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Changes of γδT cell subtypes during pregnancy and their influences in spontaneous abortion

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ABSTRACT

A successful pregnancy is a complicated process that involves the precisely timed regulation of endocrine as well as immune system. Despite increasing knowledge about immunology in gestation, the studies of immune cells in endometrium and decidua are still fragmented. Dynamic data is lacking on the transition of pre-pregnancy endometrial lymphocytes to initial pregnancy states as well as to second/third-trimester status. Here, we determined the composition of Gamma delta (γδ) T cells in endometrium and decidua from women with normal pregnancy and unexplained spontaneous abortion. We found that the frequency of γδT cells is fluctuating over the course of pregnancy, and these changes were regulated by progesterone. Different from peripheral blood, Vδ1$^+$ γδT cells accounted for the majority in endometrium and early-pregnancy decidua of healthy women, and endometrial stromal cells (ESCs) may involve in Vδ1/ Vδ2 shift directly. Moreover, an increase in the percentage of γδT cells with Vδ2 subset predominant in early-pregnancy decidua was associated with unexplained spontaneous abortion. Our results unraveled the precise timing of γδT cells occurring during pregnancy and the close relationship among endocrine, immune cells and pregnancy, which can further help understand and solve the problem of infertility and unexplained spontaneous abortion.

1. Introduction

To maintain the pregnancy, the maternal immune system needs to coordinate the balance between immunological tolerance towards allogeneic fetus and immune response to invading pathogens (PrabhuDas et al., 2015). The mucosa of the uterus (endometrium or decidua during pregnancy) is infiltrated by a large amount of immune cell subsets, which serve as the first line of defense against invading pathogens (Reis Machado et al., 2014). In addition, there is increasing evidence that uterine endometrial receptivity for implantation of semi-allogeneic embryo is associated with the uterine immune microenvironment which requires a compromise on the invasion of the embryo (Zenclussen and Hammerling, 2015). When the blastocyst aggressively adheres to the endometrium, endometrial stromal cells proliferate and re-differentiate into decidua (Gellersen and Brosens, 2014), and establish a healthy uterine immune milieu with specific tolerance towards foreign paternal fetal antigens without loss of the ability to fight infections which is different from un-pregnancy (Zenclussen and Hammerling, 2015). The decidua is considered to be the site where immune interactions between the mother and semi-allogeneic fetus occur (Duan et al., 2013). The largely recruited immune cells in decidua indicate that the maternal immune system somehow reacts to the fetus, however, this immune response seems to be locally suppressed (Mor et al., 2017). In a healthy maternal-fetal interface, specific innate immune response has been reported that up to 70% of decidual lymphocytes are CD16$^-$CD56^{bright} NK cells (Koopman et al., 2003).

Moreover, uterus is a crucial target for female sex hormones and directly affected by steroid hormones periodically (Wira et al., 2015). Thus, immune response during pregnancy may show dynamic variations as a consequence of hormonal changes (Mor and Cardenas, 2010; Wira et al., 2010). It has been found that in maternal-fetal interface, Th1 response may result in miscarriage through implantation failure and fetal resorption in early pregnancy (Saito et al., 2010). Therefore, to prevent fetal loss, in normal early pregnancy, there is a predominant shift towards Th2 bias accompanied by Th1 response suppression (Tan et al., 2015). This bias may be ascribed to the dramatic surge in circulating levels of estrogen and progesterone (Sykes et al., 2012). Progesterone, which has been shown to exert modulating function of

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various immune cells, is thought to be primarily responsible for the maternal-fetal tolerance (Tan et al., 2015), regardless of known progesterone receptor expression in these cells (Yao et al., 2017).

γδT cells have also been reported to contribute to the maintenance of tolerance during pregnancy (Huang et al., 2017). γδT cells, which show tissue-specific localization (Strbo et al., 2019), are largely accumulated in mucosa sites as well as in FRT and play a crucial role in epithelial barrier protection (Exley, 2011). Different from the conventional $CD4^+$ and $CD8^+$ T cells, γδT cells express heterodimeric TCRs composed of gamma (γ) and delta (δ) chains. In human peripheral blood, γδT cells represent a small subset of T lymphocytes (1–10%) (Hayday, 2009) while Vγ9Vδ2 cells (Davey et al., 2018) are predominant. By contrast, in maternal-fetal interface, decidual $CD3^+$ cells are dominated by γδT cells, and the majority of γδT cells express Vδ1 and produce high levels of TGF-β and IL-10 (Fan et al., 2011) which indicates that γδT cells might be a key player in maintaining the Th2 bias.

