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Original Article

Decidual and peripheral blood TCRαβ$^+$CD3$^+$CD4$^-$CD8$^-$ double negative regulatory T cells in early pregnancy subjects and unexplained recurrent spontaneous abortion patients

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Abstract: Accumulating evidences have demonstrated that TCRαβ$^+$CD3$^+$CD4$^-$CD8$^-$ NK1.1-double negative regulatory T cells (DN Tregs) are highly potent immune suppressors and play an important role in down regulating immune responses in autoimmunity, transplant rejection, graft-versus-host disease (GvHD). Whether human DN Tregs play a role in maternal-fetal immunological tolerance hasn't been reported yet. In the present study, to explore the possible role of human DN Tregs in unexplained recurrent spontaneous abortion (URSA), we compare the frequency and function of peripheral and/or decidual DN Tregs of 22 women with normal history of reproduction, 31 normal early pregnant women and 42 URSA patients through flow Cytometry and cell proliferation assays. The proportion of DN Tregs in decidua was significantly higher than peripheral blood both in URSA patients and normal early pregnant women. It is the proportion but not the suppressive ability of DN Tregs decreased in peripheral blood and decidua of URSA patients than normal early pregnant women. Our result suggests that human DN Tregs enrich in decidua and may contribute to the mechanisms mediating maternal-fetal immunological tolerance and may be involved in protection the fetus during pregnancy in human. Also, DN Tregs may serve as a novel target in URSA treatment.

Keywords: Decidua, DN regulatory T cells, pregnancy, recurrent, spontaneous abortion

Introduction

Recurrent spontaneous abortion (RSA), two or more consecutive pregnancy losses prior to 20 weeks, occurs in about 1% of all pregnancies [1]. Except for chromosomal, anatomic, endocrinologic, infectious and auto-immunologic abnormalities, there are 50% of RSA cases remain unexplained. The breakage of maternal-fetal immunologic tolerance have been implicated in the pathophysiology of unexplained recurrent spontaneous abortion (URSA) [2].

The mechanisms preventing the maternal immune system to reject its semi-allogeneic fetus are still poorly understood. Medawar first proposed the concept that human pregnancy is similar to a successful semi-allograft implantation and pregnancy being a state of immunological tolerance [3]. Localized mechanisms such as the expression of HLA-G may play an important role in fetal evasion of maternal immune attack [4]. Further, Th1/Th2 cytokine balance has been seen as another very important mechanism determining the survival of the fetus in the maternal uterus [5]. In 2004, Aluvihare reported that CD4$^+$CD25^{bright} forkhead box P3 (Foxp3)$^+$ Treg cells might mediate maternal tolerance to the fetus in mice [6]. Also, it has been demonstrated that adoptive transfer of Treg cells derived from normal pregnant mice could prevent fetal rejection in vivo, but transfer of Treg cells from nonpregnant mice was ineffective [7]. In humans, previous research show that CD4$^+$CD25$^+$Foxp3$^+$ Treg cells accumulate in the decidua in normal pregnancy, and the population of CD4$^+$CD25$^+$Foxp3$^+$ Treg cells is decreased in miscarriage [8-10]. The suppres-

Table 1. Characteristics of URSA patients, normal pregnant women and non-pregnant individuals

Groups	n	Age (year)	Pregnant duration (day)
URSA patients	42	29.96±3.89	56.1±8.3
Normal pregnant women	31	30.11±3.41	57.3±7.9
Non-pregnant individuals	22	29.92±3.67	

Note: Values are mean ± SD, URSA = unexplained recurrent spontaneous abortion.

sive activity of Tregs decreased in women with unexplained recurrent miscarriage [11].

Recently, a novel subset of $TCR^-\alpha\beta^+CD3^+CD4^-CD8^-$ double negative regulatory T cells (DN Tregs) has been described. It comprises 1-3% of peripheral T cells in normal mice and humans [12, 13]. It had been demonstrated that both rodent [14, 15] and human DN Tregs [16] lack expression of Foxp3. In murine, DN Tregs can suppress Ag-specific auto-, allo- or xeno-reactive $CD8^+$ [12, 17] T cells, $CD4^+$ [18] T cells, B cells [15, 19] or NK cells [20] and dendritic cells [21]. In mice, DN Tregs dominate female genital tract [22]. It plays an important role in preventing allograft rejection [18], graft-versus-host disease (GvHD) [23, 24] and autoimmune diabetes [15, 17]. In human, a remarkable increase has been made in the knowledge of the mechanisms of its function in recent years. In contrast to $CD4^+Foxp3^+$ Tregs, human DN Tregs have to be activated by allogeneic APCs or beads coated with anti-CD3 and anti-CD28 antibodies to induce their regulatory potential. Interestingly, human DN Tregs suppress proliferation of responder T cells by cell contact-dependent mechanisms and the suppression were reversible [16].

