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Review

Endometrial Immunity for Embryo Implantation and Pregnancy Establishment

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The uterus is an organ for raising the fetus, and its lumen is lined by the endometrium. The endometrium is an important site for the implantation and maturation of fertilized eggs. The endometrium undergoes repetitive proliferation, maturation (decidualization), and exfoliation changes every menstrual cycle. At the same time, the number and type of endometrial immunocompetent cells vary during the menstrual cycle. At the implantation stage, the immunocompetent cells occupy approximately half of the endometrial cells. Immunocompetent cells normally eliminate pathogenic microorganisms to protect the body; however, they also promote immune tolerance to accept the fetus during pregnancy. The immunocompetent cells in the uterus can perform both these functions. With the establishment of pregnancy, stimuli from the trophoblast (placenta) and fetus can also change the immune environment of the uterus, and pregnancy can be maintained only when the immune system is well adapted to the stimuli of some hormones and the fetus. Immunity for the establishment of pregnancy is not simple because multiple immunocompetent cells are involved in establishing and maintaining pregnancy. To understand the immune mechanisms associated with the establishment of pregnancy, we have to learn about each immune cell. This review, therefore, discusses the roles and distribution of the immunocompetent cells inside the uterus during menstruation and early pregnancy.

Keywords: decidua; implantation; lymphocytes; regulatory T cells; Th17 cells
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Introduction

The uterus is the organ that raises the fetus, and the lumen is lined by the endometrium. The endometrium is a unique organ that undergoes periodic regeneration and proliferation in response to sex steroid hormone stimuli that change during the course of the menstrual cycle; both menstruation and tissue exfoliation are avoided once pregnancy is established.

Pregnancy begins with fertilization of the egg with sperm, and the resulting blastocyst must adhere to the endometrium for implantation to get its supply of oxygen and nutrients. This process requires uterine tissue remodeling (Fig. 1). After blastocyst implantation, a specific immune tolerance to the foreign fetal antigens in the embryo must be established while maintaining the ability to fight infections (Tafuri et al. 1995). The tolerance involved in the establishment of pregnancy has been a focus of studies.

The superficial endometrial layer contains many immune cells that mature gradually through the proliferative and ovulatory phases of the menstrual cycle. These immune cells arise from the influx of leukocytes from the periphery and in response to the expression of ovarian hormone-regulated chemokines and cytokines (van den Heuvel et al. 2005; Woidacki et al. 2013; Zenclussen et al. 2013). Neutrophils, T cells, B cells, mast cells, macrophages, and uterus natural killer (uNK) cells are present in the endometrium and are involved in immunity and implantation. Native immune cells constitute a major population of leukocytes in the uterus at the time of embryo implantation. uNK cells are the most abundant decidual immune cells (70% of the local total of immune cells), but dendritic cells

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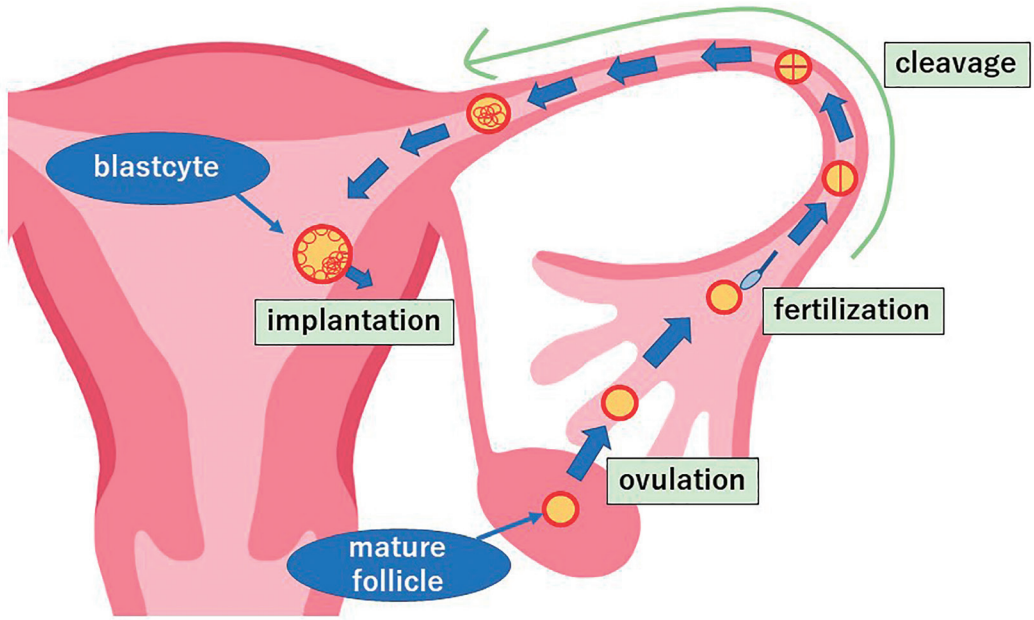

Fig. 1. Flow from ovulation to implantation.
After ovulation, the ovum is fertilized in the ampulla of the uterine tube. A fertilized egg is repeatedly divided into blastocysts, and then implanted in the womb.