So far, studies on the mechanism of γδT cells in maternal-fetal immune tolerance have mainly focused on the decidua of early pregnancy. The immune changes throughout pregnancy, and the intricate relationships among female sex hormone, γδT cells and normal pregnancy are under investigation. In this study, we reported that the proportion of γδT cells infiltrated in endometrium and decidua fluctuated during the pregnancy, and was significantly correlated to the changes of sex hormones. Besides, Increased $V\delta2^+$ γδT cells were associated with spontaneous abortion. Collectively, our findings showed that the course of pregnancy is a synergistic regulation process of both female steroid hormones and immune system. In addition, the dynamic regulation of the immune system during pregnancy had a precise time point. This timing regulation could help the successful implantation and development of embryos. Immune disorder is increasingly implicated in the pregnancy-related complications.

2. Methods and materials

2.1. Participants

Endometrial tissues were collected from relatively fit premenopausal patients who had undergone fractional curettage and provided a detailed reproductive clinical history. The pathological reports showed normal or benign lesions (n = 24, aged 27–48 years), in which proliferative endometrium and secretory endometrium were 17 and 7, respectively, while 6 patients who in proliferative phases self-reported use of progesterone medications within the last two months. Patients taking anti-inflammatory drugs or complaining menstrual disorder were excluded. Decidual tissues were collected from normal early pregnant women (n = 12, aged 24–43 years, gestational age at 6–12 weeks, termination of pregnancy voluntary or because of fetal DNA abnormalities) and unexplained early spontaneous abortion patients (n = 14, aged 22–40 years, gestational age at 6–12 weeks). In addition, second-trimester decidual tissues were also collected (n = 11, aged 26–41 years, gestational age at 13–24 weeks, induced abortion due to fetal malformation). All samples were collected under sterile conditions.

2.2. Endometrial immune cells (EICs) and endometrial stromal cells (ESCs) Isolation

Endometrial tissues were obtained and kept in ice-cold Dulbecco's Modified Eagle Medium (DMEM)/F12 (Hyclone, Logan, UT, USA). The tissues were washed twice with 1× phosphate-buffered saline (PBS; Hyclone, Logon, UT, USA), then were cut into small pieces < 1 mm^3 and digested with DMEM/F12 containing 0.1% collagenase type IV (Sigma, San Francisco, CA, USA) for 40 min at 37 °C with gentle agitation. The final suspension was filtered through 400-, 200- and 100-μm nylon strainers in turn, collected the filtrate, centrifuged at 1200 r.p.m. for 10 min at 4℃. Re-suspended the sediment with DMEM/F12 supplemented with 10% fetal bovine serum (FBS; Hyclone), 10000U/L penicillin(Sigma) and 10,000μg/L streptomycin (Sigma), cultured the cells at 37℃, 5% CO_2 overnight. The suspension cells are the EICs, and the adherent cells are the ESCs.

2.3. Decidual immune cells (DICs) isolation

The previous processing steps of decidual tissues were consistent with that of endometrium. After being cut into small pieces, the decidual tissues were digested by RPMI1640 (Hyclone, Logan, UT, USA) supplemented with 0.1% collagenase type IV and 150U/ml DNase I (AppliChem, Darmstadt, Germany) for 40 min at 37℃ with gentle agitation. The filtrate was re-suspended by RPMI1640, then layered over a preformed percoll gradient prepared in 1 × PBS (60%, 40%, 20%). The gradient was centrifuged at 2500 rpm for 20 min. The DICs (between 40% and 20%, density of 1.048–1.062 g/ml) was removed and re-suspended with RPMI1640 supplemented with 10% FBS, 10000U/L penicillin and 10000ug/L streptomycin, cultured at 37℃, 5% CO_2.

2.4. Human γδT cells isolation

Peripheral blood mononuclear cells (PBMC) were isolated by ficoll (huajing, Shanghai) density gradient centrifugation. γδT cells were separated from PBMC by positively selection using $TCR\gamma/\delta^+$T cells Isolation Kit, human (Miltenyi, 130-092-892) according to the manufacturer instructions. The purity of γδT cells were confirmed to be > 95% by flow cytometry, using anti-human gamma delta TCR-FITC (clone B1.1, eBioscience, 11-9959-41).

