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OUTSTANDING OBSERVATION

The majority of murine γδ T cells at the maternal–fetal interface in pregnancy produce IL-17

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Compared with lymphoid tissues, the immune cell compartment at mucosal sites is enriched with T cells bearing the γδ T-cell receptor (TCR). The female reproductive tract, along with the placenta and uterine decidua during pregnancy, are populated by γδ T cells predominantly expressing the invariant Vγ6$^+$Vδ1$^+$ receptor. Surprisingly little is understood about the function of these cells. We found that the majority of γδ T cells in the non-pregnant uterus, pregnant uterus, decidua and placenta of mice express the transcription factor RORγt and produce interleukin-17 (IL-17). In contrast, IFNγ-producing γδ T cells were markedly reduced in gestational tissues compared with uterine-draining lymph nodes and spleen. Both uterine-resident invariant Vγ6$^+$ and Vγ4$^+$ γδ T cells which are more typically found in lymphoid tissues and circulating blood, were found to express IL-17. Vγ4$^+$ γδ T cells were particularly enriched in the placenta, suggesting a pregnancy-specific recruitment or expansion of these cells. A small increase in IL-17-producing γδ T cells was observed in allogeneic compared with syngeneic pregnancy, suggesting a contribution to regulating the maternal response to paternally-derived alloantigens. However, their high proportions also in non-pregnant uteri and gestational tissues of syngeneic pregnancy imply a role in the prevention of intrauterine infection or quality control of fetal development. These data suggest the need for a more rigorous evaluation of the role of IL-17 in sustaining normal pregnancy, particularly as emerging data points to a pathogenic role for IL-17 in pre-eclampsia, pre-term birth, miscarriage and maternal immune activation-induced behavioral abnormalities in offspring.
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The mucosal surfaces of mammals are exposed to a myriad of environmental challenges not encountered by other tissues. The immune repertoire therefore differs greatly at these sites, and is particularly enriched in fast-acting cells of the innate immune system. γδ T cells, which are rare in blood and lymphoid tissues, are found at much higher proportions in the skin, lung, gut and the female reproductive tract. These unique microenvironments are largely populated by γδ T cells with homogeous Vγ-gene segments, exported from the thymus in defined waves during fetal and neonatal life.[1] Vγ5$^+$ γδ T cells, which home to the skin epidermis, are the first to arise in the thymus, followed by Vγ6$^+$ γδ T cells, which populate the tongue, lung, uterus and vagina.[2,3] Following these initial waves come Vγ4$^+$ and Vγ1$^+$ γδ T cells with increased T-cell receptor (TCR) diversity that constitute the main populations in the blood, peritoneum, spleen and lymph nodes (LNs).[1] (Heilig and Tonegawa mouse Vγ nomenclature used throughout[4]).

The immune cells of the uterus must both protect against infection and tolerate an allogeneic fetus during pregnancy. The fetus itself never directly contacts the maternal immune system. Instead, the site at which maternal immune cells come into contact with fetal-derived tissue is at the maternal–fetal interface; where the fetal-derived placenta is embedded within the maternal uterine decidua, a specialised stromal tissue that differentiates from uterine stromal cells at the site of embryo implantation. This interface allows for maternal immune cells integrated within the decidual stroma and those in maternal blood to directly contact placental trophoblast cells.[5]

Similar to the non-pregnant uterus, γδ T cells are found at the maternal–fetal interface during pregnancy. Indeed, γδ T cells are enriched at the maternal–fetal interface compared with non-pregnant uteri and spleen, and are higher in allogeneic compared with syngeneic matings, suggesting that they may play a specific role during pregnancy.[6–8] However the nature and significance of this role is far from understood. As with other immune cells at this site, their actions are implicated in protection from infection, prevention of immunological rejection of the fetus and modulation of the extent of trophoblast invasion into the uterus. In addition, the immune system likely has a function in quality control assessment of pregnancy progression, and selective termination of reproductive opportunities when fetal development or environmental conditions are inappropriate.[9] However, the existing data on uterine/decidual γδ

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T cells do not present a cohesive picture. In the uterus of pregnant women and ewes, γδ T cells are large granular cells, which in humans appear to express cytolytic molecules including perforin, granzyme B and FasL, indicating cytotoxic potential.[10,11] At the same time, a proportion of human and mouse decidual γδ T cells in early pregnancy have been found to express IL-10 and TGFβ, suggesting a role in regulating the anti-fetal response.[7,12–14] In addition, there is some evidence for a minor TNFα- and IFNγ-producing population in the peri-implantation period.[13,14] However, the proportion of cells producing these cytokines was either undefined or constituted only a small proportion of the γδ T cells present, leaving the function of γδ T cells in gestational tissues unclear.