(DCs), macrophages, neutrophils, and mast cells also exist. Immune cells may reach 30 to 40% of the total number of cells in the uterus during early pregnancy (Du et al. 2014). Native immune cells and other cell lineages in the uterus communicate with each other. Immune cells are regulated by the environment and acquire uterine-specific profiles. Depletion of any one lineage of these cells changes the environment and may disturb implantation. For example, maternal $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells (Treg) contribute to maintaining immune tolerance during pregnancy by suppressing the mother's alloreactive immune response to father antigens in the fetus (Mold et al. 2010; Shima et al. 2010; Samstein et al. 2012). Also, infertile patients have significantly lower endometrial Foxp3 mRNA levels than fertile ones, providing evidence for the importance of $Foxp3^+$ Treg for pregnancy establishment (Jasper et al. 2006).

Estrogen and progesterone promote the transformation of endometrial cells to decidual stromal cells (DSCs) during blastocyst adhesion and endometrial implantation (Matson and Caron 2014). The blastocyst differentiates into a functionally unique extravillous trophoblast (EVT) in the microenvironment created by the DSCs. EVTs infiltrate the endometrium and control maternal blood flow by spiral artery remodeling. These EVTs express not only native HLA but also chemokine receptor (CXCR) and corresponding chemokines (CXCL) during early pregnancy. This complex chemokine-chemokine receptor expression system promotes EVT invasion into the decidua and also attracts immune cells into the decidua to generate immune tolerance at the fetal-maternal interface. Thus, immunocompetent cells on the fetal-maternal interface are key to the success of pregnancy. This review outlines the role of each immune cell in the endometrium for implantation and pregnancy maintenance.

Distribution and Origin of Endometrial Leukocytes

Different hypotheses regarding the origin of endometrial leukocytes have been formulated. One hypothesis states that leukocytes are brought into the endometrium with the circulating blood (Johansson et al. 1999). Another hypothesis proposes in situ proliferation of resident immune cells (van den Heuvel et al. 2005; Kitaya et al. 2007). And, yet another hypothesis has hematopoietic progenitors being recruited and differentiated in the endometrium (Keskin et al. 2007; Manaster and Mandelboim 2010). Immune cells recruited from the circulation are likely to undergo tissue-specific differentiation in the local microenvironment, gaining new properties (Keskin et al. 2007).

The proportion of immunocompetent cells in the endometrium changes during the menstrual cycle (Fig. 2) (Lee et al. 2015). The average number of $CD45^+$ cells in the endometrium remains low from the early follicular phase to the early secretory phase; however, it increases approximately 5-fold during the secretory phase (Flynn et al. 2000). As a result, the total number of leukocytes peaks during the premenstrual period (during the late secretory phase) (Salamonsen and Lathbury 2000).

uNK cells

NK cells are a type of cytotoxic lymphocyte that acts as a key member of innate immunity and adaptive immune feature. The main phenotype of the uNK cell is $CD3^-$ $CD56^{bright}CD16^-$ and this subset is different from the $CD3^-$

Menstrual cycle	Proliferative phase (day 10 - 12)	Middle secretory phase (day 22 - 23)	Late secretory phase ～Menstrual phase (day 26 - 28)
			
Natural Killer cells	+/−	+/++	+++
Macrophages	+	++	+++
Neutrophils	+/−	+/−	+++
Eosinophils	−	−	++
Mast cells	++	++	++
T cells	+	+	+
B cells	+/−	+/−	+

Fig. 2. Periodic changes of immune cells in the endometrium.
The density of the leukocyte population is expressed as –, +, ++, and +++, representing 0%, 1-2%, 3-5%, and 6-15% of total number of endometrial cells, respectively. The relative rationale of each white blood cell in the endometrium was estimated using flow cytometry. Data were collected from Flynn et al. (2000), and Givan et al. (1997)

$CD56^{dim}CD16^{+}$ NK cells in peripheral blood (King 2000; Lee et al. 2011). The $CD56^{bright}$ NK cells of the peripheral blood arrive at the uterus and undergo tissue-specific differentiation in there. Although NK cells lie scattered in the endometrium during the proliferative phase, they increase in abundance after ovulation and continue increasing until a few days before menstruation. During the latter secretory phase, uNK cells increase up to 30%-40% of the number of cells in the stromal area and to 70% of the number of endometrial leucocytes (King 2000; Lee et al. 2011).

uNK cells surround endometrial arteries and glands. An association is thought to exist between the decrease in the serum progesterone levels and uNK apoptosis based on the decreased numbers of uNK cells in the menstruation and proliferative phases. Given the absence of progesterone receptors in uNK cells and other leucocytes, progesterone may have indirect effects via the production of cytokines and other soluble factors in the stroma. Stromal cells express both estrogen and progesterone receptors. Interestingly, a nuclear morphology reminiscent of NK cell apoptosis is found in the decidua after miscarriage as well as in the endometrium of patients who discontinue progesterone treatment.