2.5. Cell co-culture systems

Freshly isolated ESCs were seeded in 6-well plates at a density of 4×10^5 cells/ml overnight. The supernatant was then discarded and the cells were washed twice with 1 × PBS. Isolated γδT cells from healthy PBMC were added to the 24-well plates with or without ESCs at a γδT cells/ESCs ratio of 1:1 at 37℃ with 5% CO_2 for 48 h. The suspension cells were collected and detected by flow cytometry.

2.6. Antibodies and flow cytometry

For flow cytometric analysis, cells were incubated with fluorochrome-conjugated mAb for 20 min at 4℃ and washed with 1xPBS twice. Human-specific antibodies used in all follow-cytometric phenotyping included Fixable viability dye eFluor 506 (eBioscience, 65-0866-18), brilliant violet 421™ anti-human CD45 antibody (clone HI30, biolegend, 304032), PerCP/Cy5.5 anti-humanCD3 antibody (clone HIT3a, biolegend, 300328), anti-TCR-Vδ1-PE (Miltenyi, 130-100-536), anti-TCR-Vδ2-APC (Miltenyi, 130-099-664), anti-human gamma delta TCR-FITC (clone B1.1, eBioscience, 11-9959-41). Flow cytometry was performed on an LSRFortessa (BD Biosciences), and data were analyzed using Flowjo Software.

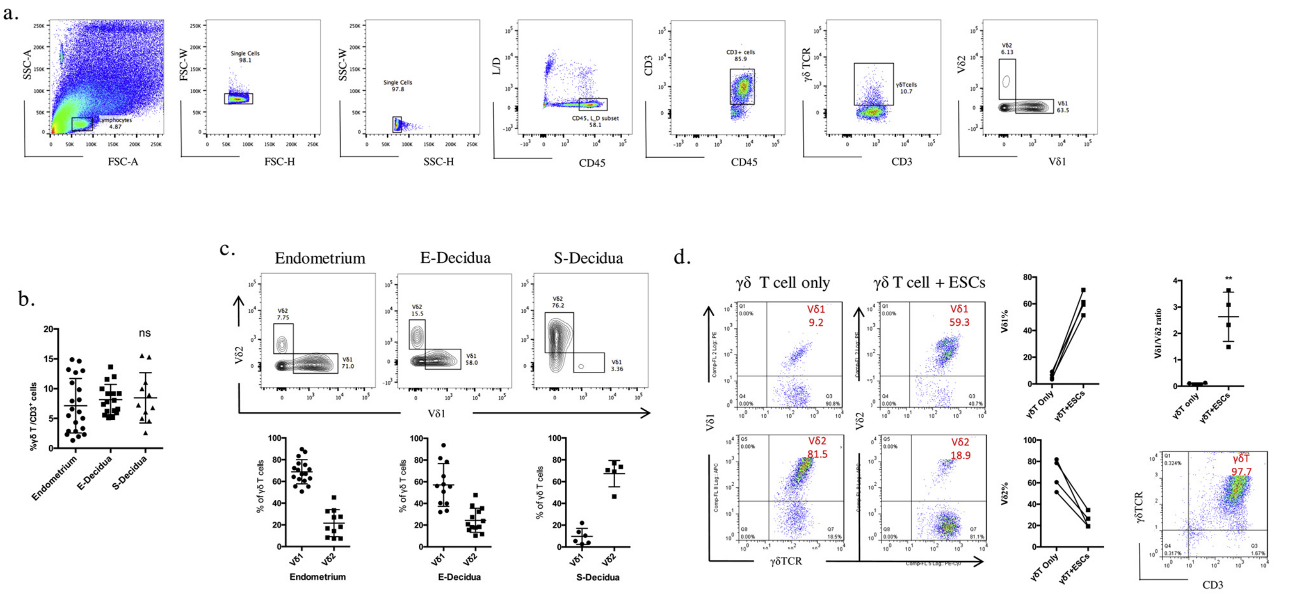
2.7. Statistical analysis

Data were shown as Mean ± SD. Statistical analysis was carried out using unpaired *t*-test or one-way ANOVA followed by Tukey test. P values of < 0.05 are considered to be statistically significant.

