Expansion of decidual CD45RO$^+$ T cells with high expression of CEACAM1 in the early stage of pregnancy

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Abstract. The aim of the present study was to investigate the mechanism involved in the expansion of CD45RO$^+$ T cells in the decidual microenvironment, and in the expression of the inhibitory carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) on the surface of decidual CD45RO$^+$ T cells. Twenty-one healthy nonpregnant females and seventeen healthy pregnant females in the first trimester were included in the study. Peripheral blood samples from nonpregnant and pregnant females, and decidual tissues from pregnant females following elective abortion, were obtained and analyzed by flow cytometry. The percentages of CD45RO$^+$ T cells and CEACAM1-expressing CD45RO$^+$ T cells were significantly higher in first trimester human decidua than in the peripheral blood. Conditioned medium from the coculture of monocytes and the human trophoblast HTR8/SVneo cell line (MHM) was added to the model for the generation of CD45RO$^+$ T cells *in vitro*. MHM caused an increase in the percentage of CD45RO$^+$ T cells in a monocyte chemoattractant protein-1 (MCP-1)-dependent manner and an increase in the percentage of CEACAM1-expressing CD4$^+$CD45RO$^+$ T cells in the model. In conclusion, our results implied that trophoblast cells and monocytes may be involved in the increase of decidual CD45RO$^+$ T cells and the high expression of CEACAM1 on their surfaces.

Introduction

The establishment of the placenta in humans begins with the invasion and migration of fetal trophoblast cells into the maternal decidua (1). During this process, the decidual environment is infiltrated by a large number of leukocytes, which predominantly include CD56$^+$CD16$^-$ NK cells (~70%), macrophages (~10%) and T cells (~10%) (2,3). Previous studies have demonstrated that T cells have an important physiological role in early pregnancy (4). T cells may be subdivided into CD45RA$^+$ T cells and CD45RO$^+$ T cells according to the surface molecules that are expressed. The percentage of CD45RO$^+$ T cells has been demonstrated to increase in first trimester human decidua (5,6). However, the expansion mechanisms of decidual CD45RO$^+$ T cells remain unclear. The expansion of leukocytes in first trimester human decidua may be explained by two mechanisms: i) decidual leukocytes are recruited from the peripheral blood by hormones, cytokines and chemokines (7-10); and ii) the generation of decidual leukocytes occurs in the decidual microenvironment (2,11). Trophoblast cells are primary fetal cells, which are in close contact with maternal immune cells in human decidua (1). Although the effects of trophoblast cells on the recruitment of immune cells have been extensively investigated (8,12,13), the effect of trophoblast cells on the expansion and differentiation of decidual leukocytes remains unknown (14). A previous study demonstrated that the interactions between trophoblast cells and monocytes significantly increased the secretion and production of cytokines and chemokines (15), including monocyte chemoattractant protein-1 (MCP-1), which is important in the generation and survival of CD45RO$^+$ T cells (16). Whether the crosstalk between trophoblast cells and monocytes participates in the expansion of decidual CD45RO$^+$ T cells remains to be elucidated.

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), is a multifunctional cellular adhesion molecule, and is a member of the carcinoembryonic antigen family and the immunoglobulin superfamily (17). It is well-established that CEACAM1 is significant in regulating the functions of T cells (18). Numerous studies have demonstrated that CEACAM1 may be expressed at low levels on the surface of resting T cells. However, the expression of CEACAM1 may be rapidly upregulated following T cell activation, implying that CEACAM1 acts as an activation-induced cell surface molecule of T cells (18-21). Previous studies have demonstrated that a fraction of T cells infiltrating the lamina propria of the small intestine in celiac disease and the large intestine in inflammatory bowel disease express CEACAM1. This suggests

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that CAECAM1 may be expressed at inflammatory sites and participate in modulating the immune response *in vivo* (21,22). Studies have also indicated that the first trimester of pregnancy is a pro-inflammatory phase (23,24). T cells in first trimester human decidua have been demonstrated to express cell surface activation markers, including CD69 and HLA-DR, which implies that decidual T cells are regionally activated (25). To date, the mechanism of CEACAM1 expression on the surface of decidual T cells at an early stage of pregnancy remains unknown. We therefore investigated the expression of CEACAM1 on the surface of T cells in first trimester human decidua, and the effect of the crosstalk between trophoblast cells and monocytes on the expression of CEACAM1 on the surface of $CD45RO^+$ T cells.