Macrophages and dendritic cells

Macrophages are antigen-presenting phagocytes that are present in almost all tissues. They have various functions and play an important role in preventing infection and maintaining homeostasis in the body. $CD68^{+}$ macrophages are detected in endometrium throughout the menstrual cycle (Salamonsen and Lathbury 2000) scattered around the glands (Song and Fraser 1995). The number of macrophages increases during the secretary phase, especially at the implantation site (King 2000; Salamonsen et al. 2002).

Dendritic cells are antigen-presenting cells with a unique morphology of dendritic processes. They are distributed in various tissues and play an important role in initiating an antigen-specific immune response. Because of the small number of DCs that can be isolated from the endometrium, they were thought to exist only at low densities. However, microscopic examinations have demonstrated the abundance of DCs in the endometrial specimens. In addition, uterine DCs (uDCs) are arranged in a clustered manner along the uterus and may point toward the future implantation site (Zenclussen et al. 2013). The density of endometrial $CD1a^{+}$ immature DCs is significantly higher than that of $CD83^{+}$ mature DCs throughout the menstrual cycle (King 2000). The density of the basal endometrial $CD1a^{+}$ DCs reaches its lowest point in the proliferative phase and peaks during the menstrual phase (Schulke et al. 2008). The density of $CD1a^{+}$ DC is higher in the basal layer than in the functional layer only during the secretory phase. Endometrial $CD83^{+}$ DCs are more abundant in the basal layer than in the functional layer throughout the menstrual cycle (Schulke et al. 2008). The percentages of $CD1a^{+}$ and $CD83^{+}$ DCs are similar in the fundus and uterine isthmus.

Mast cells

Mast cells (MCs) are bone marrow-derived cells that contain a large number of cytoplasmic granules and play an important role in biological defense mechanisms such as inflammation and immune response. Usually, they do not exist in the circulating blood but are distributed in the site adjacent to the blood vessel in the skin and digestive tract etc. MCs are found in the endometrium throughout the menstrual cycle and do not vary significantly throughout the cycle (Jeziorska et al. 1995). However, MC activation is most pronounced immediately before menstruation (Jeziorska et al. 1995).

Endometrial T and B cells

T cells are lymphocytes that have differentiated after progenitor cells produced in the bone marrow have been selected in the thymus. Effector T cells activated by antigen stimulation exert various immune functions. $CD3^+$ T cells are a minor population in the endometrium, accounting for only 1%-2% of all lymphoblast cells (Salamonsen and Woolley 1999). These T cells are aggregated into basal lymphocytes and scattered throughout stromal and epithelial sites (King 2000). In contrast to peripheral blood $CD3^+$ T cells, endometrial $CD3^+$ T cells comprise a high proportion of $CD8^+$ cells (66%) and a small proportion of $CD4^+$ cells (33%) (Salamonsen and Lathbury 2000). The cytolytic activity of endometrial $CD8^+$ T cells is maintained during the proliferative phase, but this activity gets weakened during the secretory phase (although the number of $CD8^+$ cells remains constant) (Salamonsen and Lathbury 2000; Wira et al. 2014). The suppression of $CD8^+$ cell cytolytic activity has been found only in the fallopian tube and endometrium, but not in the cervix or vagina (Wira et al. 2014). B cells differentiate into plasma cells upon antigen stimulation and secrete immunoglobulins as antibodies. Few $CD45RA^+$ B cells have been found in endometrium throughout the menstrual cycle.

The Roles of Endometrial Leukocytes in Embryo Implantation and Pregnancy Establishment

uNK cells

uNK cells are one of the most important decidual immune cells for establishing pregnancy. In the pre-ovulation phase of the menstrual cycle, few uNK cells are found in the endometrium. As the level of progesterone increases during the secretory phase, the number of uNK cells increases abruptly. If pregnancy is established, the number of uNK cells increases further and accounts for 60%-90% of the decidual immune cells (Vacca et al. 2011). During early pregnancy in humans, uNK cells become a major immune actor at the fetomaternal interface and then decrease during the middle and late stages of pregnancy. Therefore, uNK cells are thought to play important immune regulatory roles during early pregnancy.

uNK cells and peripheral blood NK (pbNK) cells display different phenotypes (Fu et al. 2011). Ninety to 95% of the peripheral blood NK cells are $CD56^{dim}$ $CD16^+$ NK cells, whereas most uNK cells are $CD56^{bright}$ $CD16^-$ NK cells (Li et al. 2015). CXCL9 and CXCL10 secreted by DSCs can further interact with CXCR3 expressed on $CD56^{bright}$ $CD16^-$ NK cells, and they allow uNK cells to remain on site (Hanna et al. 2003; Wu et al. 2005). DSCs and EVTs can also produce TGF-β and IL-15 to transform $CD56^{dim}$ $CD16^+$ NK cells into uNK cells in the decidual environment thereby maintaining the high volume of uNK cells (Keskin et al. 2007).