3. Results

3.1. The characteristics of γδT cells in the mucosa of FRT

In order to understand whether γδT cells are cyclically fluctuating during pregnancy, we first analyzed γδT cells that infiltrate in endometrium or decidua (both early pregnancy and second-trimester)



Purity of γδ T cell >95%

Fig. 1. Enumeration of the frequency of γδT cells in the endometrium and decidua. (a) γδT cells (Live CD45$^+$CD3$^+$ γδTCR$^+$) gating strategy and representative scatter plots. (b) Summary data showing percentage of γδT cells from endometrium (n = 21), early pregnancy decidua(E-decidua) (n = 17), and second-trimester decidua(S-decidua) (n = 11). One-way ANOVA analysis followed by Tukey test, $^{ns}P > 0.05$. (c) Quantification and representative figures displaying frequencies of Vδ1 and Vδ2 subsets out of γδT cells from endometrium, E-decidua and S-decidua. (d) The percentage of Vδ1 and Vδ2 subset in co-culture system was identified by flow cytometry. All analyses were performed in quadruplicate. This experiment was repeated twice and one representative sample was shown. The purity of γδT cells > 95%. Data are represented as Mean ± SEM. $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$.

from healthy gestational age women with or without pregnancy by flow cytometry. γδT cells were identified as live CD45$^+$CD3$^+$ γδTCR$^+$ cells, Vδ1 and Vδ2 represent the two main sub-populations (Fig. 1a). Surprisingly, the frequencies of γδT cells out of CD3$^+$ cells show no obvious difference among endometrium (M = 7.126%, SD = 4.56%), early decidua (E-decidua) (M = 8.161%, SD = 2.54%) as well as second-trimester decidua (S-decidua) (M = 8.456%, SD = 4.22%) (Fig. 1b). Consistent with reported data (Huang et al., 2017), majority of decidual γδT cells from first-trimester express Vδ1 (Fig. 1c) which may participate in Th2 bias for maintaining a successful pregnancy. Like early pregnancy, Vδ1 subset dominates in endometrial γδT cells isolated from healthy women (Fig. 1c). As a Th2-type cytokine profile could promote maternal-fetal tolerance (Duan et al., 2013), the specific endometrial immune microenvironment with Vδ1 subset dominant may be more conducive to the implantation of fertilized eggs. However, in second-trimester decidua, Vδ2 subset was largely accumulated (Fig. 1c) which may indicate that in second-trimester decidua Th1 response begins to recover. Notably, the decidua of second-trimester can only be obtained through parturition using a prostaglandin analog (Mifepristone or Misoprostol), so the accompanied inflammation associated with medical abortion may be related to the up-regulation of Vδ2 subset. To investigate whether subpopulation switching or selective recruitment leads to the Vδ1 subset dominant in endometrium, we co-cultured endometrial stromal cells (ESCs) with γδT cells isolated from autologous peripheral blood in a 1:1 ratio for 48 h and detected Vδ1 and Vδ2 subsets by flow cytometry. We found that Vδ1 subset as well as Vδ1/Vδ2 ratio increased obviously after co-cultured with ESCs compare to the control group without ESCs (Fig. 1d) which indicating that ESCs may be involved in Vδ1 and Vδ2 subset shift directly. Whether selectively recruitment is involved in Vδ1/ Vδ2 ratio inversion in endometrium still requires further verification. Collectively, we found that the proportion of total γδT cells show no obvious difference in endometrium and decidua during pregnancy, and endometrium microenvironment promote Vδ1 subset accumulation which may be more beneficial to the implantation of oosperm.

