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Increased IL-10-competent regulatory B cells in the decidua of early human pregnancy

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Abstract

Regulatory B cells (Bregs) may play a pivotal role in maintaining human pregnancy. For the first time, to the best of our knowledge, this study noted that cell percentages of $CD24^{hi}CD38^{hi}$ Bregs and $CD24^{hi}CD27^{+}$ Bregs, which can potentially produce IL-10, are increased in human decidua compared with the mid-luteal phase endometrium. In each case of decidua, the correlation between Bregs and dendritic cell (DC) or natural killer (NK) cell expression was further explored. A positive correlation between the percentage of $CD24^{hi}CD38^{hi}$ Bregs and $CD123^{-}CD11c^{+}$ myeloid DCs (mDCs) was noted. Furthermore, a significant positive correlation was also observed between the percentage of $CD24^{hi}CD27^{+}$ Bregs and $CD94^{+}CD56^{bright}CD16^{-}$ suppressive NK cells. These findings regarding decidual Bregs deepen the understanding of the harmonious immunological microenvironment that sustains early human pregnancy.

Keywords

decidua; dendritic cells; early human pregnancy; natural killer cells; regulatory B cells

1. Introduction

B lymphocytes are effector cells of the adaptive immune system, as they differentiate into memory B cells and plasma cells that present antigens and secrete antibodies, respectively. However, increasing evidence supports the existence of a subpopulation of B cells with immunosuppressive capacity, known as regulatory B cells (Bregs) (Mauri and Bosma, 2012). The primary function of Bregs is maintaining an immune balance required for tolerance and prevention of autoimmunity. There is no consensus about the specific phenotypic marker of Bregs, and they are defined as any B cell exhibiting an immunosuppressive function (Guzman-Genuino et al., 2021). Different phenotypes of Bregs include IL-35^{+} Bregs (Slawek et al., 2020) and transforming growth factor-beta^{+} Bregs (Lee KM et al., 2014); however, the capacity for the production of IL-10, a potent anti-inflammatory cytokine proposed to counteract tumor necrosis factor-alpha, remains a hallmark for Breg identification in humans and mice (Guzman-Genuino et al., 2021).

In the phenomenon of pregnancy, the percentage of CD24^{hi}CD27^{+} Bregs was reported to be significantly higher in the peripheral blood of women undergoing normal pregnancies than in non-pregnant women or those who experienced a miscarriage (Rolle

et al., 2013; Liu et al., 2019). Jensen et al. reported that a reduced frequency of splenic $CD19^{+}CD5^{+}CD1d^{hi}$ regulatory B10 cells and decreased levels of IL-10 mRNA in the spleen of early mouse pregnancy were associated with miscarriage, and passive transfer of splenic $CD19^{+}IL\text{-}10^{+}$ cells could prevent pregnancy failure by inhibiting dendritic cell (DC) maturation and expanding regulatory T cells (Jensen et al., 2013). In mouse uterus, a significant expansion of B cells and alteration in the B cell phenotype was observed from 2.5 to 8.5 days post-copulation, and these uterine B cells, which were enriched with subsets of $CD80^{+}CD86^{+}$ B cells and $IL\text{-}10^{+}$ B cells, suppressed the proliferation and activation of $CD4^{+}$ effector T cells (Guzman-Genuino et al., 2019). IL-10 production by B cells was reported to be essential for suppressing immune cells including natural killer (NK) cells, and inflammatory T cells (Neves et al., 2010). However, all of these studies were performed in mice or human peripheral blood, and, to the best of our knowledge, the study of Bregs in the uterus during early human pregnancy has not been reported. Therefore, it attracts our interest whether Bregs do exist in the deciduas of early human pregnancy and have any relevance to other immune cells in the fetomaternal interface.

This study aimed to explore Bregs that have the capacity for IL-10 production in the deciduas of early human pregnancy, and the correlation between Breg expression and the phenotypes of DCs or NK cells.

2. Methods

2.1. Patient characteristics

In this prospective, cohort study, endometria were obtained from 19 healthy volunteers in the mid-luteal phase. They had a history of one or more deliveries without a history of miscarriage. Deciduas were obtained from 24 consecutively presenting women whose pregnancies ended in induced abortion (IA) with the presence of a fetal heartbeat at 6–9 weeks of gestation. Chromosomal karyotyping of the villi was not performed. Peripheral blood samples were obtained from 6 of the 24 women at the time of IA.

All participants were appropriately informed of the purpose of the research, and they all provided written consent before sampling.

2.2. Flow cytometric analysis

The endometrium was obtained by pipetting with a disposable Pipette IV (MedGyn Products, Inc., Addison, IL, USA). Deciduas from the IAs were obtained via dilatation and evacuation. The tissue was suspended in phosphate-buffered saline (PBS)

containing 0.2% bovine serum albumin and 0.1% sodium azide. It was then minced using surgical scissors and strained through a nylon mesh (59 μm). A solution containing NH4Cl and ethylendiaminetetraacetic acid (EDTA) was added for 10 min at room temperature to lyse the erythrocytes. Cells obtained from the endometrium and the decidua were washed twice with PBS and then re-suspended with 1 mL of PBS before flow cytometric analysis. Peripheral blood cells were suspended in PBS containing 0.2% bovine serum albumin and 0.1% sodium azide, and subsequently processed using the same procedures as with the endometrial or decidual cells.

Bregs were analyzed using the following monoclonal antibodies: phycoerythrin (PE)-conjugated anti-CD19 (BD Biosciences, San Jose, CA, USA), brilliant violet (BV) 421-conjugated anti-IL-10 (BioLegend, Inc., San Diego, CA, USA), allophycocyanin (APC)/Cy7-conjugated anti-CD24 (BioLegend), APC-conjugated anti-CD27 (BD Biosciences), and fluorescein isothiocyanate (FITC)-conjugated anti-CD38 (Pharmingen, San Diego, CA, USA). The stimulation experiments were performed by adding a cell stimulation mixture containing phorbol 12-myristate 13-acetate (PMA) (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), ionomycin (Sigma-Aldrich, St.Louis, MO, USA), and brefeldin A (Fujifilm Wako Pure Chemical Corporation) for 4 h before staining them for intracellular IL-10 expression.

The following monoclonal antibodies were used for analyzing DCs: FITC-conjugated Lineage Cocktail 1 (BD Biosciences), APC-H7-conjugated anti-HLA-DR (BD Biosciences), Alexa Fluor 647-conjugated anti-CD123 (BD Biosciences), and BV421-conjugated anti-CD11c (BD Biosciences). NK cells were analyzed using PE-conjugated anti-CD56 (BD Biosciences), V450-conjugated anti-CD16 (BD Biosciences), FITC-conjugated anti-CD44 (BD Biosciences), and APC-conjugated anti-CD94 (Pharmingen). Since the population size of natural killer T (NKT) cells was reported to be much smaller than NK cells in the decidua of induced abortion (Tsuda et al., 2001), we did not use anti-CD3 antibody to exclude NKT cells in this study.

Three-color flow cytometric analyses were performed using a FACSCantoII flow cytometer (BD Biosciences) and CellQuest Pro Software ver. 6.0 (BD Biosciences).

2.3. Statistical analysis

P-values < 0.05 were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0). This is a modified version of R

commander (version 1.6-3) that was designed to add statistical functions frequently used in biostatistics.

The Mann–Whitney U test was used to analyze differences between endometrium and decidua. Correlation analyses were performed using the Spearman rank correlation test.

2.4. Ethical approval

This prospective cohort study was approved by the Institutional Review Board of Kobe University Graduate School of Medicine.

3. Results

3.1 Characteristics of the subjects

Endometria were provided from 19 healthy, fertile volunteers in the mid-luteal phase (5–9 days post ovulation). The median age was 34 (27–36) [median (range)] years, with an average of 2 pregnancies (1–4) and 2 live births (1–4). None of the women had a history of miscarriage.