Whether human DN Tregs contribute to the maintaining of maternal-fetal tolerance has not been reported yet. Furthermore, current studies of DN Tregs in disease models are largely based on murine models. For human being, few researches have been done. In the present study, we compared the proportion of peripheral and/or decidual DN Tregs in the setting of women with normal history of reproduction, normal early pregnancy women and URSA patients. In addition, we performed functional analysis on peripheral and decidual DN Tregs to evaluate their anti-proliferation effects on activated $CD4^+$ and $CD8^+$ autologous T cells.

Materials and methods

Subjects

All the subjects enrolled were outpatients at the Department of Gynecology or Physical Examination Center, Provincial Hospital affiliated to Anhui Medical University, Hefei, China. 42 patients who had at least three consecutive spontaneous early miscarriages of unexplained etiology with the same partner were recruited to participate in the study. The diagnosis of "unexplained" miscarriage was made as the one defined by the Practice Committee of the American Society for Reproductive Medicine [1]. No URSA subjects had risk factors such as uterine malformation, chromosomal abnormality, infection (chlamydia, ureaplasma and TORCH syndrome), endocrinal disorders (luteal function defect, hyperprolactinemia, polycystic ovary syndrome and hyperandrogenemia), metabolic diseases (diabetes, insulin resistance, hyperthyroidism and hypothyroidism), maternal thrombophilias and antiphospholipid syndrome. All recruited URSA abortuses had been karyotyped, to exclude any chromosome problems. At the same time, 31 healthy early pregnant women who were undergoing elective termination during the first trimester and 22 non-pregnant healthy women with normal history of reproduction aged between 23 and 35 years old who were undergoing their physical examination were randomly selected in this study. In all 31 normal pregnant cases, fetal heart activity had been identified through B ultrasonography. There were no significant differences in age and pregnancy duration among these three groups (**Table 1**).

The study was conducted in compliance with the Declaration of Helsinki and was approved by the Ethics Committee of Provincial Hospital affiliated to Anhui Medical University (2011 Ethics 83rd). Signed informed consent was obtained from all women.

Sample preparation

Heparinized venous blood was obtained from all of the subjects according to protocols approved by the Institutional Review Board (IRB) of Provincial Hospital affiliated to Anhui Medical University. For the analysis of the proportion of DN Tregs, the peripheral blood samples were used directly, while for the in vitro suppression

Table 2. Proportion of DN Tregs in $CD3^+$ cells in peripheral blood and decidual lymphocytes in URSA patients, normal pregnant women and non-pregnant individuals

Group	n	DN Treg (%) in PBL	DN Treg (%) in decidual lymphocytes
URSA patients	42	0.92±0.68[a]	1.82±0.85[b,c]
Normal pregnant women	31	1.56±1.07	5.26±3.58[d]
Non-pregnant individuals	22	1.21±0.63	

Note: Values are mean ± SD, URSA = unexplained recurrent spontaneous abortion, PBL = peripheral blood lymphocytes; [a]P<0.05 vs. normal pregnant women PBL; [b]P<0.05 vs. normal pregnant women decidua; [c]P<0.01 versus PBL; [d]P<0.01 versus PBL.

assays of DN Tregs, peripheral blood mononuclear cells (PBMCs) were isolated by the standard Ficoll-Hypaque density centrifugation. Decidual samples were obtained from patients with induced abortion, representing early pregnant deciduas, and immediately from patients with spontaneous abortion, representing abortion samples. None of the decidual samples showed any evidence of necrosis or acute inflammation. The decidual mononuclear cells (leukocytes) were purified by the Ficoll-Hypaque method after mechanical disruption and filtration through a 32-μm nylon mesh, as previously reported [25].

Flow cytometry

The peripheral blood sample and decidual leukocytes were stained directly with the following monoclonal antibodies (all purchased from BD Biosciences): TCR-αβ-FITC, CD4-PE, CD8-PerCP-Cy5.5, CD3-APC or isotype IgG as controls. After incubating for 30 minutes at room temperature in the dark, samples were incubated with of Red Blood Cell lysis buffer (BD Biosciences) in the dark at room temperature for 10 minutes. Then, samples were centrifuged for 5 min at 1000 rpm at 20°C, and supernatant was aspirated. After washing with phosphate buffered saline (PBS), the cells were analyzed on a fluorescence-activated cell sorting (FACS) Calibur system by using Cell Quest software (Becton Dickinson). For the analysis, a real-time gate was set around the viable lymphocytes on the basis of their forward- and side-scatter profile. The proportions of $CD3^+CD4^-CD8^-$ T cell and $TCR\alpha\beta^+CD3^+CD4^-CD8^-$ DN Tregs in $CD3^+$ T lymphocytes were calculated.