3.2. The fluctuation of γδT cell percentage is associated with progestogen

Given that steroid hormones are reported to be involved in regulating immune cells in maternal-fetal interface (Wira et al., 2010), we analyzed the frequency of γδT cells in proliferative phase and secretory phase endometrium respectively. The definition of the phase of the menstrual cycle at the day of surgical procedure was determined by reproductive clinical history and pathological report. The detail information was provided in Table 1. We observed that γδT cells in secretory phase (M = 10.692%, SD = 4.29%) were higher than those in proliferative phase significantly (M = 4.95%, SD = 3.65%) (unpaired *t test*, p = 0.0077) (Fig. 2a), which suggested that the fluctuation of γδT cells in endometrium may be affected by the steroid hormones during the menstrual cycle. Then we compared the changes of the sex hormones and γδT cells in proliferative phase and secretory phase, and found that γδT cells and progesterone share similar changing trend during the menstrual cycle (Fig. 2b). To further demonstrate the relationship between progesterone and endometrial γδT cells, we collected several specimens that were in proliferative phase and have been treated with progesterone in the preceding two month (Table 1), and compared the proportion of γδT cells in proliferative phase of the endometrium with or without progesterone treatment. Consistently, the percentage of γδT cells in proliferative phase with progesterone treatment (M = 11.589%, SD = 3.45%) was significantly higher than those in patients without progesterone treatment (unpaired t test, p = 0.0024) (Fig. 2c.). The increased percentage of γδT cells in secretory phase and proliferative phase with progesterone treatment strongly indicated that the infiltration of γδT cells in endometrium are regulated by progesterone. The observation that the percentage of decidual γδT cells in early pregnancy when the progesterone show high level, was higher than that of γδT cells in proliferative phase of the endometrium (Fig. 2d.) further confirmed the above conclusion.

Table 1
Composition of the patients and the percentage of $\gamma\delta$T cells in menstrual cycle phases.

Age	Menstrual Cycle	Drug Use (Last two month)	Diagnosis	Pathology Result	$\gamma\delta$T/CD3$^+$ (%)
47	Proliferative Phase	NO	IUD Removal	Endometrial Hyperplasia	4.32
46	Proliferative Phase	NO	Endometrial Hyperplasia	Proliferative Phase	1.95
44	Proliferative Phase	NO	IUD Removal	Proliferative Phase	2.88
41	Proliferative Phase	NO	IUD Removal	Simple Endometrial Hyperplasia	3.33
45	Proliferative Phase	NO	Endometrial Hyperplasia	Simple Endometrial Hyperplasia	14.6
26	Proliferative Phase	NO	Endometrial Hyperplasia	Simple Endometrial Hyperplasia	1.33
48	Proliferative Phase	NO	IUD Removal	Simple Endometrial Hyperplasia	7.93
37	Proliferative Phase	NO	Menstrual Disorder	Simple Endometrial Hyperplasia	4.20
35	Proliferative Phase	NO	Information Shortage	Proliferative Phase	5.45
46	Proliferative Phase	NO	Information Shortage	Simple Endometrial Hyperplasia	4.28
39	Proliferative Phase	NO	Menstrual Disorder	Proliferative Phase	4.16
42	Proliferative Phase	Duphaston 10days	Endometrial Hyperplasia	Proliferative Phase	14.87
43	Proliferative Phase	Duphaston 3days	Menstrual Disorder	Simple Endometrial Hyperplasia	12.7
27	Proliferative Phase	Luteosterone 14days	Menstrual Disorder	Simple Endometrial Hyperplasia	5.02
42	Proliferative Phase	Duphaston 20days	Menstrual Disorder	Proliferative Phase	10.97
45	Proliferative Phase	Duphaston 20days	Endometrial Hyperplasia	Proliferative Phase	13.2
29	Proliferative Phase	Luteosterone Unknown	Endometrial Hyperplasia	Simple Endometrial Hyperplasia	12.78
40	Secretory Phase	NO	Endometrial Hyperplasia	secretory Phase	6.57
28	Secretory Phase	NO	Endometrial Hyperplasia	secretory Phase	5.475
47	Secretory Phase	NO	Endometrial Hyperplasia	secretory Phase	8.15
45	Secretory Phase	NO	Hysteromyoma	secretory Phase	9.82
30	Secretory Phase	NO	Menstrual Disorder	secretory Phase	13.28
31	Secretory Phase	NO	Menstrual Disorder	secretory Phase	16.35
45	Secretory Phase	NO	IUD Removal	secretory Phase	15.2

3.3. Abnormal frequency and composition of $\gamma\delta$T cells are associated with abnormal pregnancy outcome

Abnormal immune regulation of the maternal-fetal interface can lead to adverse pregnancy outcomes (Liu et al., 2019). To study the role of $\gamma\delta$T cells in unexplained abortion, we also collected decidua from unexplained spontaneous abortion patients and found that the frequency of $\gamma\delta$T cells out of CD3$^+$ cells in spontaneous abortion decidua (AD) was up-regulated compared to normal early pregnancy decidua (ND) (Fig. 3a). Moreover, in spontaneous abortion decidua, Vδ2 subset was significantly increased (Fig. 3b). This result confirms that Vδ1 subset, but not Vδ2 subset, contributes to Th2 bias in maternal-fetal interface, the disruption of this balance can lead to poor pregnancy outcomes.