The cytokine milieu at the maternal–fetal interface is vital for the establishment and maintenance of pregnancy. Previously regarded as a matter of Th2-immunity presiding over Th1-immunity, it is becoming clear that successful pregnancy is not this simple. For while Th2 polarization does occur, and excess Th1 cytokines are detrimental to pregnancy[15–17], uterine NK (uNK) cell-derived IFNγ is important for the vascular remodeling required for successful implantation.[18] Furthermore, it is now known that Tregs and Th17 cytokines can influence pregnancy outcome. While a role for Tregs in tolerating the allogeneic fetus is recognized[19], the part played by Th17 cells and cytokines is more controversial. Th17 cells have been found in the decidua of normal healthy pregnancy, and appear to support the survival and invasion of trophoblast cells.[20] In addition, an IL-17-producing innate lymphoid cell has also recently been described in first trimester decidua, although the role of this cell type has yet to be explored.[21] However an excess of Th17 cells has been reported in the blood of women with pre-eclampsia[22] and in the blood and decidua of pre-term birth[23] and recurrent abortion cases.[24,25] While it remains unclear whether increased Th17 cells are a cause or a consequence of these pathological outcomes, the transfer of purified Th17 cells into pregnant mice dramatically increases the proportion of fetal loss.[26] Further to this, a recent study found that IL-17 has a unique contribution to maternal immune activation-induced behavioral abnormalities in offspring.[27] So while excess Th17 cells and cytokines can undoubtedly yield a negative pregnancy outcome, their role when in normal proportions in healthy pregnancy is still far from understood.

In this study, we examined the γδ T cells in the placenta, decidua and uterus of pregnant mice, and compared these with γδ T cells in the spleen, blood and uterine-draining LN, along with non-pregnant uterus. We found that γδ T cells in gestational tissues are large, granular cells and well over half of them are positive for the transcription factor RORγt and produce IL-17A (referred to as IL-17). IL-17 is produced by both uterine-resident Vγ6$^+$ γδ T cells and Vγ4+ γδ T cells, with a particular influx of Vγ4$^+$ cells into the placental tissue. These data suggest the need for a careful evaluation of the role of IL-17 in healthy pregnancy, and point to actions beyond simply a deleterious role in pre-eclampsia, pre-term birth and miscarriage.

RESULTS AND DISCUSSION

Enrichment of γδ T cells at the maternal–fetal interface

Initial characterization of the γδ T cells in the uterus and the maternal–fetal interface was achieved via sequencing γδ T-cell hybridomas in the 1990s. It was deduced that both sites are dominated by γδ T cells expressing the invariant Vγ6/Vδ1 receptor, with only a small proportion of Vγ4$^+$ and Vγ1$^+$ cells detected.[3,6] There is very little data about the functional attributes of uterine γδ T cells, and their purpose in gestational tissues remains speculative. As such, we set out to further characterize these cells.

Cells from the non-pregnant mouse uterus, along with placenta, decidua and uterine tissue from gestation day (g.d.) 15.5 pregnant mice were assessed. We analyzed both DBA/2J × DBA/2J and C57BL/6 × C57BL/6 syngeneic pregnancies, along with DBA/2J × C57BL/6 allogeneic pregnancy. We found that γδ T cells in gestational tissues of mice are large, granular cells compared with their counterparts in the spleen (Figure 1a and Supplementary Figure 1a), a finding similar to that observed in humans and sheep.[10,11] As a proportion of both lymphocytes and T cells, γδ T cells in the uterus and at the maternal–fetal interface are greatly enriched compared with that found in the uterus-draining para-aortic LN, spleen and blood (Figures 1b and c and Supplementary Figures 1b and c), confirming previous observations.[6] Interestingly, the proportion of γδ T cells in the non-pregnant uterus varies depending on the stage of the estrous cycle, being highest in diestrus and lowest in proestrus (Figure 1d). Fluctuations have been observed in other leukocyte populations over the course of the estrous cycle[28], indicating regulation by hormonal fluctuations and suggesting a potential role in preparing the uterus for implantation.

Unlike the initial studies by Heyborne *et al.*[6], we separated the placenta and decidua so that these two components of the maternal–fetal interface could be analyzed separately. Although placental cell preparations contain maternal blood, the placenta contains a significantly higher proportion of γδ T cells compared with peripheral blood (Figures 1b and c), demonstrating that cells are specifically recruited and/or retained in this site. Interestingly, it was here and not the decidua, where we observed an increase in γδ T cells as a result of allogeneic pregnancy (Figure 1e).

Pregnancy is associated with accumulation of Vγ4$^+$ γδ T cells in the uterus and placenta

Analysis of gestational tissues by flow cytometry consistently revealed two γδ T-cell populations as delineated by CD3 and γδTCR (Figure 2a). The γδTCR^{lo}CD3^{bright} subset recapitulates that recently discovered within lung and skin γδ T cells.[29] Designated as CD3^{bright} γδ T cells in this study, the cells were found to uniformly express the canonical germline encoded Vγ6/Vδ1$^+$ TCR, offering a convenient surrogate for detection of Vγ6$^+$ cells, which lack a commercially available antibody. We also confirmed that this CD3^{bright} (Vγ6$^+$) subset is negative for Vγ1 and Vγ4 in gestational tissues (Figure 2b). We found that decidual and uterine γδ T cells contain a very high proportion of CD3^{bright} (Vγ6$^+$) γδ T cells (~60%) (Figure 2c and Supplementary Figure 1d), much higher than that reported in lung and skin γδ T cells (~25%).[29] This proportion did not change between non-pregnant and pregnant uteri (Figure 2c). Interestingly, the placenta displays a phenotype intermediate between the blood and other gestational tissues (Figure 2c and Supplementary Figure 1d), with CD3^{bright} (Vγ6$^+$) cells representing between 5–70% of γδ T cells. The presence of Vγ6$^+$ cells in the placenta itself suggests that these cells may be 'seeded' from the decidua as it differentiates from uterine tissue, and that the remaining Vγ6$^-$ cells are recruited from maternal circulation.