Deciduas were collected from 24 consecutively seen women who underwent IA. The mean gestational age at evacuation was 7 weeks (6–9). Mean age was 27 (18–39) years which were not statistically different from the women who provided endometria ($p = 0.107$). The average number of pregnancies was 1 (0–4) ($p = 0.0064$), and the average number of live births was 0 (0–4) ($p = 0.0066$), both of which were smaller than the women providing endometria. None of the women had a history of miscarriage.

3.2 Endometrial and decidual B cells

3.2.1 Detection and characterization of mid-luteal endometrial B cells and early human pregnancy decidual B cells

Figure 1-A shows the procedure for the detecting and characterizing mid-luteal endometrial B cells and early human pregnancy decidual B cells. The results of each representative case are shown. B cells were detected as $CD45^{+}CD19^{+}$ cells and further subjected to analysis using CD24, CD27, and CD38 antibodies by the method reported by Hasan et al. (2019). $CD24^{hi}CD38^{hi}$ (P1) and $CD24^{dim}CD38^{hi}$ cells (P2)

were detected, and $CD38^{-}$ and $CD38^{dim}$ cells were further analyzed by CD24 and CD27. This resulted in detection of $CD24^{hi}CD27^{+}$ (P3) and $CD24^{+}CD27^{-}$ cells (P4).

3.2.2 Comparative analysis of endometrial and decidual B cells

The endometria from 19 mid-luteal phase women and the deciduas from 24 women with IA were analyzed, and their composition of B cells was compared.

Decidual B cells had a significantly higher percentage of $CD24^{hi}CD38^{hi}$ cells (P1) (4.04 (2.36–5.07) [median (25–75 percentile)] vs. 1.52 (0.93–2.49); p = 0.000325); $CD24^{dim}CD38^{hi}$ cells (P2) (3.34 (1.90–5.24) vs. 1.36 (0.88–2.28); p = 0.000612), and $CD24^{hi}CD27^{+}$ cells (P3) (40.6 (33.7–49.5) vs. 22.8 (18.7–28.7); p = 0.0000254) among $CD19^{+}$ cells compared with endometrial B cells. The percentage of $CD24^{+}CD27^{-}$ cells (P4) among the $CD19^{+}$ cells was significantly lower in decidual than in endometrial B cells (35.9 (32.9–45.0) vs. 45.4 (40.5–48.9); p = 0.0378).

3.3 Detection and characterization of IL-10-competent Bregs in the deciduas of early human pregnancy

3.3.1 Capacity for B cell IL-10 production in peripheral blood and deciduas of early human pregnancy

B cell IL-10 expression in the deciduas and peripheral blood of 6 pregnant women was explored following stimulation with PMA and ionomycin for 4 h. A representative result is shown in Figure 2-A. A small but certain population of IL-10^{+} Bregs was most clearly confirmed in the decidua after stimulation.

3.3.2 Characterization of decidual IL-10-competent Bregs in early human pregnancy

The decidual IL-10^{+} Bregs stimulated by PMA and ionomycin were further subjected to analysis using CD24, CD27, and CD38 antibodies. A representative result is shown in Figure 2-B. $CD24^{hi}CD38^{hi}$ cells (P1) (30.8% of $CD19^{+}$IL-10^{+} cells) and $CD24^{hi}CD27^{+}$ cells (P3) (60.4% of $CD19^{+}$IL-10^{+} cells) were proved to be the major population of Bregs in decidua of early human pregnancy, which produced IL-10 after stimulation.

3.4 Characterization of endometrial and decidual DCs and the correlation between Bregs and DCs in deciduas

3.4.1 Detection and characterization of endometrial DCs in mid-luteal phase and decidual DCs in early human pregnancy

The mid-luteal phase endometrium from 19 women and the deciduas from 14 women with IA were analyzed, and their characterization of DCs was compared. Figure 3-A shows the procedure for detecting endometrial and decidual DCs. A representative result is shown. DCs were further analyzed using CD11c and CD123 antibodies. Myeloid DCs (mDCs) were detected as $CD123^{-}CD11c^{+}$ cells. Plasmacytoid DCs (pDCs) were detected as $CD123^{+}CD11c^{-}$ cells with a small but identifiable population.

3.4.2 Percentage of DCs and DC subsets in endometria and deciduas

The percentage of decidual DCs in $CD45^{+}$ white blood cells (3.44 (2.98–4.61)) were significantly lower than that of the endometrial DCs (6.28 (4.40–8.57); p = 0.0111) (Figure 3-B). The percentage of $CD123^{-}CD11c^{+}$ mDCs among DCs was relatively higher in deciduas (44.2 (34.5–58.7)) than in endometria (34.0 (23.5–40.2); p = 0.071), and the percentages of $CD123^{+}CD11c^{-}$ pDCs among DCs was lower in deciduas (1.85 (1.11–3.67)) than in endometria (2.71 (1.00–5.12); p = 0.577),

1 although not statistically significant. The ratio of pDCs/mDCs was lower in deciduas
2 (0.06 (0.03–0.09)) than in endometria (0.10 (0.05–0.11); $p = 0.0653$), but again
3 without statistical significance.

4

5 3.4.3 Correlation between percentages of Bregs and DCs in the deciduas of early
6 human pregnancy

7 The correlation between percentages of Bregs and DCs in the decidua of each
8 patient was analyzed. Figure 3-C shows the correlation between percentage of
9 $CD24^{hi}CD38^{hi}$ Bregs or $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ cells and DCs among
10 $CD45^{+}$ white blood cells, $CD123^{-}CD11c^{+}$ mDCs, and $CD123^{+}CD11c^{-}$ pDCs among DCs,
11 as well as the ratio of pDCs/mDCs.
12 A significant positive correlation was observed (Spearman's rank correlation
13 coefficient (r_s) = 0.626; $p = 0.0192$) between the percentage of $CD24^{hi}CD38^{hi}$ Bregs
14 among $CD19^{+}$ cells and the mDCs among DCs. A significant negative correlation (r_s =
15 -0.684; $p = 0.00894$) between the $CD24^{hi}CD38^{hi}$ Bregs among $CD19^{+}$ cells and the ratio
16 of pDCs/mDCs was noted.

No statistically significant correlation was confirmed between the percentage of $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ cells and the percentages of DCs, mDCs, pDCs, or the ratio of pDCs/mDCs.

3.5 Characterization of endometrial and decidual NK cells and the correlation between Bregs and NK cells in deciduas

3.5.1 Detection and characterization of endometrial NK cells in mid-luteal phase and decidual NK cells in early human pregnancy

The mid-luteal phase endometria from 17 women and the deciduas from 13 women with IA were analyzed, and their characterization of NK cells was compared. A representative result of the procedure for the detection of endometrial and decidual NK cells is shown in Figure 4-A. NK cells were detected as $CD56^{+}$ cells and subclassified as $CD56^{dim}CD16^{+}$ NK cells and $CD56^{bright}CD16^{-}$ NK cells. They were then further analyzed using CD44 and CD94 antibodies.

3.5.2 Percentage of NK cells and NK cell subsets in endometria and deciduas and the correlation between percentages of Bregs and NK cells or NK cell subsets in the deciduas of early human pregnancy

The percentage of decidual NK cells in $CD45^+$ white blood cells (68.3 (60.4–71.8)) was relatively higher than that of the endometrial NK cells (58.9 (38.0–80.7)) (Figure 4-B), albeit without statistical significance ($p = 0.157$). The percentage of $CD56^{dim}CD16^+$ NK cells among NK cells was significantly higher in deciduas (6.55 (3.83–9.88)) than those in endometria (2.11 (1.22–5.79); $p = 0.00798$). The percentage of $CD56^{bright}CD16^-$ NK cells among NK cells was relatively lower in deciduas (84.8 (76.8–88.4)) than in endometria (89.6 (78.7–93.9)), again without statistical significance ($p = 0.385$).