4. Discussion

Although the role of $\gamma\delta$T cells in maternal-fetal interface during

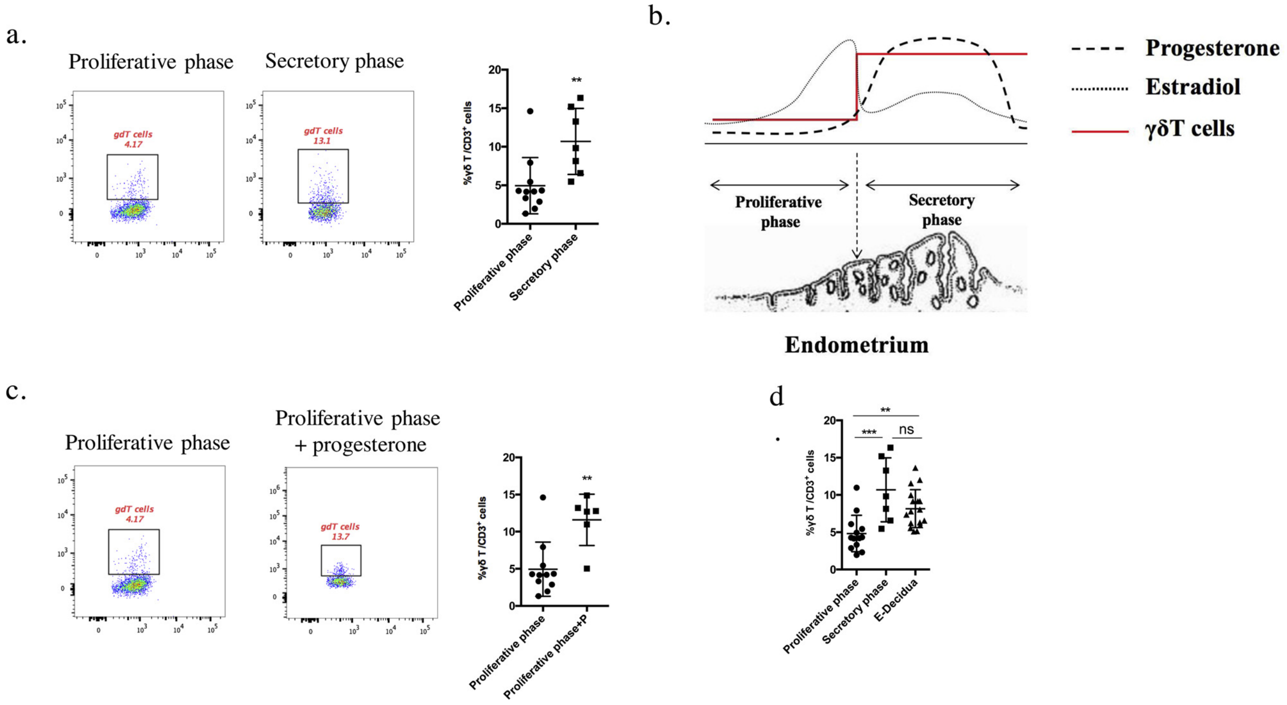


Fig. 2. The frequency of $\gamma\delta$T cells in menstrual cycle phases. (a) Representative figures and quantification of the percentage of $\gamma\delta$T cells in proliferative phase and secretory phase during the menstrual cycle. Unpaired *t*-test **P < 0.01. (b) Schematic diagram of changes in sex hormones and $\gamma\delta$T cells during the menstrual cycle. (c) Representative figures and quantification of the frequency of $\gamma\delta$T cells in proliferative phase with or without progestogen(P) treatment. (d) Summary data showing frequency of $\gamma\delta$T cells in endometrium from different phases of menstrual cycle and in healthy early pregnancy decidua. One-way ANOVA analysis followed by Tukey test, ^{ns}P > 0.05, **P < 0.01, ***P < 0.0001. Data are represented as Mean ± SEM. ^{ns}P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.0001.