T cells expressing Vγ1$^+$ and Vγ4$^+$ TCRs, which are typically found in the lymphoid tissues, blood and lung, have been described as only minor populations in the uterus.[3,6] Vγ1$^+$ cells have been shown to recognize trophoblast cells via their TCR in *in vitro* cultures[30], and may produce TNF or TGF-β2 in early pregnancy[31], while no role has been ascribed to Vγ4$^+$ cells at the maternal–fetal interface. To determine the Vγ-usage of the remaining γδ T cells in non-pregnant and pregnant mice, we examined the CD3^{lo} (Vγ6$^-$) subset for Vγ1 and Vγ4 expression. In the non-pregnant uterus, we found that the CD3^{lo} subset is comprised of equivalent proportions of Vγ1$^+$ and Vγ4$^+$ γδ

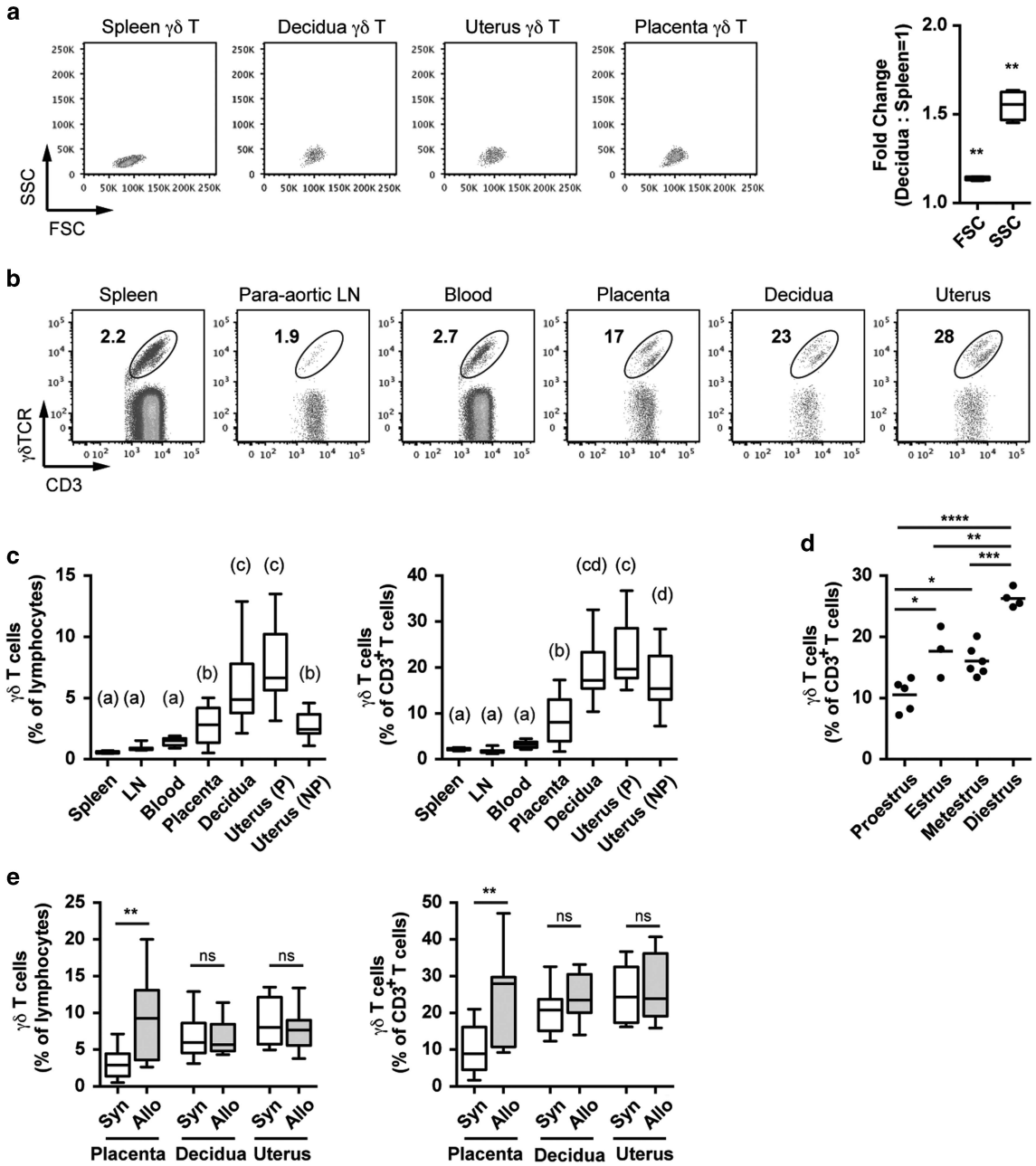


Figure 1 Enrichment of γδ T cells in gestational tissues. Spleen, para-aortic LN, blood, placenta, decidua and uterus were recovered on g.d. 15.5 from pregnant DBA/2J female mice mated with syngeneic males. Uterus was also recovered from age-matched virgin non-pregnant DBA/2J females. (**a**) FSC and SSC of γδ T cells ($CD3^+\gamma\delta TCR^+$) from the spleen, decidua, uterus and placenta and the fold change (right panel) of FSC and SSC between spleen (=1) and decidual γδ T cells. (**b**) Proportion γδ T cells of $CD3^+$ T cells as shown by representative flow cytometry plots. (**c**) Proportion γδ T cells of lymphocytes (left panel) and $CD3^+$ T cells (right panel) from lymphoid and gestational tissues and non-pregnant uterus. (**d**) Proportion γδ T cells of $CD3^+$ T cells from the non-pregnant uterus of mice at different stages of the estrous cycle. (**e**) Proportion γδ T cells of lymphocytes (left panel) and $CD3^+$ T cells (right panel) from tissues from DBA/2J mice mated with DBA/2J (Syn) or C57BL/6 (Allo) males. Box and whisker graphs in **a**, **c** and **e** display 25–75% (box) and minimum to maximum (whisker). The line in the box represents the median value. Data in **a** are representative of γδ T cells from syngeneically mated DBA/2J ($n=4$) females. Data in **b**, **c** are from DBA/2J females ($n=12$) mated with DBA/2J males, and non-pregnant DBA/2J females ($n=18$). Data in **d** are from non-pregnant DBA/2J females ($n=18$), with each data point representing an individual mouse. Data in **e** compares DBA/2J females mated with DBA/2J males ($n=12$) or C57BL/6 males ($n=10$). Data from DBA/2J×DBA/2J matings are collated from 12 independent experiments, data from DBA/2J×C57BL/6 matings are collated from 10 independent experiments and data from non-pregnant mice are collated from 4 independent experiments. Uterus (NP), non-pregnant uterus; uterus (P), pregnant uterus. Data in **a** are analyzed by paired Student's *t*-test. Data in **c**, **d** are analyzed by one-way analysis of variance with Tukey's post test, with significant differences ($P\leqslant0.05$) denoted by different letters in **c**. Data in **e** are analyzed by unpaired Student's *t*-test with Welch's correction. $^*P\leqslant0.05$, $^{**}P\leqslant0.01$, $^{***}P\leqslant0.001$, $^{****}P\leqslant0.0001$. NS, not significant. A full color version of this figure is available online at the *Immunology and Cell Biology* website.