The isolation of $TCR\alpha\beta^+CD3^+CD4^-CD8^-$ DN Tregs from PBMC and decidual leukocytes was performed using FACS also. Fluorochrome labeled mouse anti-human monoclonal antibodies (mAb) against TCRαβ-FITC, CD4-PE, CD8-PerCP-Cy5.5 and CD3-APC were used together with appropriate isotype controls to allow isolation of positive and negative cell populations. Isolated DN Tregs were reanalyzed after cell sorting and showed more than 95% purity. DN Tregs were then cultured at a density of 1×10^6/mL in RPMI 1640 medium plus 10% fetal bovine serum (FBS).

In vitro activation of DN Tregs

Isolated DN Tregs (1×10^5/well) were cocultured with anti-CD3/CD28-coated beads (2.5×10^4/well; Dynabeads CD3/CD28, Invitrogen) in 96-well U-bottom plates in complete medium. The total volume was 200 μl per well. Functional assay was performed 6 days later.

CFSE labeling and cell proliferation assays

Fresh isolated PBMCs from the same subject with DN Tregs were resuspended in RPMI 1640, After incubation for 2 h at 37°C in 5% CO_2, non-adherent cells were harvested and washed once and suspended in PBS at a density of 1×10^7/ml. Equal volume PBS containing 5 μmol/L CFSE was added into the lymphocytes suspension and incubated for 10 min at 37°C. CFSE-labeled lymphocytes were cultured in medium containing RPMI-1640 with added 10% FBS at a density of 1×10^6/ml.

CFSE-labeled lymphocytes (5×10^4/well) were stimulated in 96-well U-bottom plates with anti-CD3/CD28 beads (2.5×10^4/well, Invitrogen/Dynal, Oslo, Norway) in complete medium containing RPMI-1640 with added 10% FBS (200 ul/well) in the presence or absence of activated DN Tregs (5×10^4/well). After 5 days of culture, cells were harvested and stained with anti-CD8-PerCP-Cy5.5 and anti-CD4-PE mAb. Proliferation of cells was determined by flow cytometry. Cell Quest software (Becton Dickinson) was used for Flow cytometry analysis. For the analysis, a real-time gate was set around the viable lymphocytes based on their forward scatter/side scatter profile, avoiding monocyte