No significant correlation was observed (Figure 4-C) between the percentage of $CD24^{hi}CD38^{hi}$ Bregs or $CD24^{hi}CD27^+$ Bregs among $CD19^+$ cells and the ratio of NK cells in $CD45^+$ white blood cells, $CD56^{dim}CD16^+$ NK cells, or $CD56^{bright}CD16^-$ NK cells among NK cells in the deciduas of early human pregnancy.

3.5.3 Suppressive phenotypes of $CD56^{bright}CD16^{-}$ NK cells in endometria and deciduas and their correlation with Bregs percentages in the deciduas of early human pregnancy

Suppressive states of $CD56^{bright}CD16^{-}$ NK cells were detected as $CD44^{-}$ cells, $CD94^{+}$ cells, or $CD44^{-}CD94^{+}$ cells. The percentage of $CD44^{-}$ cells among $CD56^{bright}CD16^{-}$ NK cells was significantly higher in deciduae (76.4 (65.8–91.0)) than in endometria (65.2 (44.1–68.4); $p = 0.0197$) (Figure 4-D). The percentage of $CD94^{+}$ cells among $CD56^{bright}CD16^{-}$ NK cells was significantly higher in deciduas (96.1 (93.2–97.1)) than in endometria (91.7 (86.4–95.3); $p = 0.0197$). Finally, the percentage of $CD44^{-}CD94^{+}$ cells among $CD56^{bright}CD16^{-}$ NK cells was significantly higher in deciduas (72.3 (60.3–87.2)) than in endometria (55.0 (36.1–61.6); $p = 0.00284$).

The correlation between percentages of Bregs and suppressive NK cells in the decidua of each patient was analyzed. A significant positive correlation was observed ($r_s = 0.654$; $p = 0.0183$) between the percentage of $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ cells and the percentage of $CD94^{+}$ cells among $CD56^{bright}CD16^{-}$ NK cells (Figure 4-E).

The $CD56^{dim}CD16^{+}$ NK cells were a minor NK population in the deciduas. The percentage of $CD44^{-}$, $CD94^{+}$, or $CD44^{-}CD94^{+}$ suppressive NK cells among $CD56^{dim}CD16^{+}$ NK cells was relatively lower in the deciduas, although not statistically significant, than in the endometria (data not shown). The percentage of $CD44^{-}$, $CD94^{+}$, or $CD44^{-}CD94^{+}$ suppressive NK cells among $CD56^{dim}CD16^{+}$ NK cells did not show a correlation with the percentage of $CD24^{hi}CD38^{hi}$ Bregs or the $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ cells.

4. Discussion

Here, this study demonstrated that $CD24^{hi}CD38^{hi}$ cells (P1) and $CD24^{hi}CD27^{+}$ cells (P3) are the Bregs possessing the capacity to produce IL-10 in the decidua of early human pregnancy. The amount of Bregs in the decidua was significantly higher than that in non-pregnant endometrium.

$CD24^{hi}CD38^{hi}$ cells are reported to be early immature transitional B cells and one of the phenotypes of IL-10-producing Bregs in human blood (Blair et al., 2010). $CD24^{hi}CD27^{+}$ cells are memory B cell populations, the human equivalent of mouse B10 cells, and are also reported to be IL-10-producing Bregs in human blood (Iwata et al., 2011). The percentage of $CD24^{hi}CD27^{+}$ Bregs was reported to be significantly higher in

the peripheral blood of women undergoing normal pregnancies than in non-pregnant women (Rolle et al., 2013; Liu et al., 2019). In the current study, not only $CD24^{hi}CD27^{+}$ Bregs but also $CD24^{hi}CD38^{hi}$ Bregs increased in the fetomaternal interface, decidua of early human pregnancy.

This study noted a positive correlation between the percentage of $CD24^{hi}CD38^{hi}$ Bregs and mDCs in the deciduas of early human pregnancy. A negative correlation was shown between the percentage of $CD24^{hi}CD38^{hi}$ Bregs and the ratio of pDC/mDC. In early human pregnancy, the decidua has more mDCs and fewer pDCs than peripheral blood (Miyazaki et al., 2003), and the results of most studies have shown that the ratio of pDC/mDC in the peripheral blood of pregnant women is lower than that of non-pregnant women (Wei et al., 2021). Therefore, the possibility was demonstrated that the increase in $CD24^{hi}CD38^{hi}$ Bregs establishes a decidual microenvironment favorable to the maintenance of pregnancy with a rise of mDCs.

CD94/NKG2 family expressed on the surface of NK cells consists of seven members: NKG2A, B, C, D, E, F and H (Plougastel and Trowsdale, 1997; Borrego et al., 2006; Lanier, 2008). NKG2A and NKG2B receptors transmit inhibitory signal; on the other hand NKG2C, NKG2D, NKG2E and NKG2H are activating receptors. King et al. have reported that human trophoblast cells express HLA-E on their cell surface and

interact CD94/NKG2 receptors on decidual NK cells (King et al., 2000). They demonstrated that the vast majority of decidual NK cells (approximately 95%) express CD94/NKG2A inhibitory receptors and the overall dominant effect of CD94/NKG2 interaction with HLA-E is inhibition of cytotoxicity by decidual NK cells. It is also reported the rates of NKG2A-positive cells are significantly higher for decidual $CD56^{bright}$ NK cells than for peripheral $CD56^{dim}$ NK cells in early human pregnancy, but the rates of NKG2C-positive cells are comparable between the two cell types (Kusumi et al., 2006). Therefore, we regarded decidual $CD94^{+}CD56^{bright}CD16^{-}$ cells as suppressive NK cells with comparative dominancy of inhibitory signal.

We previously reported that a high dose of intravenous immunoglobulin therapy (IVIg) in patients with recurrent pregnancy loss (RPL) increased the expression level of CD94 on NK cells in peripheral blood (Shimada et al., 2009). When IVIg is administrated in the RPL model mice, it decreases the spontaneous abortion rate by suppressing the increase of $CD44^{bright}$ uterine NK cells (Tanaka et al., 2016). In the current study, the percentage of $CD44^{-}$, $CD94^{+}$, and $CD44^{-}CD94^{+}$ suppressive NK cells among $CD56^{bright}CD16^{-}$ NK cells was higher in the deciduas of early human pregnancy than in the mid-luteal phase endometrium. Moreover, a positive correlation was

observed between the percentages of $CD24^{hi}CD27^{+}$ Bregs and $CD94^{+}CD56^{bright}CD16^{-}$ NK cells in the deciduas of early human pregnancy.

Taken together, $CD24^{hi}CD38^{hi}$ Bregs and $CD24^{hi}CD27^{+}$ Bregs, which can potentially produce IL-10, were significantly increased in the decidua of early human pregnancy. A positive correlation between Bregs and mDCs and between Bregs and suppressive NK cells was found in the decidua. These findings provide direct evidence that Bregs are involved and play a role for the maintenance of local environment, and help to understand the orchestrated immune suppressive system during early human pregnancy.

Declaration of competing interest

The authors report no declarations of interest.

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Figure legends

Figure 1

Endometrial and decidual B cells. (A) Gating strategy for dividing B cell subsets P1 ($CD24^{hi}CD38^{hi}$), P2 ($CD24^{dim}CD38^{hi}$), P3 ($CD24^{hi}CD27^{+}$), and P4 ($CD24^{+}CD27^{-}$) in the endometrium of healthy volunteers and in the decidua of women with induced abortion (IA). Representative data is shown. (B) Comparison of cell percentages for each B cell subset (P1, P2, P3, and P4) among $CD19^{+}$ B cells seen in the mid-luteal phase endometrium of 19 volunteers and in the decidua of 24 fertile women who underwent IA. Box and whisker plots show the following: bar (horizontal line), median; boxes, 25th and 75th percentiles; whiskers, the lowest point greater than the lower 1.5*interquartile range (IQR) and the highest point less than the upper 1.5*IQR.