Some researchers believe uNK cells are not directly involved in embryo implantation but contribute by stimulating changes in endometrial vessels and spiral arterial structures (Croy et al. 2010). During the early stages of pregnancy, human uNK cells secrete various strong angiogenic factors such as VEGF-C, IL-8, IP-10, placenta growth factor antibody, and angiopoietin, and they promote infiltration into the trophoblast (Vacca et al. 2013). This creates a low resistant uterine circulation, which promotes trophoblast infiltration and the transport of blood, nutrition, and oxygen between the maternal placenta and fetus.

uNK cells are not thought to respond to fetal cells that coexist in the uterus. This conclusion is based on the fact that decidual leukocytes that contain both NK cells and macrophages do not respond at all in cell killing assays. However, NK cells can kill extravillous cytotrophoblasts and semiallogeneic fetal placental cells (Co et al. 2013). A similar situation (failure to respond in cell killing assays) occurs in primary trophoblast cultures, despite using NK cells taken from the same pregnancy. These results suggest a suppression mechanism exists in the uterus. Macrophages play this role, because their removal enables targeted NK cell killing (and the killing is suppressed after adding the macrophages back). Regarding the molecular basis of this phenomenon, decidual macrophages produce significant amounts of TGF-β1, and removal of TGF-β1 increases the killing of primary trophoblast cells by decidual leukocytes. This mechanism may normally induce quiescence of maternal NK cell attacks in the pregnant uterus.

The effects of NK cells on trophoblasts have also attracted attention. Research has shown that both NK cells and EVTs target the extracellular matrix and smooth muscle cells of the extrauterine spiral artery, altering vascular regulation and facilitating nutrient delivery (Soares et al. 2014). EVTs get into the endothelium of the uterine artery in a process called pseudo angiogenesis. NK cells limit EVT activity, thereby protecting the mother and preventing rapid and excessive invasion and remodeling of the uterine spiral artery. NK and trophoblast cells communicate directly and via extracellular mediators, regulating actions on the uterine spiral artery and oxygen delivery. In normal pregnancies, these cells balance the needs of both the mother and the fetus. However, abnormal NK and/or EVT functions (as in many pregnancy-related diseases) interfere with nutrient delivery to the developing placenta, jeopardizing maternal

and fetal health.

NK cells are also involved in other factors for establishing pregnancy. A report discovered that in the tgε 26 mice that lack NK cells, many abnormalities appear in uterine glands, decidua, and blood vessels, and abortion occurs at a high rate of 64% in the middle stage of pregnancy (Guimond et al. 1998). In this model, this pregnancy disorder can be reversed by adoptive transfer of bone marrow from severe combined immunodeficient (SCID) mice (T cells and B cells are deficient, but NK cell activity is maintained). Thus, NK cells appear to have critical functions during pregnancy that promote decidual health, appropriate vascularization of implantation sites, and placental size. In addition, NK cells regulate trophoblast cell infiltration and vascular remodeling by secretion of angiogenesis regulatory molecules, cytokines, and chemokines (Hanna et al. 2006; Lima et al. 2014). Incomplete remodeling of uterine helical arteries due to abnormalities in NK cells is associated with increased risk of premature labor and preeclampsia (Fraser et al. 2012).

Adrenomedullin is a potent vasodilatory peptide that has been reported to induce and activate uNK cells during uterine spiral artery remodeling in early pregnancy (Li et al. 2013). It stimulates the secretion of numerous chemokines, cytokines, and MMPs from uNK cells in a dose-dependent manner and induces the apoptosis of vascular smooth muscle cells. Therefore, adrenomedullin is considered essential for the functioning of uNK cells in early pregnancy.

Macrophages

Macrophages are the second most common type of immune cells in decidual tissues (~20% of the immune cells). During embryo implantation, human DSCs, uNK cells, and glandular epithelial cells have the capacity to produce CSF1, which promotes macrophage proliferation and differentiation. Meanwhile, CCL2 (MCP-1), CCL 7 (MCP-3), CCL12 (MCP-5), and monocyte chemokines are induced to express macrophages. These chemokines continue to attract circulating monocytes to the decidua where they differentiate into macrophages (Repnik et al. 2008; Houser et al. 2011). Decidual macrophages have been shown to be involved in tissue remodeling, repair of apoptotic cells, induction of local tolerance microenvironment during early pregnancy and onset of labor during late pregnancy (Erlebacher 2013).

Specific ablation of CD11b macrophages results in implantation failure in mice (Care et al. 2013). Decidual macrophages (dMφs) can present antigens to T cells as well as to peripheral macrophages (Petroff et al. 2002). They can secrete cytokines resembling M2 macrophages, an immunosuppressive phenotype, including transforming growth factor (TGF)-beta and interleukin-10 (IL-10) (Heikkinen et al. 2003; McIntire and Hunt 2005). In addition, they produce other immune tolerance-related molecules such as indoleamine 2,3 dioxygenase (IDO) (Renaud and Graham 2008). IDO is involved in maintaining pregnancy by suppressing the proliferation of immune cells induced by fetal antigens. Leukemia inhibitory factor (LIF) is secreted from endometrial gland cells, and increased LIF expression prior to implantation is important for embryo implantation. Although LIF-deficient mice are infertile due to implantation failures, LIF administration on the fourth day of pregnancy can save the pregnancies (Chen et al. 2000). The early pregnancy cell populations of mated LIF knockout mice were investigated to delineate the cellular changes associated with implantation failure, and macrophages were halved in number in LIF knockout versus wild-type mice (Schofield and Kimber 2005). This supports the role of macrophages in implantation (Renaud and Graham 2008).