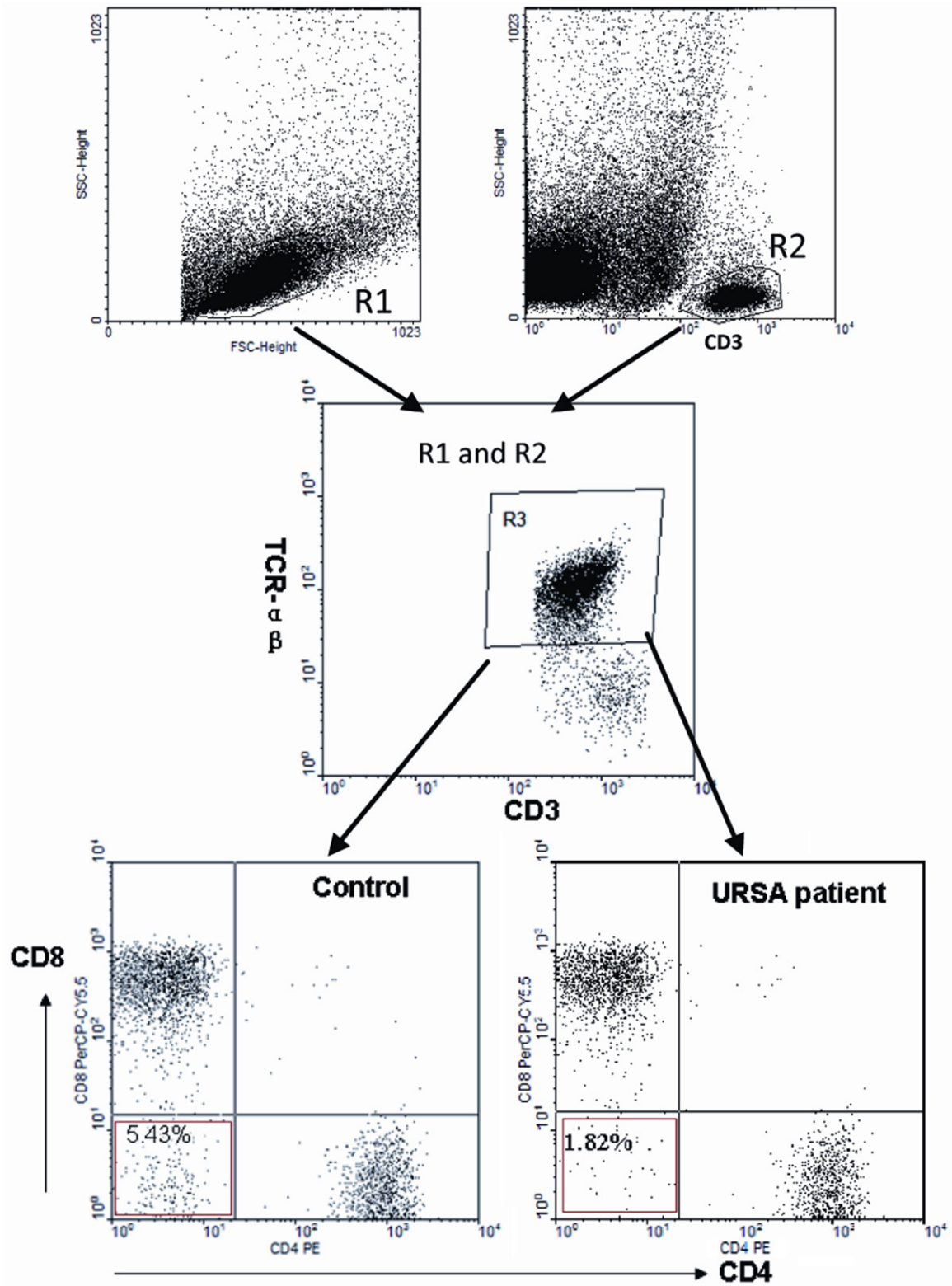

Figure 1. Flow cytometric analysis of TCR-αβ$^+$CD3$^+$CD4$^-$CD8$^-$ DN Tregs in decidual in normal early pregnant individuals and URSA patients. Decidual leukocytes were stained with anti-CD3-APC, anti-CD4-PE, anti-CD8-PerCP-Cy5.5, and anti-TCR-αβ-FITC mAbs and analyzed by flow cytometry. Regions were set on the CD3$^+$ T cells based on their forward and side scatter properties and their CD3 expression. The frequency of TCRαβ$^+$CD3$^+$CD4$^-$CD8$^-$ DN Tregs was determined as the percentage of TCRαβ$^+$CD4$^-$CD8$^-$ cells on gated CD3$^+$ T cell population. Representative results of flow cytometric analysis on decidual leukocytes from a normal early pregnant individual and an URSA patient are presented.