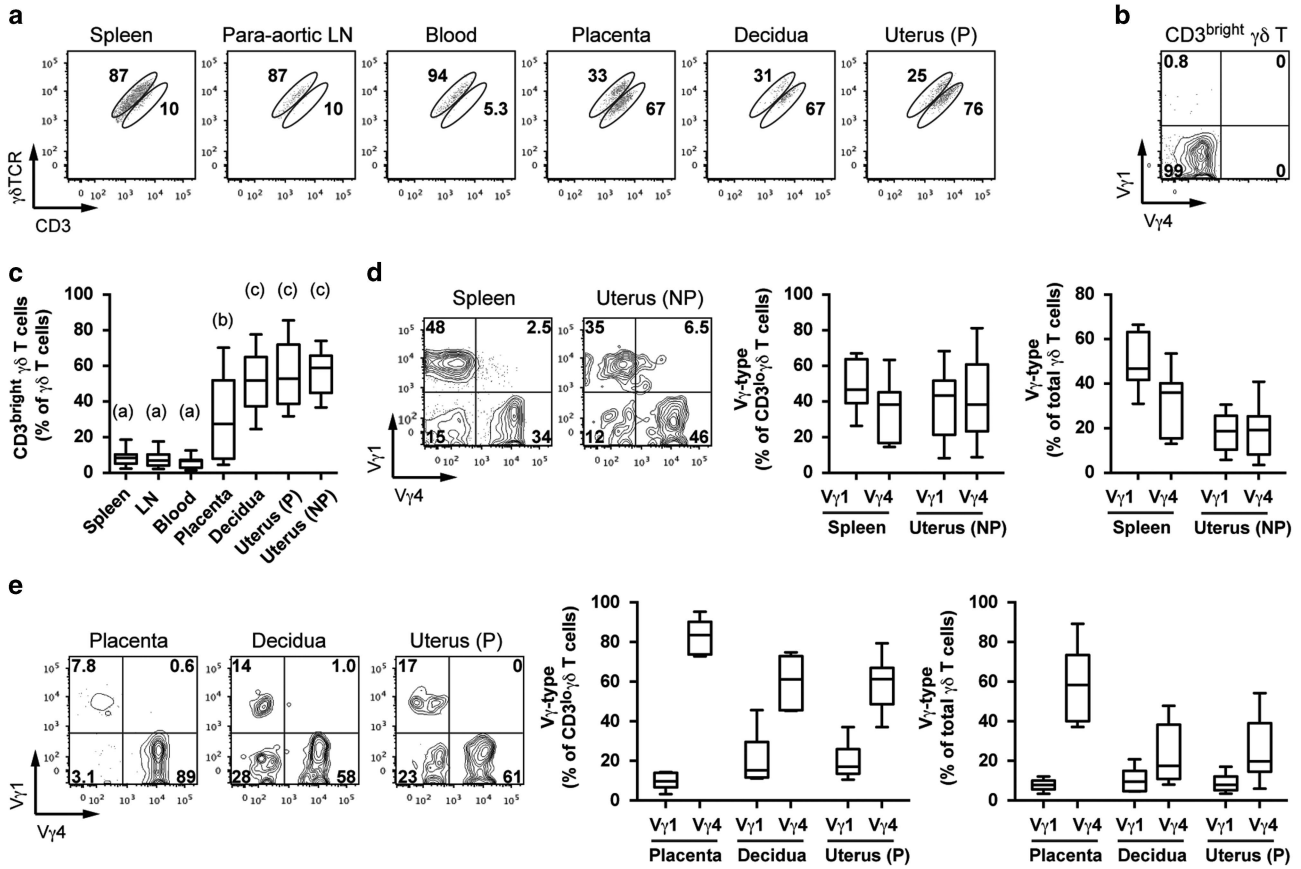
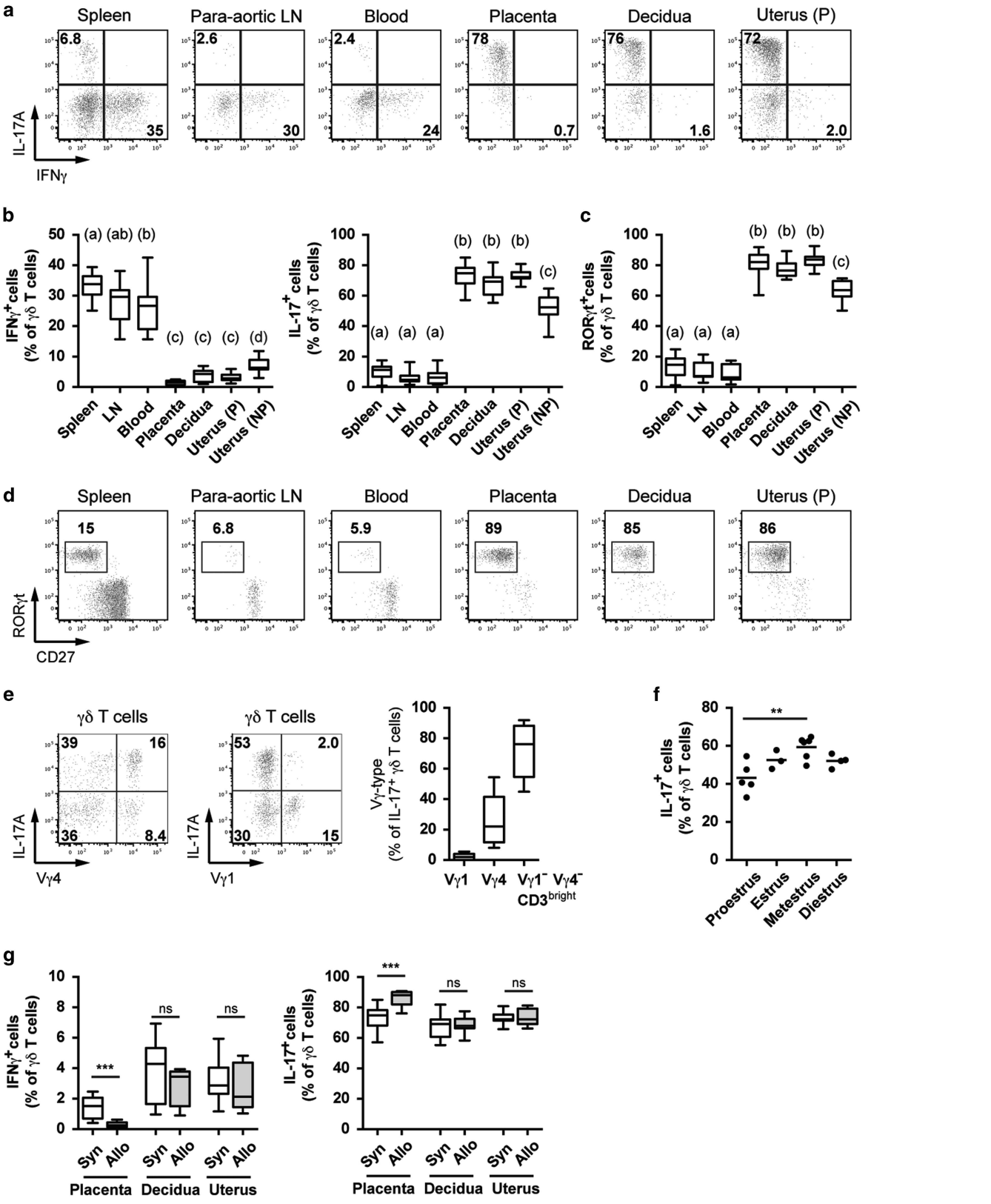


Figure 2 CD3^{bright} (Vγ6$^+$) and Vγ4$^+$ cells dominate γδ T cells in gestational tissues. Spleen, para-aortic LN, blood, placenta, decidua and uterus were recovered on g.d. 15.5 from pregnant DBA/2J and C57BL/6 female mice mated with syngeneic males. Uterus was also recovered from age-matched virgin non-pregnant DBA/2J females. (**a**) Proportion of CD3^{bright} and CD3^{low} cells of γδ T cells as shown by representative flow cytometry plots. (**b**) Percentage of Vγ1$^+$ and Vγ4$^+$ of CD3^{bright} γδ T cells from pregnant uterus shown as representative flow cytometry plot. (**c**) Proportion of CD3^{bright} (Vγ6$^+$) cells of γδ T cells from lymphoid and gestational tissues and non-pregnant uterus. (**d**) Percentage of Vγ1$^+$ and Vγ4$^+$ γδ T cells in the spleen and uterus of non-pregnant mice shown as representative flow cytometry plots (gated on CD3^{low} γδ T cells) (left panel), as a proportion of CD3^{low} γδ T cells (middle panel) and as a proportion of total γδ T cells (right panel). (**e**) Percentage Vγ1$^+$ and Vγ4$^+$ γδ T cells in the placenta, decidua and pregnant uterus shown as representative flow cytometry plots (gated on CD3^{low} γδ T cells) (left panel), as a proportion of CD3^{low} γδ T cells (middle panel) and as a proportion of total γδ T cells (right panel). Box and whisker graphs in **c**–**e** display 25–75% (box) and minimum to maximum (whisker). The line in the box represents the median value. Data in **a**, **c** and **d** are from DBA/2J females ($n=12$) mated with DBA/2J males, and non-pregnant DBA/2J females ($n=15$) and non-pregnant C57BL/6 females ($n=6$). Data in **e** are from syngeneically mated DBA/2J ($n=2$) and C57BL/6 ($n=4$) females. Data from pregnant mice are from 12 independent experiments, and data from non-pregnant mice are from three independent experiments. Uterus (NP), non-pregnant uterus; uterus (P), pregnant uterus. Data in **c** are analyzed by one-way analysis of variance with Tukey's post test, with significant differences ($P\leqslant0.05$) denoted by different letters. A full color version of this figure is available online at the *Immunology and Cell Biology* website.