Two unique human dMφs subsets have been identified with different gene expression patterns that suggest potentially different functions during implantation (Houser et al. 2011). The two populations of dMφ can be identified based on high and low expressions of CD11c ($CD11c^{HI}$ and $CD11c^{LO}$ dMφ), a classification differing from the conventional one of proinflammatory (M1) and anti-inflammatory (M2) macrophages. $CD11c^{HI}$ dMφs secrete a potent anti-inflammatory cytokine IL-10, the amount of which increases nearly four-fold after LPS stimulation. $CD11c^{LO}$ dMφs do not secrete IL-10 prior to stimulation, and once stimulated they secrete only enough IL-10 to reach the low basal levels seen in $CD11c^{HI}$ dMφ. On the other hand, both dMφ subsets can produce proinflammatory cytokines such as TNF-α and IL-1β. This finding contradicts the notion that the fetal-maternal interface is an anti-inflammatory environment. But it is consistent with the hypothesis that immune activation is required to promote trophoblast infiltration and establishment of fetal and maternal tolerance.

Human dMφs are localized in close proximity to invasive trophoblasts and are thought to contribute to trophoblast invasion and placental development (Helige et al. 2014). In pregnancy disorders, macrophage-related molecule levels have been reported to decrease (e.g., granulocyte-macrophage colony stimulating factor [GMCSF]) (Huang et al. 2010). In humans, $CD163^{high}$ endometrial macrophages constitutively secrete both proinflammatory and anti-inflammatory cytokines (Jensen et al. 2012). There are studies of fractalkine that highlight the relevance of macrophages in human pregnancy. The addition of fractalkine has been shown to significantly impair the ability of monocytes to attach to the trophoblast (Siwetz et al. 2015). Fractalkine is upregulated during pregnancy complications such as chorioamnionitis (Szukiewicz et al. 2014), suggesting that it may interfere with communication between the trophoblast and monocytes.

With the onset of labor, a number of dMφs gather locally in the decidual membrane, secretion of IL-10 decreases and secretion of inflammatory cytokines increases. This inflammation may help initiate labor (Hamilton et al. 2012; Gomez-Lopez et al. 2014).

Dendritic cells

DCs are immune cells that function as antigen presenting in mammals. Antigen presentation is an important determinant of the success or failure of pregnancy, as it determines the fate of the immune response that determines blastocyst tolerance or rejection.

Like macrophages and uNKs, uDCs display different characteristic phenotypes from circulating DCs or DCs present in other tissues. uDCs in endometrial tissues of mice during the embryo-receptive phase have a characteristic phenotype (Hsu et al. 2012). DC-SIGN$^+$ CD14$^+$ CD83$^-$ DC found in the uterus represent a unique subpopulation that can activate inducible Tregs (Hsu et al. 2012). Heme oxygenase-1 (HO-1), which is necessary for stress response and iron recycling, and the pregnancy molecule human chorionic gonadotropin (hCG) are important modulators of uDC maturation, and GM-CSF is also an important regulator of DC maturity (Moldenhauer et al. 2010).

Implantation fails in CD11c diphtheria toxin receptor (DTR) mice (DC-depleted mice) because of their inability to regulate uterine receptivity. DCs are thought to be critical for uterine tissue remodeling and angiogenesis, which are necessary for embryo implantation (Plaks et al. 2008; Blois et al. 2011). Thus, DCs appear to be directly involved in the decidual process by promoting decidual proliferation and differentiation.

A bidirectional communication between DCs and the microenvironment of the uterus regulates their phenotype. IL-10 produced from the placenta suppresses DC-mediated T cell stimulation in vitro (Steinbrink et al. 1997). DCs are involved in communication between trophoblasts and decidual Tregs. Depletion of 33D1$^+$ DCs (which shifts Th1/Th2 balance to Th2 type) during the perinatal period causes fetal losses. These findings show the importance of balancing DC subsets for a successful pregnancy.

uDCs are affected by gonadal hormones. Estrogen produced from the ovary and placenta suppresses the T cell proliferative capacity of DCs and the cytokine production of helper T cells (Xiao et al. 2004). In addition, the fact that progesterone can rescue mice abortions induced by DC depletion confirms the importance of the hormonal environment for a balanced immune response, as well as the effects of immune cells on the ovarian steroidogenic function (Pate et al. 2010; Negishi et al. 2012). The interactions between hormones and cytokines seem to regulate DC maturation in the pregnant uterus.

Mast cells (MCs)

MCs exist in the endometrium (Shelesnyak 1963; Norrby 2002; Bytautiene et al. 2008). Their effects for the establishment of pregnancy are unclear and conflicting reports have been published.