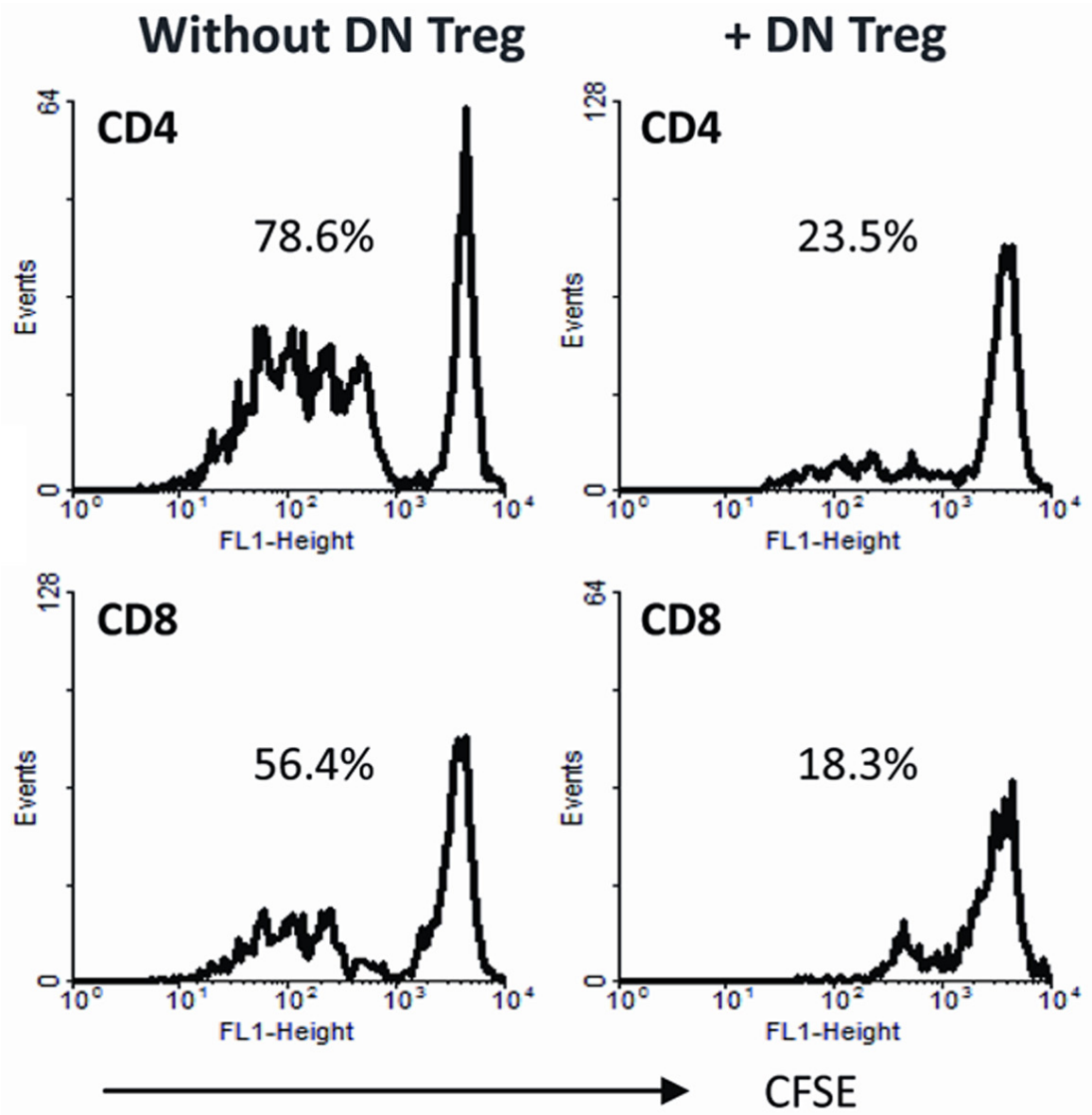

Figure 2. DN Treg cells in peripheral blood are able to suppress proliferation of autogenetic $CD4^+$ and $CD8^+$ T cells in URSA. FACS profiles are from 1 experiment of 3 performed.

and granulocyte populations. The in vitro proliferation of $CD4^+$ T cells or $CD8^+$ T cells in the presence or absence of DN Tregs was measured by calculating the percentage of CFSE dim cells in $CD4^+$ T cell or $CD8^+$ T cell population.

Statistical analysis

Student's t-test and one-way ANOVA were used for data with homogeneous variance. While Mann Whitney U test and Kruskal-Wallis test were used for heterogeneous variance data. All data analyses were processed by SPSS 15.0 statistical software. $P<0.05$ was considered significant.

Results

DN Tregs accumulate in the decidua in human in case of pregnancy

The proportions of DN Tregs in decidua were significantly higher than that in peripheral blood both in URSA patients (1.82±0.85% vs. 0.92±0.68%, $P<0.01$) and normal early pregnant women (5.26±3.58% vs. 1.56±1.07%, $P<0.01$) (**Table 2**). These data indicate that