Figure 2

P1 ($CD24^{hi}CD38^{hi}$) and P3 ($CD24^{hi}CD27^{+}$) are regulatory B cells (Bregs), which have the capacity for IL-10 production. (A) Decidua and peripheral blood stimulated for 4 h with phorbol 12-myristate 13-acetate and ionomycin in the presence of brefeldin A before staining them for intracellular IL-10. (B) P1 ($CD24^{hi}CD38^{hi}$) and P3 ($CD24^{hi}CD27^{+}$) were the main populations producing IL-10 after stimulation in the

decidua of early human pregnancy. Representative data are shown from six independent experiments using cells from six donors.

Figure 3

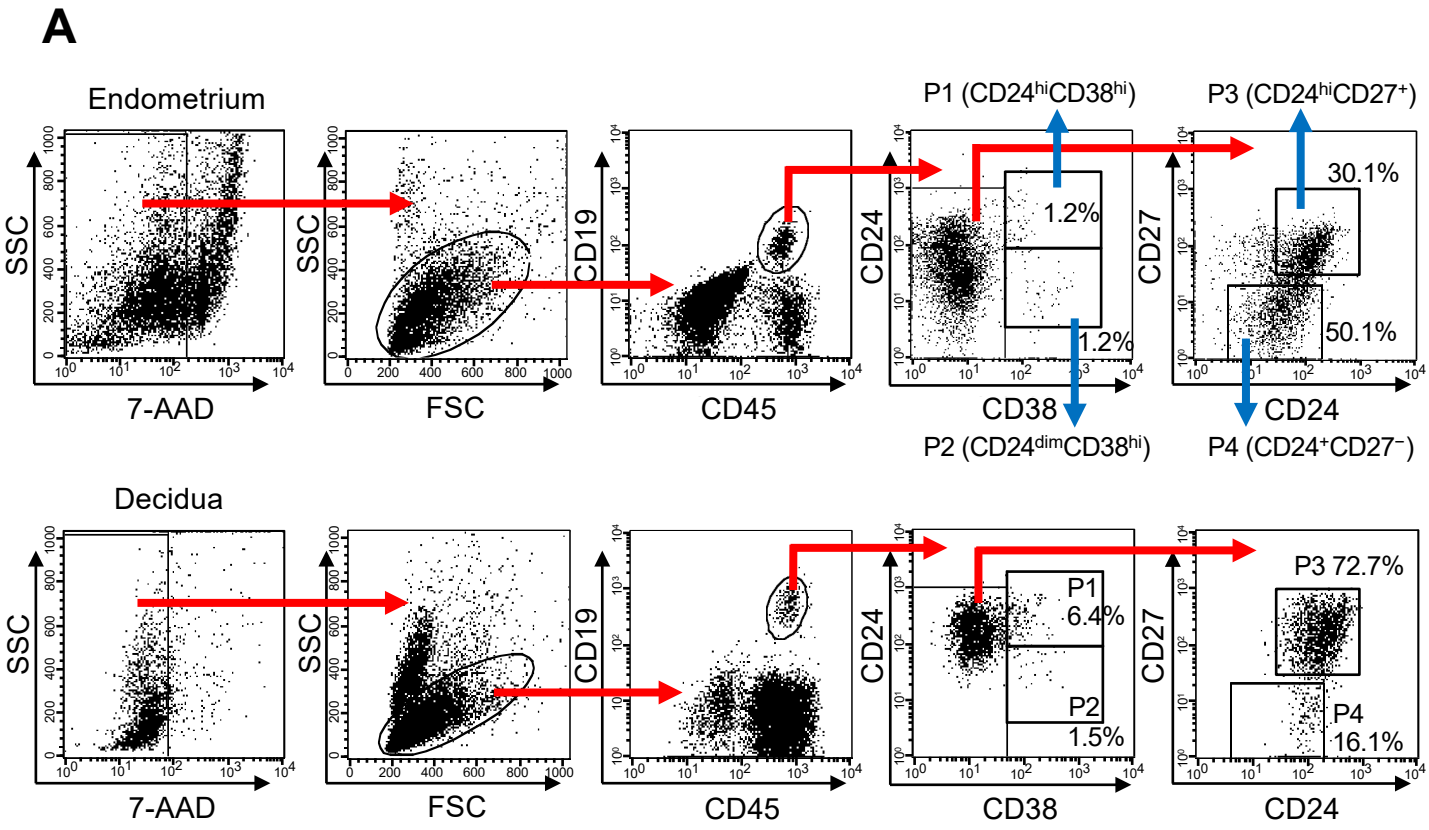
Endometrial and decidual dendritic cell (DCs) detection and their correlation with Bregs. (A) Gating strategy for detecting DCs. The expression of DCs, which are gated on lineage cocktail 1^{-}HLA-DR^{+} cells, is shown. Once DCs were gated, the expression of CD123 and CD11c was analyzed. A representative result of $CD123^{-}CD11c^{+}$ myeloid DCs (mDC), and $CD123^{+}CD11c^{-}$ plasmacytoid DCs (pDC) is shown. (B) Comparison of the percentage of DCs in $CD45^{+}$ white blood cells, mDCs, and pDCs in DCs, and the ratios of pDCs/mDCs seen in the mid-luteal phase endometriums of 19 volunteers and in the deciduas of 14 fertile women who underwent IA. (C) Correlation between the percentage of Bregs and DCs in the decidua of each woman. Correlations between the percentage of $CD24^{hi}CD38^{hi}$ Bregs or $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ B cells and the percentage of DCs among $CD45^{+}$ white blood cells, mDCs, and pDCs among DCs, and the ratio of pDCs/mDCs are shown. r_s, correlation coefficient. p, p-value using the Spearman rank correlation test.

Figure 4

Correlation between natural killer (NK) cells and Bregs in the endometrium and decidua. (A) Gating strategy for detecting NK cells and NK cell subsets. The expression of NK cells, which are gated as $CD56^{+}$ cells, is shown. Gating of NK subsets ($CD56^{dim}CD16^{+}$ NK cells and $CD56^{bright}CD16^{-}$ NK cells), the expression of CD94 and CD44 was analyzed. A representative result is shown. (B) Comparison of NK cell percrntage among $CD45^{+}$ white blood cells, $CD56^{dim}CD16^{+}$ NK cells and $CD56^{bright}CD16^{-}$ NK cells among NK cells seen in the mid-luteal phase endometria of 17 volunteers and in the deciduas of 13 fertile women who underwent IA. (C) Correlation between Bregs and NK cell percentages in the decidua of each case. Correlation between the percentages of $CD24^{hi}CD38^{hi}$ Bregs or $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ B cells and NK cells among $CD45^{+}$ white blood cells, $CD56^{dim}CD16^{+}$ NK cells, and $CD56^{bright}CD16^{-}$ NK cells among NK cells are shown. r_s, correlation coefficient. p, p-value utilizing the Spearman rank correlation test. (D) Comparison of the percentage of suppressive NK cells among $CD56^{bright}CD16^{-}$ NK cells seen in the endometria and the deciduas. Suppressive NK cells gated as $CD44^{-}$ cells, $CD94^{+}$cells, and $CD44^{-}CD94^{+}$ cells were compared using the Mann–Whitney U test. (E) Correlation between Bregs and suppressive NK cell percentages in the decidua of each woman.

Correlations between the percentage of $CD24^{hi}CD38^{hi}$ Bregs or $CD24^{hi}CD27^{+}$ Bregs among $CD19^{+}$ B cells and the percentage of $CD44^{-}$ cells, $CD94^{+}$cells, or $CD44^{-}CD94^{+}$ cells among $CD56^{bright}CD16^{-}$ NK cells are shown. r_s, correlation coefficient. p, p-value utilizing the Spearman rank correlation test.