Figure 3 The majority of γδ T cells in gestational tissues produce IL-17 and express RORγt. Spleen, para-aortic LN, blood, placenta, decidua and uterus were recovered on g.d. 15.5 from pregnant DBA/2J and C57BL/6 female mice mated with syngeneic males. Uterus was also recovered from age-matched virgin non-pregnant DBA/2J females. (**a**) Proportion of IL-17A$^+$ and IFNγ$^+$ cells of γδ T cells as shown by representative flow cytometry plots. (**b**) Proportion of IFNγ$^+$ cells (left panel) and IL-17A$^+$ cells (right panel) of γδ T cells from lymphoid and gestational tissues and non-pregnant uterus. (**c**) Proportion of RORγt$^+$ cells of γδ T cells from lymphoid and gestational tissues and non-pregnant uterus. (**d**) Proportion of RORγt$^+$CD27$^-$ cells of γδ T cells as shown by representative flow cytometry plots. (**e**) Proportion of IL-17-producing γδ T cells that are Vγ1$^+$, Vγ4$^+$ or are Vγ1$^-$ Vγ4$^-$CD3^{bright} (presumed Vγ6$^+$) in non-pregnant uterus as shown by representative flow cytometry plots (left and middle) and box and whisker plot (right). (**f**) Proportion IL-17A$^+$ cells of γδ T cells from the non-pregnant uterus of mice at different stages of the estrous cycle. (**g**) Proportion IFNγ$^+$ cells (left panel) and IL-17A$^+$ cells (right panel) of γδ T cells from tissues from DBA/2J mice mated with DBA/2J (Syn) or C57BL/6 (Allo) males. Box and whisker graphs in **b**, **c**, **e** and **g** display 25–75% (box) and minimum to maximum (whisker). The line in the box represents the median value. Data in **a**–**d** are from DBA/2J females ($n=12$) mated with DBA/2J males, and non-pregnant DBA/2J females ($n=10$ for IFNγ and $n=18$ for IL-17). Data in **f** are from non-pregnant DBA/2J females ($n=18$) analyzed in four independent experiments, with each data point representing an individual mouse. Data in **g** compares DBA/2J females mated with DBA/2J males ($n=12$) or C57BL/6 males ($n=10$). Data from DBA/2J×DBA/2J matings are collated from 12 independent experiments, data from DBA/2J×C57BL/6 matings are collated from 10 independent experiments and data from non-pregnant mice are collated from four independent experiments. Uterus (NP), non-pregnant uterus; uterus (P), pregnant uterus. Data in **b**, **c** and **f** are analyzed by one-way analysis of variance with Tukey's post test, with significant differences ($P\leqslant0.05$) denoted by different letters. Data in **g** are analyzed by unpaired Student's *t*-test with Welch's correction. $^{**}P\leqslant0.01$, $^{***}P\leqslant0.001$. NS, not significant. A full color version of this figure is available online at the *Immunology and Cell Biology* website.

T cells (Figure 2d). In pregnancy, this changes. We found that Vγ4$^+$ cells outweigh Vγ1$^+$ in the pregnant uterus and decidua (Figure 2e). Interestingly, rather than the expected intermediate pattern between decidua and blood, the placenta was seen to contain an even greater proportion of Vγ4$^+$ cells, at around 80% of the CD3^{lo} (Vγ6$^-$) population, and around 50% of γδ T cells overall (Figure 2e). These data suggest that Vγ4$^+$ γδ T cells are specifically recruited to, or expand within, the maternal–fetal interface during pregnancy.



The majority of γδ T cells in gestational tissues express RORγt and IL-17

The effector function of γδ T cells generally correlates with their expression of Vγ-gene segments and their tissue localization.[32] Vγ1$^+$ γδ T cells appear to be largely skewed towards Th1-type cytokines, producing IFNγ in infection and tumor models, although some IL-4 production has been reported.[33–35] Vγ6$^+$ γδ T cells have been found to produce IL-17 in the lung, peritoneal cavity, non-pregnant uterus, liver, inflamed joints and lymphoid tissues.[33,36–38] Vγ4$^+$ γδ T cells can produce IL-17 in the skin, lung, peritoneal cavity, liver and lymphoid tissues[36,39,40], but they can also produce IFNγ in response to peritoneal tumors[33] and both Th1 and Th2 cytokines during pulmonary inflammation.[41] Very little data are available on the cytokine-producing potential of γδ T cells at the maternal–fetal interface during pregnancy.