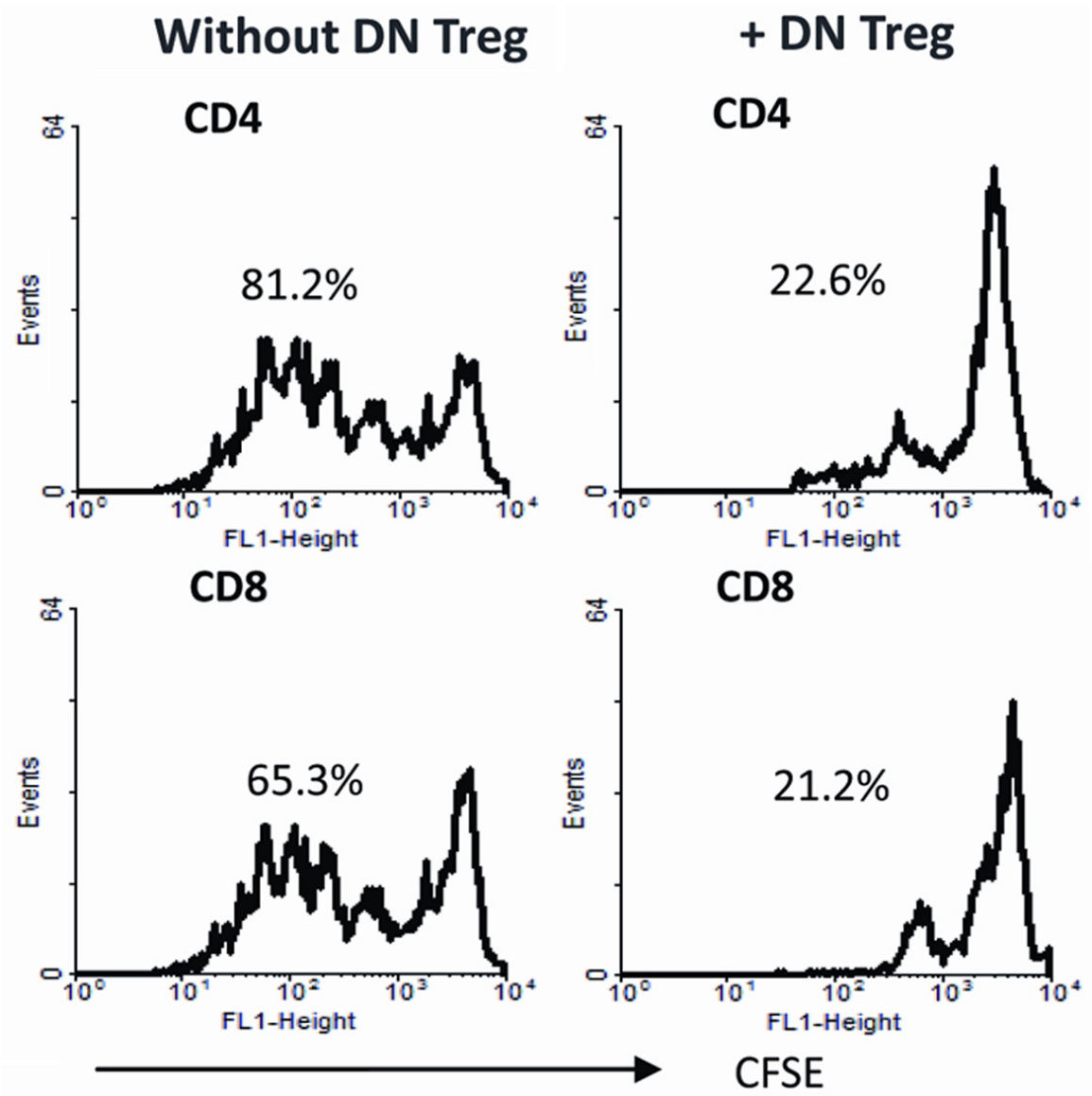

Figure 3. Decidual DN Tregs are able to suppress proliferation of autogenetic $CD4^+$ and $CD8^+$ T cells in URSA. FACS profiles are from 1 experiment of 3 performed.

decidua is one of the favorite tissue localizations of DN Tregs in human in case of pregnancy.

Frequency of DN Tregs from patients with URSA is decreased

The population of DN Tregs as a percentage of total $CD3^+$ T cells was evaluated by flow cytometric analysis using the gating strategy shown in **Figure 1**. The findings in this study indicated a decreased proportion of DN Tregs both in the peripheral blood lymphocytes (0.92±0.68% vs. 1.56±1.07%, $P<0.05$) and in deciduas leukocytes (1.82±0.85% vs. 5.26±3.58%, $P<0.05$) of patients with URSA than their normal early pregnant counterpart (**Table 2**).

DN Tregs have a suppressive effect on $CD4^+$ and $CD8^+$ T cells during early pregnancy

To investigate the suppressive potential of human decidual and peripheral blood DN Tregs, coculture experiments were performed. CFSE-labeled decidual and peripheral blood lymphocytes were cocultured with anti-CD3/CD28 beads in the presence or absence of allo-activated DN Tregs and proliferation of decidual

and peripheral blood lymphocytes cells was measured by flow cytometry. After 5 days, decidual and peripheral blood lymphocytes cells revealed a strong proliferation, which was significantly suppressed by the addition of DN Tregs (**Figures 2** and **3**). The suppressive effect of peripheral blood DN Tregs was similar among non-pregnant, normal pregnant and URSA patients. Also, the suppressive effect of decidual DN Tregs in URSA patients was similar with that of normal pregnant women.

Discussion

Suppression of immune responses by regulatory T (Treg) cells is one of the major mechanisms for the induction and maintenance of immuno-tolerance [26, 27]. Tregs have been shown to play an important role in a wide range of immune processes, including autoimmune disease [26, 28], transplantation tolerance [29, 30], GvHD [31] and cancer [32]. It has been confirmed that regulatory T cells consist of many distinct T cell subsets, including $CD4^+CD25^+Foxp3^+$ T cells, T-regulatory type 1 (Tr1) cells, T-helper 3 (Th3) cells, $CD8^+CD28^-$ T cells, and $TCR\alpha\beta^+CD3^+CD4^-CD8^-$ T cells [33, 34]. The group of L. Zhang was the first to identify and characterize $TCR\alpha\beta^+CD3^+CD4^-CD8^-$ NK1.1-T cells as antigen-specific double negative regulatory T cells (DN Tregs) in vitro and in vivo [12]. Subsequent reports from various laboratories confirmed and extended these findings in different disease models [13, 14, 18, 19]. Our study is the first in exploring the probably role of human DN Tregs in maternal-fetal tolerance.