In female adult mice, a transient population of MCs in the endometrium appears periodically (Woidacki et al. 2013). Estradiol and progesterone promote MC migration from the periphery to the uterus in ovariectomized mice, and they promote subsequent maturation of these cells in situ. MC numbers peak during fertilization of the estrous cycle in mice. They remain present in large numbers with the establishment of pregnancy. uMCs and other immune cells represent different MC population from those found in other tissues. uMCs are divided into three subtypes: connective tissue MCs (which has heparin-rich granules with high levels of histamine), mucosal MCs (which has granules rich in chondroitin sulfate but poor in histamine), and a transitional mixed population that shares characteristics of both MC types. MCs express CD117 and FcεRIα, but only a small number of them express mast cell protease (Mcpt) 5 and Mcpt8.

KitW-sh/W-sh mice (which have a mutation in the promoter region of the tyrosine receptor c-kit and lack MCs) have been reported as being fertile (Lyon and Glenister 1982). However, KitW-sh/W-sh colonies often show irregular birth rates and high mortality at and after birth. MC-deficiency causes severely impaired implantation in most allogeneic KitW-sh/W-sh female mice (Woidacki et al. 2013). Transplantation of wild-type bone marrow-derived MCs (BMMCs) into these mice completely recovers the implantation ability. The transplanted MCs have been shown to move to the fetal-maternal interface (Woidacki et al. 2013). Therefore, MCs act locally in the uterus and are involved in normal pregnancy.

T cells

Human fetuses are semi allogeneic transplants (foreign bodies for mothers). Maternal-fetal immune tolerance plays an important role in establishing and maintaining the success of pregnancy. CD4$^+$ T cells are important in this process. CD4$^+$ T cells can be divided into Th1 cells, Th2 cells, regulatory T cells (Treg) and Th17 cells (Fig. 3). CD3$^+$ T cells account for 10%-20% of all decidual immune cells (30%-45% of T cells are CD4$^+$ T cells). CD4$^+$ T cells are divided into Th1 cells (5%-30%) and Th2 cells (5%). Treg and Th17 cells account for 5% and 2% of CD4$^+$ T cells, respectively (Tilburgs et al. 2010). In spite of these small proportions for Treg and Th17 cells, they are believed to be important for establishing pregnancies.

Th1 and Th2 cells

Th1 cytokines (generally considered to be proinflammatory) include interferon-γ, tumor necrosis factor (TNF) α, and interleukins (IL) 1, 2, 12, 15, and 18. On the other hand, Th2 cytokines such as IL4, 5, 6, 10, and 13 and granulocyte-macrophage colony stimulating factor control the proinflammatory action of Th1 cytokines. A dominant Th2 state is considered important for the establishment of pregnancy (Wegmann et al. 1993). In mice, the stimulation of proinflammatory cytokines, such as IFN-γ and TNF-α, induces miscarriages; however, these miscarriages can be reversed by the administration of Th1 cytokine inhibitors or anti-inflammatory IL-10 (Th2 cytokines) (Chaouat et al. 1995).

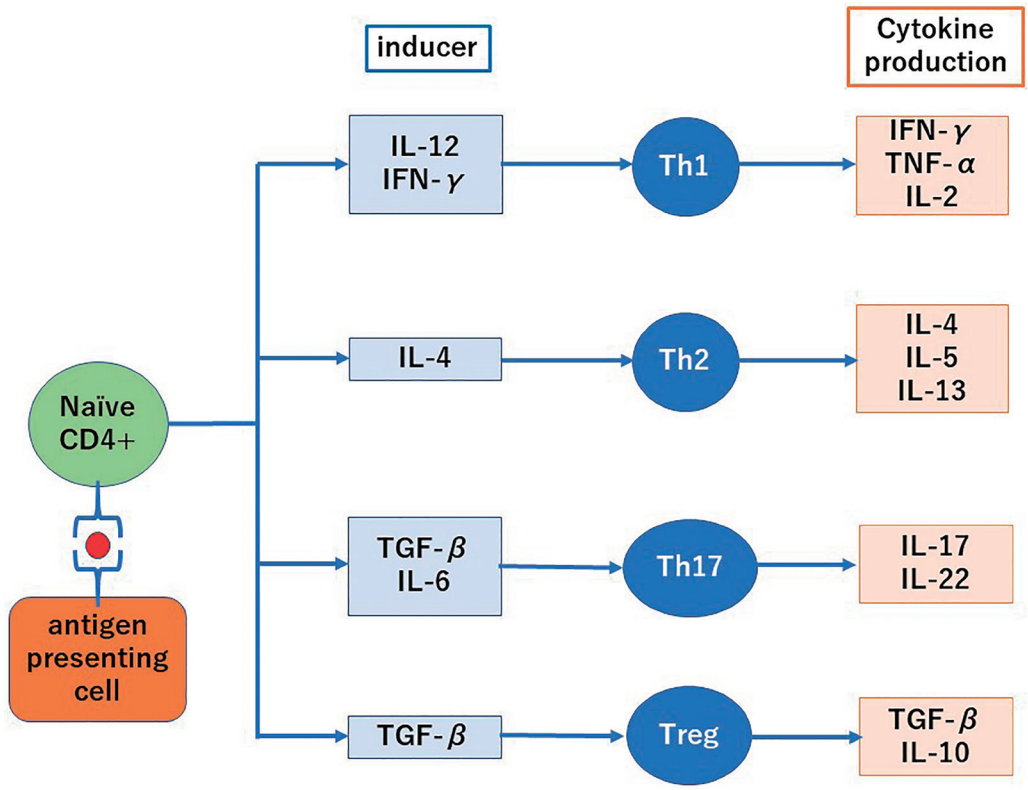

Fig. 3. Differentiation of helper T cells.
Naive $CD4^+$ cells that have received the antigen are differentiated into Th1, Th2, Th17, and Treg under cytokine stimulation.