We examined the production of IFNγ and IL-17 by γδ T cells in pregnant mice. As in non-pregnant mice, IFNγ-producing cells made up about 30% of γδ T cells in the blood and lymphoid tissues, while IL-17-producing γδ T cells were a minor population (Figures 3a and b and Supplementary Figure 1e). Surprisingly, however, IFNγ$^+$ γδ T cells were scarce in gestational tissues, while IL-17$^+$ cells constituted an overwhelming majority (~70%) of γδ T cells at these sites (Figures 3a and b and Supplementary Figure 1e). These cells were also RORγt$^+$ and CD27^{neg} (Figures 3c and d and Supplementary Figure 1f), hallmarks of IL-17-producing γδ T (γδT17) cells in peripheral sites[39,42,43], and were composed of both CD3^{bright}Vγ1$^-$Vγ4$^-$ (Vγ6$^+$) and Vγ4$^+$ γδ T cells (Figure 3e). Although IL-17 was also the dominant cytokine in the non-pregnant uterus, it was found at significantly lower proportions than in the pregnant uterus (Figure 3b). Instead a small enrichment in IFNγ was observed (Figure 3b). This most likely reflects the changing balance of Vγ1$^+$ to Vγ4$^+$ γδ T cells from the non-pregnant to pregnant uterus (Figures 2d and e), as the proportion of CD3^{bright} (Vγ6$^+$) cells did not change (Figure 2c). Interestingly, the proportion of IL-17-producing γδ T cells was lower in proestrus compared with metestrus (Figure 3f). This, coupled with the overall percentage of γδ T cells being lower in proestrus (Figure 1d) suggests a potential contribution to preparing the uterus for implantation. In pregnant mice, the placenta was the only site of difference in allogeneic compared with syngeneic pregnancy. Here we observed an even greater decrease of IFNγ$^+$ γδ T cells and a significant increase in IL-17$^+$ γδ T cells compared with syngeneic pregnancy (Figure 3g).

Considering the emerging role of IL-17 in pre-eclampsia, pre-term birth and miscarriage, it was surprising to find that IL-17-producing cells make up the overwhelming majority of γδ T cells in the gestational tissues of normal pregnant mice. Our data suggests that along with uterine-resident Vγ6$^+$, Vγ4$^+$ γδ T cells also make a significant contribution to IL-17 production, particularly within the placenta. Considering that the proportion of Vγ4$^+$ cells increases from non-pregnant to pregnant tissues, and is very high in the blood-rich placenta, we suggest that this population in particular may be recruited specifically for the period of gestation. CCL2 is expressed by decidual stromal cells, and has been found to recruit Th17 cells to first trimester human decidua, which in turn support trophoblast invasion.[20] As the CCL2–CCR2 axis contributes to the recruitment of Vγ4$^+$ γδT17 cells into inflamed skin[44], it may also be the means by which these cells come to be enriched in the placenta and associated gestational tissues. In addition, their presence at these sites may depend on cytokines such as IL-23, IL-1β or IL-7, all of which support the function or homeostasis of γδT17 cells in lymphoid tissues.[43,45] However, since elevated IL-1β is associated with placental inflammation[46] and IL-23 does not appear to be highly expressed by placenta or decidual macrophages[47], their contribution to γδT17 cells in gestational tissues is uncertain.

The high proportion of γδT17 cells suggests that they play an important biological role during normal pregnancy. As mentioned, immune cells in gestational tissues can protect from infection, prevent rejection or modulate the extent of trophoblast invasion. While it is possible that γδT17 cells aid trophoblast invasion in early pregnancy, as has been suggested for CD4$^+$ Th17 cells[20], we detected γδT17 cells in both non-pregnant uteri and in day E15.5 gestational tissues, suggesting an ongoing role at these sites, not just one restricted to early pregnancy. Furthermore, no breeding difficulties are reported for TCRδ- and IL-17A-deficient animals housed in specific-pathogen-free conditions, implying that implantation is not significantly affected in their absence. To protect from an immunological rejection of the fetus typically requires recognition of paternal alloantigens, and some decidual γδ T cells can recognize trophoblast antigens. In humans, some Vδ2 cells recognize HLA-E, a phenomenon believed to inhibit maternal anti-fetal responses.[48] In mice, Vγ1$^+$ cells are reported to recognize a conserved mammalian molecule, yet no such recognition for Vγ1$^-$ cells was found[30], implying that Vγ4$^+$ and Vγ6$^+$ γδT17 cells may not play this role. However, we did observe an increase in the proportion of IL-17-producing γδ T cells in the placenta of allogeneic compared with syngeneic pregnancy, and as such cannot rule out a contribution towards controlling fetal tolerance. In addition there is evidence that under some circumstances, T cells contribute to activating pro-inflammatory responses that terminate pregnancy[49], potentially in an active mechanism of immune-mediated quality control to prevent progression of infected or aberrantly developing fetuses, or otherwise inappropriate reproductive investment.[9] It seems possible that γδT17 cells in gestational tissues might exert cytotoxic activity and contribute to an immune surveillance role, but further work is required to investigate whether such an effector function exists.

Considering the functions of IL-17, it seems likely that γδT17 cells in gestational tissues would contribute to host defence. The placenta is the site of nutrient and gas exchange between maternal and fetal circulations, and where blood-borne pathogens can gain access to the fetus. The combination of immune cells and the highly infection-resistant syncytiotrophoblasts (placental cells that directly contact maternal blood), makes the placenta a formidable barrier to pathogens.[50] However, there is still much to understand about how the immune system protects against invasion without producing excess inflammation that can trigger pre-term birth and other pregnancy complications such as preeclampsia. Systemically, IL-17 is crucial for host defence against a range of bacterial and fungal infections, and promotes pathogen clearance by induction of granulopoiesis and neutrophil recruitment.[51] While this could also occur in gestational tissues, neutrophil influx causes membrane rupture in both term and pre-term labor[52], and as such is not desirable until term.