DN Tregs seem to preferentially dwell in specific organs or tissues [35]. A previous study showed that DN Tregs were the dominant lymphocyte population (70%-90%) in the genital tract of naive, pregnant, or Chlamydia trachomatis-infected C57BL/6 mice [30]. Some previous studies demonstrate that DN Tregs can home into tolerant allografts to suppress immune responses locally and prevent the rejection of donor-specific allografts [36-38]. Our study showed for the first time that either in normal early pregnant woman or for URSA patients, the proportion of DN Treg in decidua was significantly higher than peripheral blood. The molecular mechanism leading to the selective accumulation of DN Tregs to deciduas was unknown. More researches should focus on the identification of molecules that are critical for DN Tregs migration.

In 2000, DN Tregs was first described having the ability in the induction of donor-specific transplantation tolerance to MHC-mismatched allografts [12]. Subsequent studies also showed that DN Tregs could induct transplantation tolerance to skin [39] and islet [14] allografts as well as cardiac xenografts [40] in donor-specific manner. Human pregnancy represents a condition of semiallograft to maternal host because of the presence of paternally derived antigens. However, mechanisms preventing the maternal immune system to reject its semiallogeneic fetus are still poorly understood. URSA is thought to be caused by the allorejection of the fetus by the mother. In this study, we demonstrated for the first time that the proportion of DN Tregs both in peripheral blood and in decidua decreased in URSA patients than normal early pregnant women. Moreover, in decidual, the significance was more obvious. The results suggest that the decreased proportion of DN Tregs in peripheral blood and decidua may play an important role in the pathogenesis of URSA in human.

In this study we have examined the suppressive function of human DN Tregs in non-pregnant woman, normal early pregnant woman and URSA patients. We demonstrate that human DN Tregs are highly potent suppressor cells of $CD4^+$ and $CD8^+$ T cell responses in these patients. However, our data reveal that the suppressive effects of DN Tregs in peripheral blood and decidual were similar among these three groups.

Conclusions

In summary, our data show that human DN Tregs enrich in decidua during pregnancy. Its proportion decreased in women with URSA, although it suppressive capacity did not change. The results suggest that DN Tregs may contribute to the mechanisms mediating maternal-fetal immunological tolerance and may be involved in protection the fetus during pregnancy in human. It also suggests that DN Tregs may serve as a novel target in URSA treatment.

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Disclosure of conflict of interest

None.

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References

[1] Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril 2013; 99: 63.

[2] Christiansen OB. Reproductive immunology. Mol Immunol 2013; 55: 8-15.

[3] Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 1953; 7: 320-38.

[4] Nakamura O. Children's immunology, what can we learn from animal studies (1): Decidual cells induce specific immune system of feto-maternal interface. J Toxicol Sci 2009; 34 Suppl 2: SP331-39.

[5] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993; 14: 353-6.

[6] Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004; 5: 266-71.

[7] Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, Kotsch K, Leber J, Volk HD. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. Am J Pathol 2005; 166: 811-22.

[8] Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod 2004; 10: 347-53.

[9] Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH. Evidence for a selective migration of fetus-specific CD4+CD25^{bright} regulatory T cells from the peripheral blood to the decidua in human pregnancy. J Immunol 2008; 180: 5737-45.

[10] Mjösberg J, Berg G, Jenmalm MC, Ernerudh J. FOXP3+ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. Biol Reprod 2010; 82: 698-705.

[11] Wang WJ, Hao CF, Qu QL, Wang X, Qiu LH, Lin QD. The deregulation of regulatory T cells on interleukin-17-producing T helper cells in patients with unexplained earlyrecurrent miscarriage. Hum Reprod 2010; 25: 2591-6.

[12] Zhang ZX, Yang L, Young KJ, DuTemple B, Zhang L. Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. Nat Med 2000; 6: 782-9.

[13] Fischer K, Voelkl S, Heymann J, Przybylski GK, Mondal K, Laumer M, Kunz-Schughart L, Schmidt CA, Andreesen R, Mackensen A. Isolation and characterization of human antigen-specific TCR alpha beta1CD4(-)CD8-double-negative regulatory T cells. Blood 2005; 105: 2828-35.

[14] Zhang D, Yang W, Degauque N, Tian Y, Mikita A, Zheng XX. New differentiation pathway for double-negative regulatory T cells that regulates the magnitude of immune responses. Blood 2007; 109: 4071-9.

[15] Hillhouse EE, Beauchamp C, Chabot-Roy G, Dugas V, Lesage S. Interleukin-10 limits the expansion of immunoregulatory CD4-CD8-T cells in autoimmune-prone non-obese diabetic mice. Immunol Cell Biol 2010; 88: 771-80.

[16] Voelkl S, Gary R, Mackensen A. Characterization of the immunoregulatory function of human TCR-alphabeta1CD4-CD8-double-negative T cells. Eur J Immunol 2011; 41: 739-48.

[17] Ford MS, Chen W, Wong S, Li C, Vanama R, Elford AR, Asa SL, Ohashi PS, Zhang L. Peptide-activated double-negative T cells can prevent autoimmune type-1 diabetes development. Eur J Immunol 2007; 37: 2234-41.