The switch from Th1 to Th2 is more pronounced at the maternal-fetal interface. Th2 cells accumulate in the decidua, and uDCs can drive naive T cells to become Th2 cells (Thellin et al. 2000; Sykes et al. 2012). Therefore, switching to the Th2 phenotype is caused by both Th2 cell migration as well as cell induction. The Th2 dominance seen in early pregnancy is caused by elevated progesterone concentrations. In addition to increasing the secretion of Th2 cytokines, progesterone inhibits the secretion of Th1 cytokines. An association between loss of Th2 dominance in early pregnancy and poor clinical outcome exists, and Th2 and Treg reductions can be found in the basal deciduas of women with abortions and normal fetal chromosomes (Michimata et al. 2003; Saito et al. 2011).

Increases in Th2 cytokines IL-4, IL-10, and monocyte colony stimulating factor in the peripheral blood and at the maternal-fetal interface are associated with successful pregnancy. The trophoblast, decidua, and amnion contribute to a predominance of Th2 cytokines by producing IL-4, IL-6, IL-10, and IL-13 (Bennett et al. 1996; Roth et al. 1996; Jones et al. 1997). In addition, macrophages and Tregs present in the decidua during pregnancy also produce IL-10 that promotes immune tolerance (Chen et al. 2012). The placenta also produces prostaglandin D2, which acts as a chemoattractant of Th2 cells to the maternal-fetal interface via the Th2 receptor CRTH2 (a chemoattractant receptor homologous to that expressed in Th2 cells). Women suffering from recurrent pregnancy losses have lower $CRTH2^+$ cell expressions than women undergoing selective pregnancy terminations. The anti-inflammatory cytokines IL-4 and IL-10 inhibit Th1 cells and macrophages and prevent fetal allograft rejection.

Treg

Treg is involved not only in autoimmune responses, but also in various immune reactions (tumor, transplantation, allergy, and microbial infection immunities). Tregs also play an important role in pregnancy. The nuclear transcription factor Foxp3 is a master gene for differentiation of Treg. A decrease in Foxp3 expression and a decrease in $Foxp3^+$ Treg cells can be seen in the endometrium of women with unknown infertility and repeated implantation failures, suggesting that Treg cell defects impair embryo implantation in humans (Jasper et al. 2006; Diao et al. 2017). In addition, uterine tissues become inflamed and thickened after the removal of Treg. In mice without Treg, inflammatory mediators IL-6, IL-15, chemokine receptor (CCR) 5, CXCL11, CCL19, and CXCL3 are upregulated, and fibrosis can be detected (Teles et al. 2013).

Based on studies on Treg cell depletion and implantation in pregnant mice, Treg cells are needed for embryo implantation and pregnancy success. Depletion of Treg cells before embryo implantation leads to comprehensive effector T cell infiltration that causes inflammation in the

uterine microenvironment, resulting in implantation failure (Wang et al. 2014). Foxp3$^+$ Tregs can be depleted by application of human diphtheria toxin (DT) in Foxp3 DTR mice (Kim et al. 2007). Treg-depleted Foxp3 DTR mice present uterine inflammation and fibrosis that impaired embryo implantation starting on day 9 of pregnancy, whereas PBS- and DT-treated control mice show normal implantations (Teles et al. 2013). A similar scenario occurs in CCR7-deficient mice. CCR7 induces homing of Treg to the uterus. In these mice, the number of CD4$^+$ Foxp3$^+$ Treg in the uterus decreased significantly and consistently led to implantation failure (Teles et al. 2013). CCL19, a ligand for CCR7, is present in glandular and luminal uterine epithelial cells (Guerin et al. 2011). CCR7/CCL19 are presumed to be involved in thymic Treg migration into the uterus.

In humans and mice, Treg increase at the time of ovulation in response to ovarian steroid hormones to ensure sufficient Treg for implantation (Arruvito et al. 2007; Weinberg et al. 2011; Teles et al. 2013). Arruvito et al. (2007) studied the changes in Treg cell numbers in healthy women and in women with unexplained recurrent spontaneous abortion (URSA) at various stages of the menstrual cycle to uncover associations between Treg cells and URSA. They found that the level of CD4$^+$ Foxp3$^+$ Treg in peripheral blood of women who undergo URSA was significantly lower in the follicular phase of the menstrual cycle than that in healthy women who were not pregnant. However, the number of Tregs did not change significantly in the luteal phase, and a decrease in Treg in the proliferative phase may have causes the repeated implantation failure (van Mourik et al. 2009). Treg also fluctuates at various pregnancy stages. Mjösberg et al. (2009) observed changes in the number of Treg cells during normal pregnancy with CD4$^+$ CD2^{bright} Treg cells and CD4$^+$ CD25$^+$ Treg cells increasing during early pregnancy, peaking during middle pregnancy, and getting low during late pregnancy and delivery. Ablation of murine Tregs during pregnancy results in almost complete fetal rejection and elimination of a living fetus, suggesting the importance of Treg for maintenance of pregnancy (Rowe et al. 2011). Also, Treg decreases during late pregnancy and parturition may cause the inflammatory response called labor.

