.

68. Tagliani E, Shin C, Nancy P, Tay CS, Pamer EG, Erlebacher A. Coordinate regulation of tissue macrophage and dendritic cell populations by CSF-1. J Exp Med. 2011;208(9):1901-16.

69. Miyazaki S, Tsuda H, Sakai M, Hori S, Sasaki Y, Futatani T, et al. Predominance of Th2-promoting dendritic cells in early human placentation. J Clin Invest. 2006;116(10):2643-52.

70. Yamamoto T, Takahashi K, Ito J, Kimura K, Miura H, et al. T regulatory cells mediate maternal tolerance to the fetus. J Clin Invest. 2004;114(9):1289-300.

71. Jeon H, Blacklow SC. Structure and physiologic function of the low-density lipoprotein receptor. Annu Rev Biochem. 2005;74:535-62.

72. Barral DC, Brenner MB. CD1 antigen presentation: how it works. Nat Rev Immunol. 2007;7(12):929-41.

73. Dascher CC, Brenner MB. Evolutionary constraints on CD1 structure: insights from com-
86. Briken V, Moody DB, Porcelli SA. Diversification of antigen-presenting molecules. Adv Immunol. 2003;248(8):412-8.

87. Martin LH, Calabi F, Lefebvre BA, Bilsland CA, Milstein C. Structure and expression of the human thymocyte antigens CD1a, CD1b, and CD1c. Proc Natl Acad Sci USA. 1987;84(24):9189-93.

88. Longley J, Kraus J, Alonso M, Edelson R. Molecular cloning of CD1a (T6), a human epidermal dendritic cell marker related to class I MHC molecules. J Invest Dermatol. 1989;92(4):628-31.

89. Martin LH, Calabi F, Milstein C. Isolation of CD1 genes: a family of major histocompatibility complex-related differentiation antigens. Proc Natl Acad Sci USA. 1986;83(23):9154-8.

90. Grant EP, Degano M, Rosat JP, Stenger S, Modlin RL, Wilson IA, et al. Molecular recognition of lipid antigens by T cell receptors. J Exp Med. 1999;189(1):195-205.

91. Pena-Cruz V, Ito S, Dascher CC, Brenner MB, Sugita M. Epidermal Langerhans cells efficiently mediate CD1a-dependent presentation of microbial lipid antigens to T cells. J Invest Dermatol. 2003;121(3):517-21.

92. Grassi F, Dezutter-Dambuyant C, McClroy D, Jacquet C, Yoneda K, Imamura S, et al. Monocyte-derived dendritic cells have a phenotype comparable to that of dermal dendritic cells and display ultrastructural granules distinct from Birbeck granules. J Leukoc Biol. 1998;64(4):484-93.

93. Caux C, Vanbervliet B, Massacrier C, Dezutter-Dambuyant C, de Saint-Vis B, Jacquet C, et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. J Exp Med. 1996;184(2):695-706.

94. Smith ME, Thomas JA, Bodmer WF. CD1c antigens are present in normal and neoplastic B-cells. J Pathol. 1988;156(2):169-77.

95. Small TN, Knowles RW, Keever C, Kerman NA, Collins N, O’Reilly RJ, et al. M241 (CD1) expression on B lymphocytes. J Immunol. 1987;138(10):2864-8.

96. Plebani A, Proserpio AR, Guarneri D, Buscaglia M, Cattoretti G, and CD1d molecules regulate dendritic cell CD1 expression and function. Immunology. 2008;125(3):289-301.

97. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331(6013):44-9.

98. Moretta A, Marcellino E, Sivori S, Della Chiesa M, Vitale M, Moretta L. Early liaisons between cells of the innate immune system in inflamed peripheral tissues. Trends Immunol. 2005;26(12):668-75.

99. Vivier E, Nunes JA, Vely F. Natural killer cell signaling pathways. Science. 2004;306(5701):1517-9.

100. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol. 2005;5(3):201-14.

101. Rauelt DH, Guerra N. Oncogenic stress sensed by the immune system: role of natural killer cell receptors. Nat Rev Immunol. 2009;9(8):568-80.

102. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature. 1986;319(6055):675-8.

103. Eissmann P, Davis DM. Inhibitory and regulatory immune synapses. Curr Top Microbiol Immunol. 2010;340:63-79.

104. King A, Wooding P, Gardner L, Loke YW. Expression of perforin, granzyme A and TIA-1 by human uterine CD56+ NK cells implies they are activated and capable of effector functions. Hum Reprod. 1993;8(12):3061-7.

105. Long EO. Negative signaling by inhibitory receptors: the NK cell paradigm. Immunol Rev. 2008;224:70-84.

106. Kopcow HD, Allan DS, Chen X, Rybalov B, Andzelm MM, Ge B, et al. Human decidual NK cells form immature activating synapses and are not cytotoxic. Proc Natl Acad Sci USA. 2005;102(43):15563-8.

107. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med. 2006;12(9):1065-74.

108. Dietl J, Honig A, Kammerer U, Rieger L. Natural killer cells and dendritic cells at the human fetoplacental interface: an effective cooperation? Placenta. 2006;27(4-5):341-7.

109. Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter M, et al. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. Nat Med. 1999;5(4):405-11.

110. Chiijohe O, Munz C. Interactions of human myeloid cells with natural killer cell subsets in vitro and in vivo. J Biomed Biotechnol. Epub 2011 Mar 31.

111. Carbone E, Terrazzano G, Melian A, Zanzi D, Carbone E, Terrazzano G, Melian A, Zanzi D, et al. Interactions of human feto-maternal interface: an effective cooperation? Placenta. 2006;27(4-5):341-7.