[18] Chen W, Zhou D, Torrealba JR, Waddell TK, Grant D, Zhang L. Donor lymphocyte infusion induces long-term donor-specific cardiac xenograft survival through activation of recipient double-negative regulatory T cells. J Immunol 2005; 175: 3409-16.

[19] Ma Y, He KM, Garcia B, Min W, Jevnikar A, Zhang ZX. Adoptive transfer of double negative T regulatory cells induces B-cell death in vivo and alters rejection pattern of rat-to-

mouse heart transplantation. Xenotransplantation 2008; 15: 56-63.
[20] He KM, Ma Y, Wang S, Min WP, Zhong R, Jevnikar A, Zhang ZX. Donor double-negative Treg promote allogeneic mixed chimerism and tolerance. Eur J Immunol 2007; 37: 3455-66.
[21] Gao JF, McIntyre MS, Juvet SC, Diao J, Li X, Vanama RB, Mak TW, Cattral MS, Zhang L. Regulation of antigen-expressing dendritic cells by double negative regulatory T cells. Eur J Immunol 2011; 41: 2699-2708.
[22] Johansson M, Lycke N. A unique population of extrathymically derived alpha beta TCR+CD4-CD8-T cells with regulatory functions dominates the mouse female genital tract. J Immunol 2003; 170: 1659-66.
[23] Young KJ, DuTemple B, Phillips MJ, Zhang L. Inhibition of graft-versus-host disease by double-negative regulatory T cells. J Immunol 2003; 171: 134-41.
[24] Ye H, Chang Y, Zhao X, Huang X. Characterization of CD3+CD4-CD8-(double negative) T cells reconstitution in patients following hematopoietic stem-cell transplantation Transpl Immunol 2011; 25: 180-6.
[25] Tsuda H, Michimata T, Hayakawa S, Tanebe K, Sakai M, Fujimura M, Matsushima K, Saito S. A Th2 chemokine, TARC, produced by trophoblasts and endometrial gland cells, regulates the infiltration of CCR4+ T lymphocytes into human decidua at early pregnancy. Am J Reprod Immunol 2002; 48: 1-8.
[26] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995; 155: 1151-64.
[27] Shevach EM. Mechanisms of Foxp3+ T regulatory cell-mediated suppression. Immunity 2009; 30: 636-45.
[28] Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol 2010; 10: 849-59.
[29] Kang SM, Tang Q, Bluestone JA. CD4+CD25+ regulatory T cells in transplantation: progress, challenges and prospects. Am J Transplant 2007; 7: 1457-63.
[30] Word KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nature Rev 2003; 3: 199-210.
[31] Ukena SN, Velaga S, Geffers R, Grosse J, Baron U, Buchholz S, Stadler M, Bruder D, Ganser A, Franzke A. Human regulatory T cells in allogeneic stem cell transplantation. Blood 2011; 118: e82-92.
[32] Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, Karin M. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature 2011; 470: 548-53.
[33] Chang CC, Ciubotariu R, Manavalan JS, Yuan J, Colovai AI, Piazza F, Lederman S, Colonna M, Cortesini R, Dalla-Favera R, Suciu-Foca N. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat Immunol 2002; 3: 237-43.
[34] Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997; 389: 737-42.
[35] D'Acquisto F, Crompton T. CD3(+)CD4(-)CD8(-) (double negative) T cells: saviors or villains of the immune response? Biochem Pharmacol 2011; 82: 333-40.
[36] Young KJ, Yang L, Phillips MJ, Zhang L. Donor-lymphocyte infusion induces transplantation tolerance by activating systemic and graft-infiltrating double-negative regulatory T cells. Blood 2002; 100: 3408-14.
[37] Lee BP, Mansfield E, Hsieh SC, Hernandez-Boussard T, Chen W, Thomson CW, Ford MS, Bosinger SE, Der S, Zhang ZX, Zhang M, Kelvin DJ, Sarwal MM, Zhang L. Expression profiling of murine double-negative regulatory T cells suggests mechanisms for prolonged cardiac allograft survival. J Immunol 2005; 174: 4535-44.
[38] Chen W, Ford MS, Young KJ, Zhang L. Infusion of in vitro generated DN T regulatory cells induces permanent cardiac allograft survival in mice. Transplant Proc 2003; 35: 2479-80.
[39] Ford MS, Young KJ, Zhang Z, Ohashi PS, Zhang L. The immune regulatory function of lymphoproliferative double negative T cells in vitro and in vivo. J Exp Med 2002; 196: 261-7.
[40] Chen W, Ford MS, Young KJ, Cybulsky MI, Zhang L. Role of double-negative regulatory T cells in long-term cardiac xenograft survival. J Immunol 2003; 170: 1846-53.