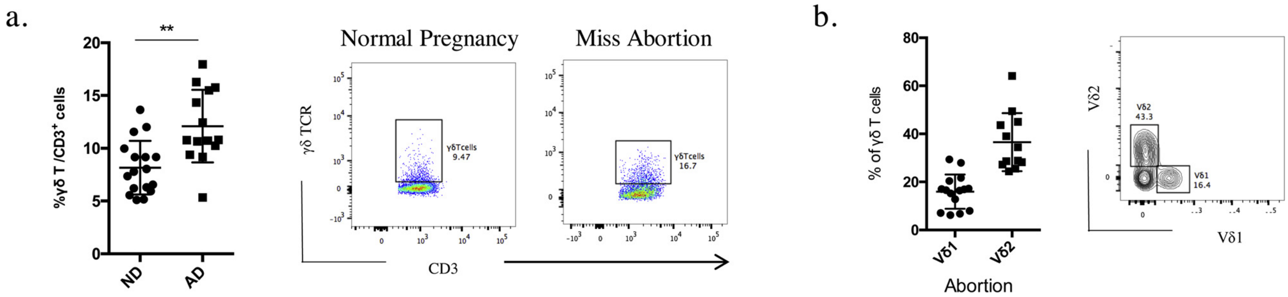


Fig. 3. γδT cells increased in decidua of spontaneous abortion patients. (a) Frequency of γδT cells from healthy early pregnancy decidua (ND) (n = 17) and miss abortion decidua (AD) (n = 11) and representative scatter plots. Unpaired *t*-test $^{**}P < 0.01$. (b) Frequencies of Vδ1 and Vδ2 subsets out of γδT cells from miss abortion decidua and representative scatter plots. Data are represented as Mean ± SEM. $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$.

human first-trimester has been reported (Fan et al., 2011), data is lacking on the transition of γδT cells throughout the whole pregnancy, from pre-pregnancy endometrium to second/third-trimester decidua, and the effect of sex hormones on γδT cells. In our study, we immunophenotyed the γδT cells isolated from endometrium and decidua for direct comparison and found that the composition of γδT cells is fluctuating over the course of pregnancy, and these changes are regulated by progesterone. Moreover, in endometrial and early-pregnancy decidual γδT cells from healthy women, Vδ1 subset accounted for the majority. Aberrant increased decidual Vδ2$^+$γδT cells in early-pregnancy are associated with unexplained spontaneous abortion. The balance of the Th1/Th2 response plays a pivotal role in a successful gestation, while a disorder of the balance may lead the poor pregnancy outcomes (Sykes et al., 2012). It has been reported that women who experienced recurrent spontaneous abortions exhibited a marked Th1 bias (Wang et al., 2016). Consistently, in our finding, Vδ2 subset was largely accumulated in abortion decidua suggesting that primary contributor to γδT cells involvement in Th2 bias should be the Vδ1 subset, while the Vδ2 subpopulation was more likely to involve in Th1 response. However, we also found that although the frequency of total γδT cells show no obviously difference from those in endometrium and early-pregnancy decidua, Vδ2 subset is predominant in the second-trimester decidual γδT cells obtained from healthy women. The subsets are functionally polarized from Th2 cells to Th1 cells, this result is contrary to the previous work which has described that immune tolerance dominates in the second-trimester and gradually revises in the third-trimester (Shah et al., 2017). Vδ2$^+$ γδT cells can be activated by recognizing natural phosphonates and synthetics phosphoantigens (Agrati et al., 2011), and response to inflammation and infection via producing inflammatory cytokines and inducing cytotoxicity in infected host cells (Li et al., 2013). A pro-inflammatory switch is reported necessary for labour (Mor et al., 2017). Different from the early pregnancy decidua obtained by curettage directly, the second-trimester decidua we got through parturition after using mifepristone combined with misoprostol. Mifepristone, a progesterone/glucocorticoid antagonist, competitively binds to the receptor of progesterone on decidua (Zhou et al., 2011), weakens the progesterone function, leads to the decidual necrosis. Therefore, whether the enrichment of Vδ2$^+$ γδT cells in second-trimester decidua is a normal physiology or the consequence of the inflammation of necrosis still needs to be further investigated.