Figure 1


A
Endometrium
SSC
7-AAD
SSC
FSC
CD19
CD45
CD24
CD38
P1 (CD24hiCD38hi)
1.2%
1.2%
P2 (CD24dimCD38hi)
CD27
CD24
P3 (CD24hiCD27+)
30.1%
50.1%
P4 (CD24+CD27−)
Decidua
SSC
7-AAD
SSC
FSC
CD19
CD45
CD24
CD38
P1
6.4%
P2
1.5%
CD27
CD24
P3 72.7%
P4
16.1%

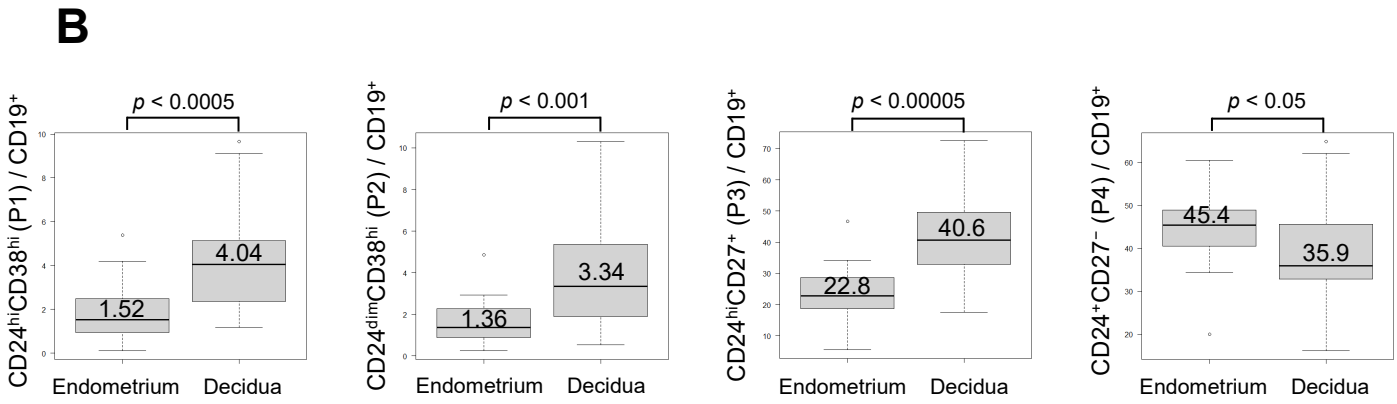
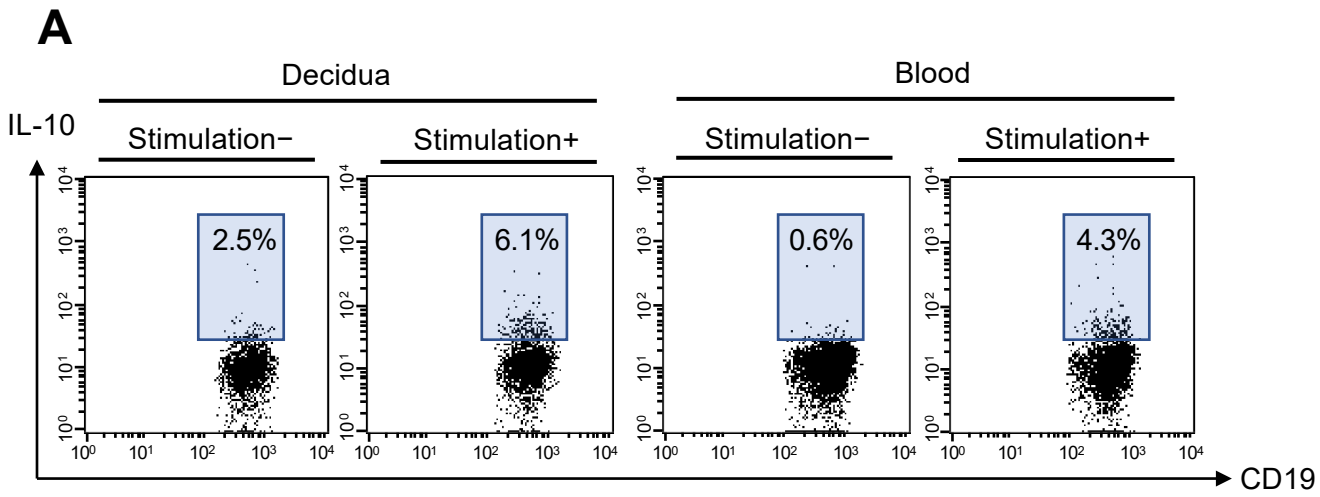

B
p < 0.0005
CD24hiCD38hi (P1) / CD19+
1.52
4.04
Endometrium
Decidua
p < 0.001
CD24dimCD38hi (P2) / CD19+
1.36
3.34
Endometrium
Decidua
p < 0.00005
CD24hiCD27+ (P3) / CD19+
22.8
40.6
Endometrium
Decidua
p < 0.05
CD24+CD27− (P4) / CD19+
45.4
35.9
Endometrium
Decidua

Figure 2


A
Decidua
Blood
IL-10
Stimulation−
Stimulation+
Stimulation−
Stimulation+
2.5%
6.1%
0.6%
4.3%
CD19

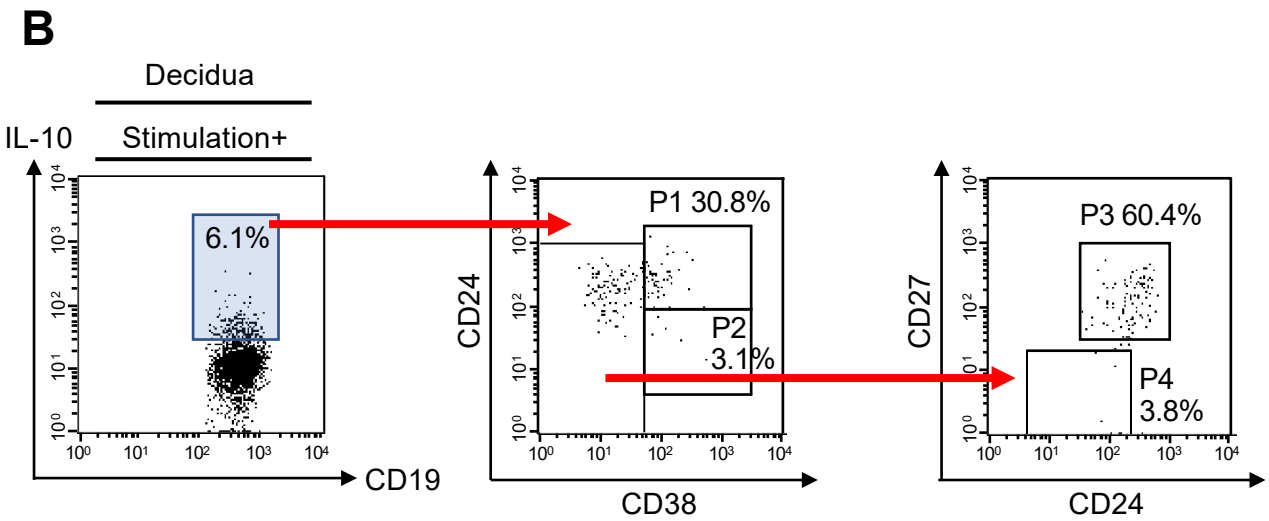

B
Decidua
IL-10
Stimulation+
6.1%
CD19
CD24
P1 30.8%
P2
3.1%
CD38
CD27
P3 60.4%
P4
3.8%
CD24