The involvement of Tregs with other immunocompetent cells has been suggested. As for uNK cells, Treg cells affect the maternal vascular adaptation required for robust placental development. Experiments in mice deficient in T and/or NK cells have shown that T cells interact with uNK cells and affect the maternal hemodynamic response to pregnancy (Burke et al. 2011; Croy et al. 2011). Women with preeclampsia have been shown to present a marked decrease in Tregs in the peripheral blood (Sasaki et al. 2007). In addition, women with preeclampsia also have high Th17 cell numbers (Santner-Nanan et al. 2009). In women with preeclampsia, suppression of Treg differentiation and induction into Th17 cells induces chronic inflammation.

Th17 cells

Th17 cells play an important role in autoimmune and defense responses to bacteria by releasing proinflammatory cytokines such as IL-6, IL-17, IL-22, and tumor necrosis factor-α (TNF-α) (Manni et al. 2014). Th17 cells are regulated by the transcription factor retinoic acid orphan nuclear receptor C (RORC) (Hayashi et al. 2012), and IL-1β and IL-23 are involved in differentiation of Th17 cells (Santarlasci et al. 2009). After differentiation, Th17 cells secrete IL-21 to increase further differentiation and activate the STAT3 signaling pathway to induce the expression of retinoic acid-related nuclear receptor-γt (RORγt). IL-23 maintains the stability and maturity of Th17 cells at the late stages of differentiation (Solt et al. 2011). In contrast, IFN-γ, IL-4, and IL-27 inhibit the formation of Th17 cells (Cosmi et al. 2008). The same naive T cells are thought to originate both inducible Treg (iTreg) and Th17 cells (Fig. 3). Naive T cells differentiate into iTregs when antigen stimulation is added in the presence of TGF-β, whereas they differentiate into Th17 cells when IL-6 is added (Chen et al. 2003, Weaver et al. 2006).

Maternal-fetal immune tolerance is regulated by the intercellular balance of Treg and Th17 cells. The incidence of repeated spontaneous abortion (RSA) increases as the level of Th17 cells increases and the level of Treg cells decreases (Wang et al. 2010). Qian et al. (2018) found that a woman with an unknown RSA history had a higher Th17/Treg ratio at the fetal-maternal interface than a woman without RSA history.

Also, uNK cells can be processed to prepare Th17 cells. uNK cells promote immune tolerance and successful pregnancy by dampening inflammatory Th17 cells via IFN-γ secreted by the CD56^{bright}CD27$^+$ NK subset (Korn et al. 2009). When the IFN-γ secreted from uNK cells is neutralized, uNK cells cannot control the polarization of Th17 cells resulting in loss of immune tolerance and decidual flares with abnormal pregnancies at the fetal-maternal interface (Fu et al. 2013). Thus, the balance between uNK cells and Th17 cells is very important for the success of pregnancy.

Discussion

During artificial fertility treatments, less than half of the fertilized eggs transferred into the uterus get implanted, more than half of them are lost shortly thereafter (Boomsma et al. 2009). This is thought to mimic the normal reproduction process in humans. Genetic analyses have revealed that about 70% of morphologically good embryos contain blastomeres with chromosomal aneuploidy. Decidual cells may recognize embryos with genomic instability to prevent invasion into the endometrium (Lucas et al. 2013; Macklon and Brosens 2014).

Differences in the number of immune cells at the maternal–fetal interface in women experiencing repeated-

miscarriages of unknown cause have been reported (Gao and Wang 2015; Qian et al. 2018). Given the role of each immunocompetent cell described above, an exquisite balance of endometrial immunocompetent cells must be maintained for successful pregnancies. However, causes of abnormalities are unclear, although reports of decreased Treg cells in the endometrium in patients with endometriosis and adenomyosis exist (Tanaka et al. 2017; Wang et al. 2018). Intrauterine and peri uterine inflammations seem to affect the endometrium.

The inflammation inside the uterus may be due to abnormalities in the endometrial microbiome. The uterine lumen has been considered sterile, but next generation sequencing results of the 16S rRNA gene have shown a lactobacillus-centered bacterial flora in the uterus (Mitchell et al. 2015; Chen et al. 2017). In the gastrointestinal tract, inflammation is due to abnormal intestinal microfloras, fluctuations in immune cells such as Treg, and inflammatory bowel diseases such as Crohn's disease (Frank et al. 2007; Machiels et al. 2014). Thus, the possibility that the intrauterine bacterial flora causes immune cells abnormalities exists.

Research is being conducted on the abnormal distribution of immune cells in patients with implantation failure and abnormal pregnancies focusing not only on endometriosis and adenomyosis but also on an abnormal endometrial microbiome.

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Conflict of Interest

The authors declare no conflict of interest.

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