γδT cells are disproportionately represented in the mucosal surface by selectively homing and retention (Vantourout and Hayday, 2013). Compare to conventional lymphocytes, γδT cells are imparted with greater mucosa-homing potential in the thymus where the epithelial signaling to γδT cells begins early (Guy-Grand et al., 2013). The expression of E- and P-selectin ligands on thymic γδT cells which bind to corresponding selectins expressed on the endothelium play a crucial role in this process (Austrup et al., 1997). Apart from adhesion molecules, a successful migration of γδT cells acquires specific chemokines receptors. Gut homing of γδT cells has been described relies on CCR9 and its ligands CC-chemokine ligand 25(CCL25) expressed by intestinal epithelial cells (Witherden and Havran, 2011). While, after arriving in mucosa, γδT cells seemingly engage in cross-talk with mucosal microenvironment to stabilize their retention. In human intestine, γδT cells repertoire undergoes dramatic changes with age and becomes oligoclonal in adult which suggest strong local selection by the surroundings (McCarthy and Eberl, 2018). However, how far mucosal milieu shape γδT cells in this process is unclear. Additionally, recent study confirmed that γδT cells function outside the thymus display remarkable plasticity. *in vitro* assay demonstrated that isopentenyl pyrophosphate (IPP) activation of Vγ9Vδ2$^+$ cells in the presence of IL-12 and anti-IL-4 antibody promotes IFN-γ- producing Th1-like cells, while IL-4 combined with anti-IL-12 antibody generating IL-4-secreting Th2-like cells (Pang et al., 2012). In our study, we co-cultured ESCs with γδT cells isolated from PBMC *in vitro* and found that the dominant Vδ2$^+$ cells bias to Vδ1$^+$ cells which supported the view that Vδ2$^+$ cells have plasticity. Whether ESCs regulates γδT cells polarization through cell-cell contact or soluble factors secretion requires further study.

The immune cells numbers, distribution and functions are tightly regulated by hormones, directly or indirectly, throughout the FRT to maintain the equilibrium between effectively fighting infection and the immune regulation that is required for successful sperm migration, fertilization, implantation and pregnancy (Wira and Fahey, 2008). The activity of the sex steroid receptors, for estrogens (ER α and ERβ) (Kovats, 2015) and for progesterone(PR) (Hall and Klein, 2017), expressed by immune cells were reported to be correlated with immune cells numbers and/or functional responses (Kadel and Kovats, 2018). Several studies demonstrated that endometrial immune cells are upregulated during the secretory phase compared with the proliferative phase of the menstrual cycle which is dominated by estrogen and characterized by the regulation of the endometrial tissue (Wira et al., 2015). During the ovulation, the peak of angiogenesis facilitates recruitment of leukocytes into the uterus, including NK cells, macrophages and neutrophils, which are necessary for pregnancy to occur (Wira et al., 2015). In our study, we found that endometrial γδT cells, which participate in Th2 bias, maintain maternal tolerance at the maternal-fetal interface, vary during the menstrual cycle, increase during the secretory and decrease during the proliferative phase, and show similar changing trends with progesterone. Decidual γδT cells was reported to express PR and become progestogen-responsive after exposed to tetoplacental antigens which stimulate PR expression, suggesting that γδT cells may be regulated by progestogen directly through PR (Shah et al., 2017).Apart from the indirect regulation of sex hormones, such as recruitment via chemokines, the direct regulation of sex hormones cannot be ruled out.

Ultimately, our research has demonstrated that the γδT cells are participated in maternal-fetal immune regulation throughout the entire pregnancy, and this orderly regulation are further regulated by progestogen precisely. The cooperation of γδT cells and progestogen promotes the receptive endometrium generation which ensures the successful implantation. Moreover, the increased γδT cells, mainly increased Vδ2 subset, in early pregnant decidua may contribute to Th1 response and promote miscarriage. Our study is of great significance to understand the physiologic mechanisms of normal pregnancy, which may help find methods to address infertility and miscarriage.

Authors' contributions

Dongli Cai carried out all the experiments, performed statistical analyses and wrote the manuscript. Yunhui Tang provided and collected the clinical specimens. Xiaoying Yao conceptualized the main idea of the project, designed the study, and supervised the whole project.

Ethical approval

This study was approved by the human ethics committee of Gynecology and Obstetrics hospital, Shanghai Medical School of Fudan University, Shanghai, China. Every participant signed a written informed consent, and all methods were carried out in accordance with the approved guidelines.

Conflict of interest

All authors declare that they have no conflict of interest

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The author(s) declare that they have no conflict of interest

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