In addition, IL-17 has been found to promote the production of antimicrobial proteins and peptides at mucosal surfaces such as the skin and lung.[53] Antimicrobials are an innate form of defence that is well-documented in gestational tissues[54], as well as being an important component of the fetal and neonate immune system.[55] It is not known whether IL-17 contributes to the expression of antimicrobials at the maternal–fetal interface, however, it is interesting to note that neonatal monocytes are skewed towards IL-23 production[55], a situation that could promote an IL-17 led induction of antimicrobials at least in newborns.

In summary, we have found that the majority of γδ T cells at the maternal–fetal interface in mice produce IL-17. Both uterine-resident invariant Vγ6$^+$ and Vγ4$^+$ γδ T cells produce IL-17, with an enrichment of the latter in the placenta, suggesting a particular recruitment of these cells to this site during pregnancy. The function of these cells is not known, however, the high proportion found even in non-pregnant uterus and syngeneic pregnancy suggests that they may contribute to host defence. Their role in preventing intrauterine infection and in sustaining normal pregnancy requires further investigation, particularly in the face of emerging data suggesting that IL-17 plays a deleterious role in pre-eclampsia, pre-term birth and miscarriage.

METHODS

Mice

DBA/2J and C57BL/6 mice were obtained from the Animal Resources Centre (Perth, WA, Australia). Animals were housed under conventional barrier protection and handled in accordance with the Garvan Institute of Medical Research and St Vincent's Hospital Animal Experimentation and Ethics Committee, which comply with the Australian code of practice for the care and use of animals for scientific purposes. For experiments, one or two 8–20-week-old virgin DBA/2J or C57BL/6 females were housed with either DBA/2J or C57BL/6 males. Mice were examined daily for vaginal plugs, with the morning of plug detection designated as g.d. 0.5, at which time pregnant females were separated from the male and housed individually. On g.d. 15.5, pregnant mice were killed by cervical dislocation and spleen, para-aortic LN and blood were collected. Pregnant uteri were removed and individual placenta and decidua were separated by blunt dissection. For the collection of non-pregnant uteri, the stage of the estrous cycle of virgin mice was first determined by examination of vaginal lavage.[56] Briefly, the vagina was flushed three times with a single volume of 10 µl endotoxin-free PBS. The final flush was deposited onto a glass microscope slide and observed under a light microscope. Proestrus was identified by the presence of nucleated epithelial cells and some cornified cells. Estrus was identified by abundant anucleated cornified epithelial cells. Metestrus was identified by the presence of leukocytes in addition to nucleated and cornified epithelial cells, while vaginal exudates at diestrus contained an almost homogeneous population of leukocytes. The mice were killed by cervical dislocation and the uterus was separated from the ovaries and cervix.

Flow cytometry

Single-cell suspensions were prepared from spleen and LN by mechanical disruption. Gestational tissues (uterus, decidua and placenta) were macerated using dissection scissors and incubated in 2 mg ml^{-1} collagenase D (Roche, Basel, Switzerland) containing 0.01% DNase at 37 °C for 40 min. Cells were passed through a 100-µm cell strainer (Becton Dikinson) and red blood cell lysis was performed if required. Cell suspensions were stained for FACS analysis using the following antibodies: fluorescein isothiocynate- (FITC) anti-Vγ2 (clone UC3-10A6; Biolegend, San Diego, CA, USA), FITC-anti-IFNγ (clone XMG1.2; Biolegend), FITC-anti-CD27 (clone LG.7F9; eBioscience, San Diego, CA, USA), phycoerythrin- (PE) anti-Vγ1.1/Cr4 (clone 2.11; Biolegend), PE-anti-RORγ(t) (clone B2D; eBioscience), PE-Cyanine7 (PE-Cy7)-anti-CD14 (clone SA14-2, Biolegend), PerCP-Cyanine5.5-anti-IL-17 (clone eBio17B7; eBioscience), allophycocyanin- (APC) anti-γδTCR (clone GL3; eBioscience), APC-eFluor 780-anti-CD45.2 (clone 104; eBioscience) and eFluor 450-anti-CD3 (clone 17A2; eBioscience). Viability was determined using 7-Aminoactinomycin D (7AAD) (BD Biosciences, San Jose, CA, USA). Samples were analyzed on a CantoII (BD Biosciences). Data were analyzed using FloJo software (Treestar Inc, Ashland, OR, USA). γδ T cells were γδTCR$^+$CD3$^+$ and analyzed within either the CD3$^+$ T-cell gate or lymphocyte gate. Lymphocyte gate was determined using FSC and SSC parameters with the exclusion of CD14$^+$ monocytes.

Cytokine measurement

For intracellular cytokine staining, single-cell suspensions were incubated in 24-well plates at 37 °C in complete RPMI-1640 medium with PMA (10 ng ml^{-1}) and ionomycin (1 µg ml^{-1}) for 4 h with monensin. Cells were surface stained, then treated with Cytofix/Cytoperm according to manufacturer's protocol (BD Biosciences, Franklin Lakes, NJ, USA) and stained with IL-17 and IFNγ.

Statistical analysis

Statistical significance was assessed using the Student's *t*-test or one-way analysis of variance with Tukey's multiple comparison test (Prism, Graphpad Software, La Jolla, CA, USA) as detailed in Figure Legends.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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