Figure 3

A

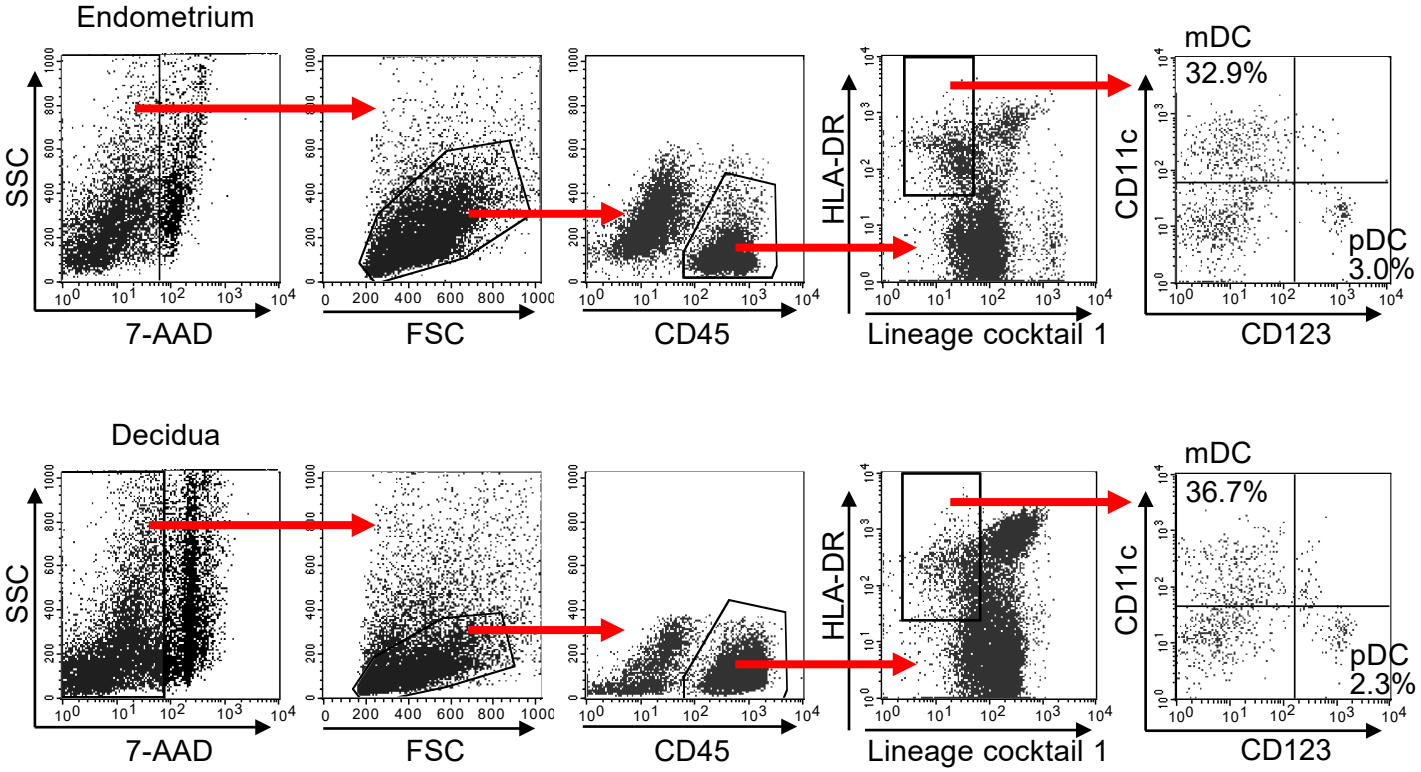

Endometrium
SSC
7-AAD
FSC
CD45
HLA-DR
Lineage cocktail 1
mDC
32.9%
CD11c
pDC
3.0%
CD123
Decidua
SSC
7-AAD
FSC
CD45
HLA-DR
Lineage cocktail 1
mDC
36.7%
CD11c
pDC
2.3%
CD123

Figure 3

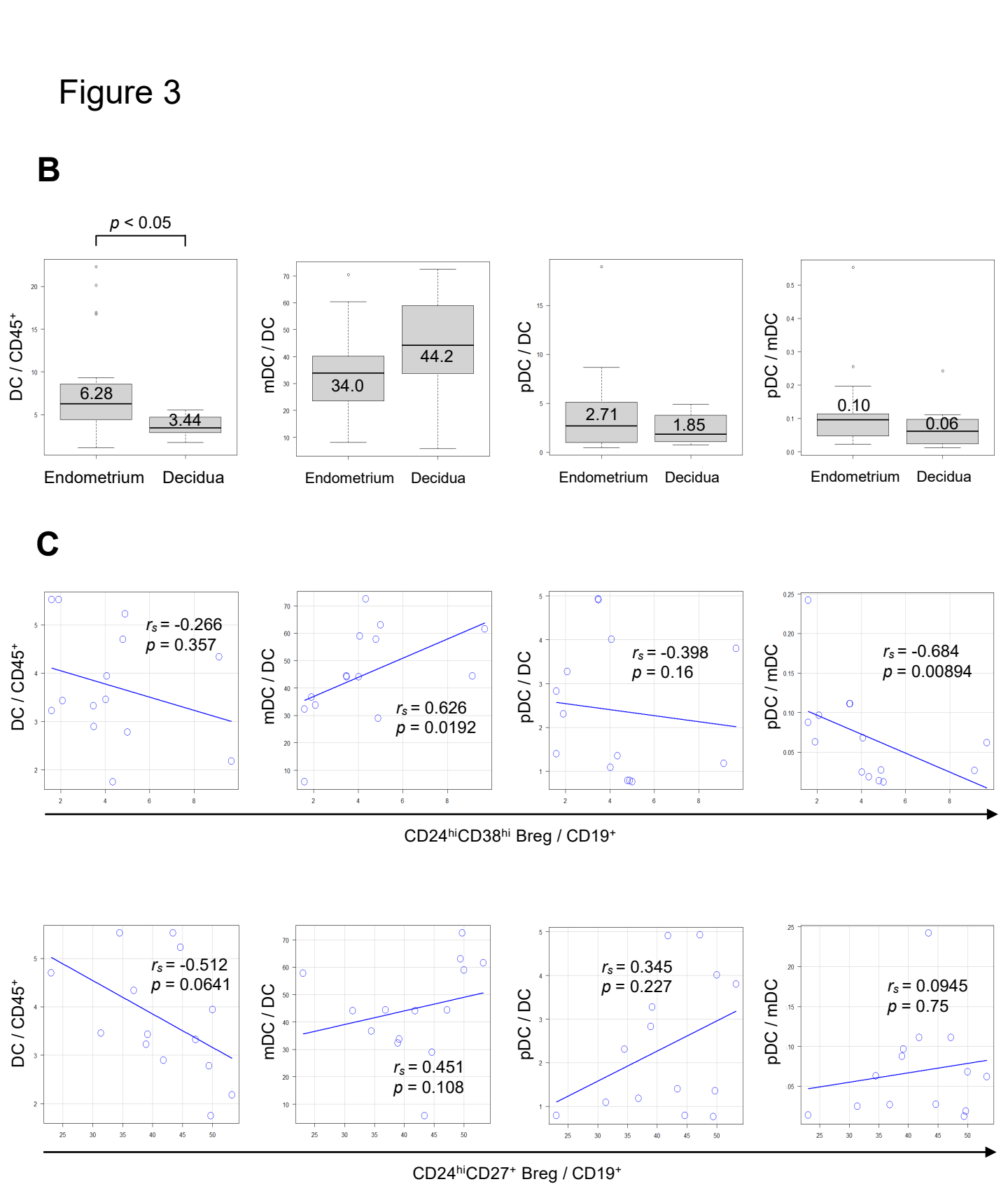
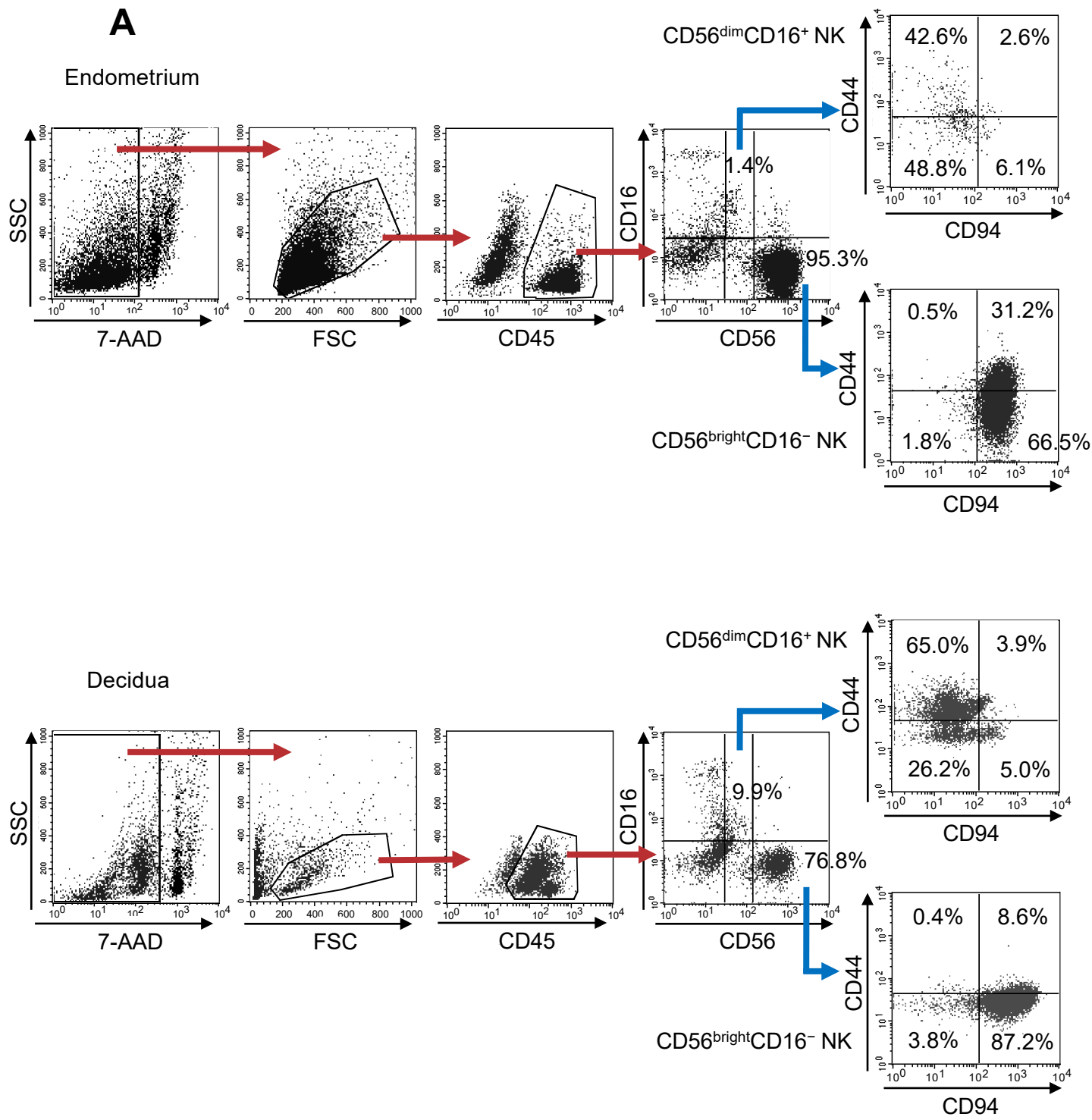
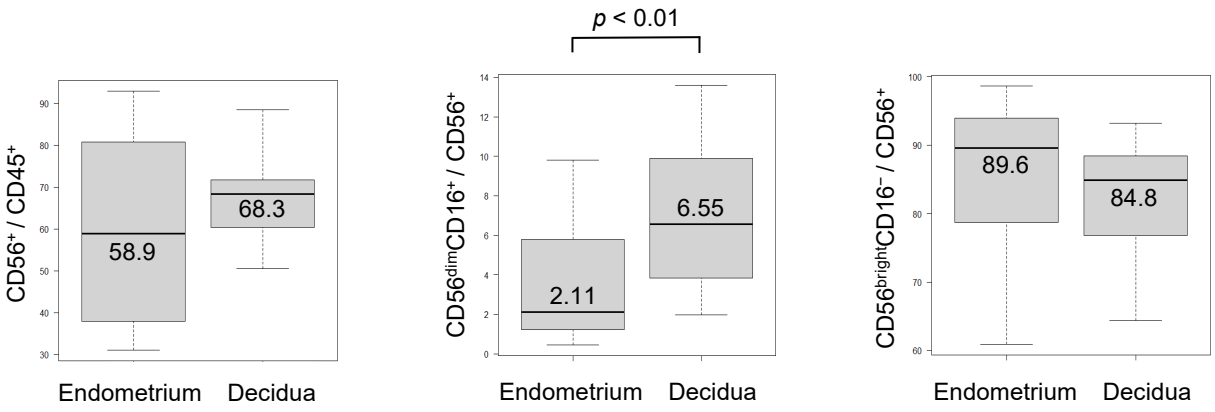

B
p < 0.05
DC / CD45+
6.28
3.44
Endometrium
Decidua
mDC / DC
34.0
44.2
Endometrium
Decidua
pDC / DC
2.71
1.85
Endometrium
Decidua
pDC / mDC
0.10
0.06
Endometrium
Decidua
C
DC / CD45+
rs = -0.266
p = 0.357
mDC / DC
rs = 0.626
p = 0.0192
pDC / DC
rs = -0.398
p = 0.16
pDC / mDC
rs = -0.684
p = 0.00894
CD24hiCD38hi Breg / CD19+
DC / CD45+
rs = -0.512
p = 0.0641
mDC / DC
rs = 0.451
p = 0.108
pDC / DC
rs = 0.345
p = 0.227
pDC / mDC
rs = 0.0945
p = 0.75
CD24hiCD27+ Breg / CD19+

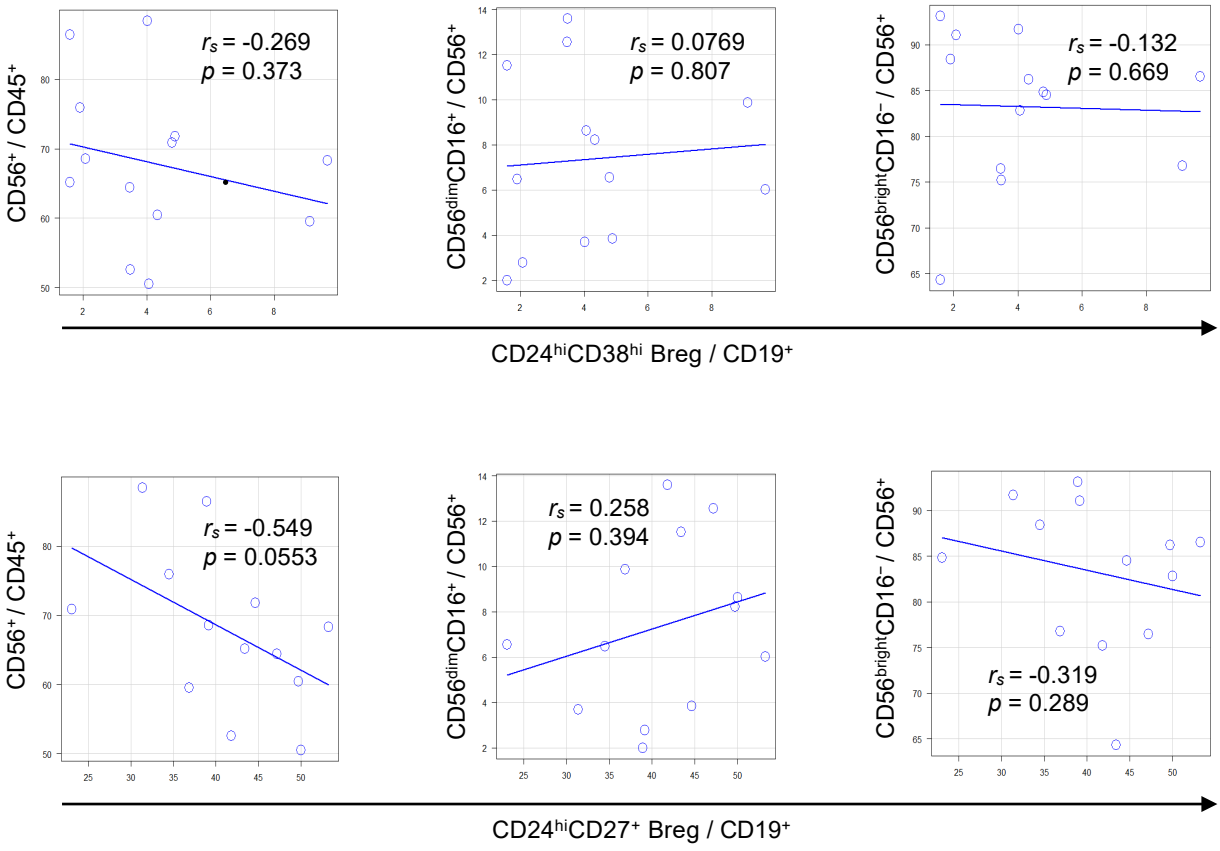
Figure 4


A
Endometrium
SSC
7-AAD
FSC
CD45
CD16
CD56
1.4%
95.3%
CD56dimCD16+ NK
CD44
CD94
42.6%
2.6%
48.8%
6.1%
CD56brightCD16− NK
0.5%
31.2%
1.8%
66.5%
Decidua
9.9%
76.8%
65.0%
3.9%
26.2%
5.0%
0.4%
8.6%
3.8%
87.2%

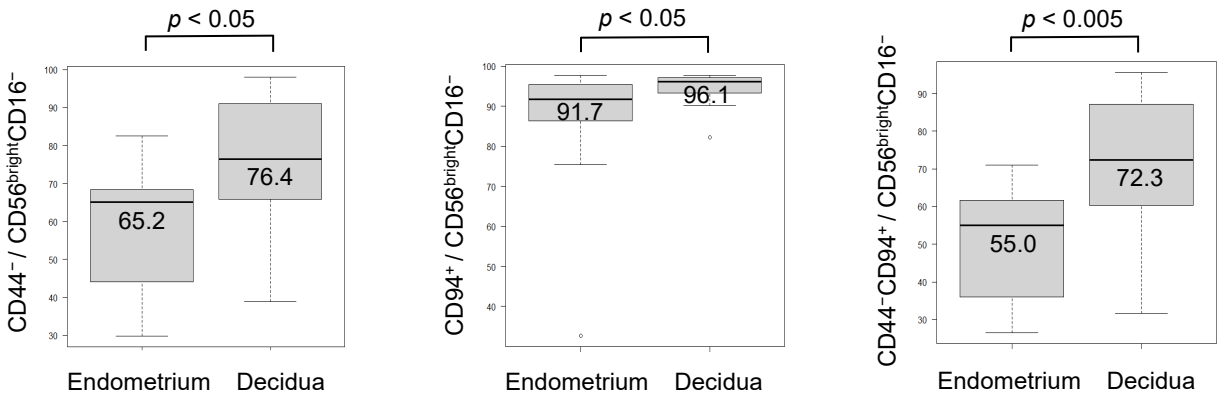
B


p < 0.01
CD56+ / CD45+
58.9
68.3
Endometrium
Decidua
CD56dimCD16+ / CD56+
2.11
6.55
Endometrium
Decidua
CD56brightCD16− / CD56+
89.6
84.8
Endometrium
Decidua

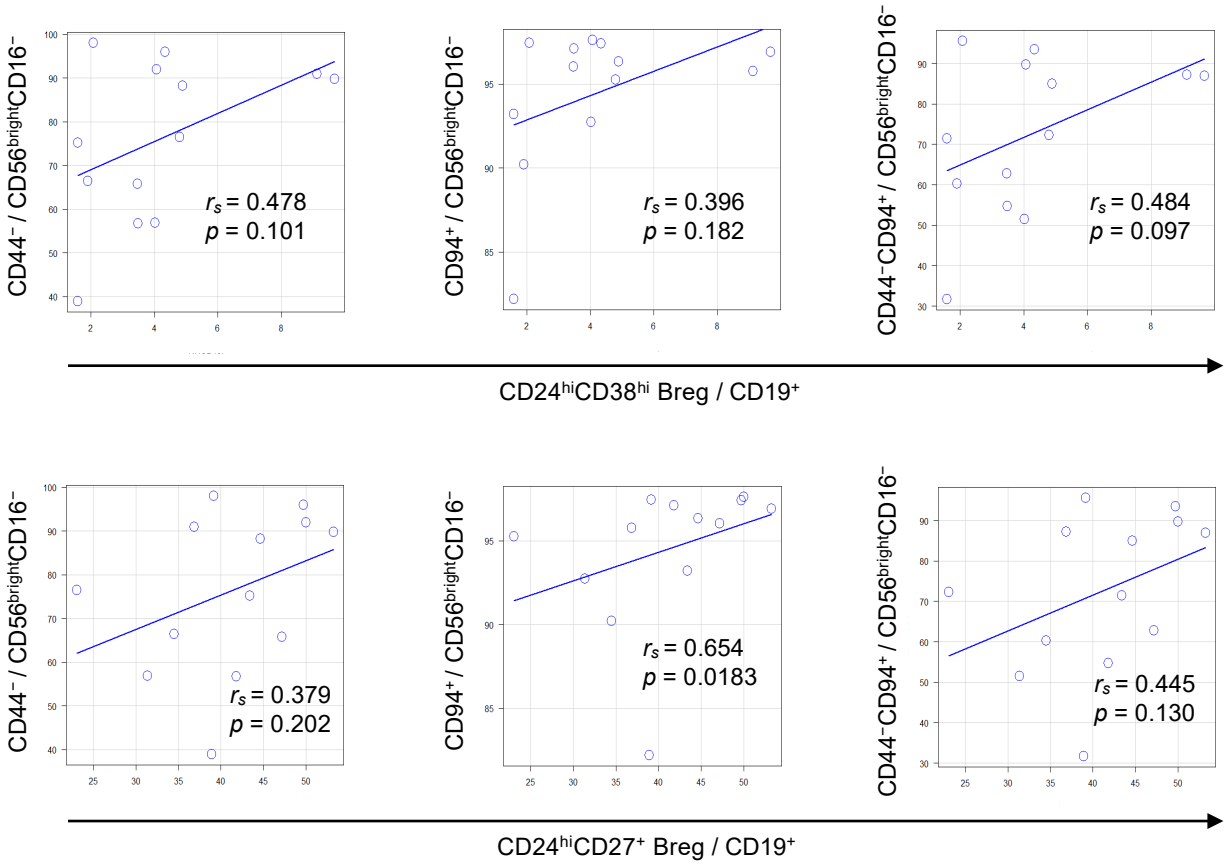
C


CD56+ / CD45+
rs = -0.269
p = 0.373
CD56dimCD16+ / CD56+
rs = 0.0769
p = 0.807
CD56brightCD16− / CD56+
rs = -0.132
p = 0.669
CD24hiCD38hi Breg / CD19+
CD56+ / CD45+
rs = -0.549
p = 0.0553
CD56dimCD16+ / CD56+
rs = 0.258
p = 0.394
CD56brightCD16− / CD56+
rs = -0.319
p = 0.289
CD24hiCD27+ Breg / CD19+

D


p < 0.05
CD44⁻ / CD56brightCD16⁻
65.2
76.4
Endometrium
Decidua
p < 0.05
CD94⁺ / CD56brightCD16⁻
91.7
96.1
Endometrium
Decidua
p < 0.005
CD44⁻CD94⁺ / CD56brightCD16⁻
55.0
72.3
Endometrium
Decidua

E


CD44⁻ / CD56brightCD16⁻
rs = 0.478
p = 0.101
CD94⁺ / CD56brightCD16⁻
rs = 0.396
p = 0.182
CD44⁻CD94⁺ / CD56brightCD16⁻
rs = 0.484
p = 0.097
CD24hiCD38hi Breg / CD19⁺
CD44⁻ / CD56brightCD16⁻
rs = 0.379
p = 0.202
CD94⁺ / CD56brightCD16⁻
rs = 0.654
p = 0.0183
CD44⁻CD94⁺ / CD56brightCD16⁻
rs = 0.445
p = 0.130
CD24hiCD27⁺ Breg / CD19⁺