In conclusion, in the present study, we analyzed the percentage of $CD45RO^+$ T cells and the expression of CEACAM1 on the surface of T cells in first trimester human decidua and in the peripheral blood, and identified that these percentages were significantly increased in the decidua. Using the model to generate $CD45RO^+$ T cells *in vitro*, we demonstrated that conditioned medium from the coculture of the extravillous trophoblast HTR-8/SVneo cell line and monocytes (MHM) increased the percentage of $CD45RO^+$ T cells in an MCP-1 dependent manner, and increased the expression of CEACAM1 on the surface of $CD4^+CD45RO^+$ T cells. These data implied that decidual $CD45RO^+$ T cells were activated in an early stage of pregnancy, and suggested that trophoblasts and monocytes may be involved in the increase of $CD45RO^+$ T cells and the high expression of CEACAM1 on their surface.

Materials and methods

Sample collection. Twenty-one healthy nonpregnant females and seventeen healthy pregnant females in their first trimester (from Qilu Hospital, Shandong University, Shandong, China) volunteered to participate in this study (Table I). The use of human tissues was approved by the Ethics Committee of Qilu Hospital (Shandong University, Jinan, China), and written informed consent was obtained from all participants. Peripheral blood samples and decidual tissues were obtained following elective termination of the pregnancy. Cases without maternal or fetal complications were selected for tissue sampling. Peripheral blood samples from nonpregnant females, who were not taking systemic hormonal contraception, were without medical complications and were in the secretory phase of the menstrual cycle, were included as controls. To obtain the decidual mononuclear cells, the decidual tissue was macroscopically separated from the villi, washed twice with phosphate-buffered saline (PBS) to remove the contaminated blood, dissected into small pieces, washed twice again, and then passed through a 120- and 75-μm stainless steel mesh. The decidual mononuclear cells were isolated using Ficoll Histopaque®-1077 (Sigma-Aldrich, St. Louis, MO, USA) by density gradient centrifugation.

Reagents. Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD4 monoclonal antibody (mAb) (Jingmei Biological, Co., Beijing, China), CD8 mAb (Becton-Dickinson, Franklin Lakes, NJ, USA), phycoerythrin (PE)-conjugated mouse anti-human CEACAM1 mAb (R&D Systems, Minneapolis, MN, USA), PE-cyanine (Cy) 5-conjugated mouse anti-human CD45RO mAb (Jingmei Biological Co.) and their isotype- and fluorochrome-matched control antibodies, were used for flow cytometry. Human recombinant MCP-1 (rhMCP-1) and monoclonal anti-MCP-1 antibodies were obtained from R&D Systems.

Conditioned medium from the coculture of monocytes and HTR8/SVneo cell line (MHM). HTR8/SVneo cells were grown in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin. $CD14^+$ monocytes were isolated from the peripheral blood with a magnetic cell sorting system [$CD14^+$ microbeads (#130-050-201) and an LS column (#130-042-401)] according to the manufacturer's instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). The HTR8/SVneo cells were cocultured at a ratio of 1:1 ($5x10^5/5x10^5$ cells). The conditioned medium was collected after 40 h.

Enzyme-linked immunosorbent assay (ELISA). The MCP-1 levels in the culture supernatant were measured with the Human CCL2/MCP-1 Quantikine ELISA kit (R&D Systems), and assays were conducted according to the manufacturer's instructions. All measurements were performed in triplicate to avoid technical error and intra-assay variants.

Flow cytometric analysis. The expression of CD4, CD8, CD45RO and CEACAM1 on the surface of T cells was determined by extracellular staining with a specific monoclonal antibody. The background fluorescence was assessed using the appropriate isotype-and fluorochrome-matched control mAbs. The FACSCalibur flow cytometer and the CellQuest software program of the FACSCalibur system (Becton-Dickinson) were used for the measurement and analysis of the stained cells.

Model to generate $CD45RO^+$ T cells. Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood of healthy donors using gradient centrifugation over Histopaque®-1077 (Sigma-Aldrich). PBMCs from one donor were treated with 25 mg/ml mitomycin C (Roche, Basel, Switzerland) for 20 min, and washed twice with warmed RPMI-1640 supplemented with 10% FBS. PBMCs were reseeded into 24-well plates at a density of 500 μl/well and a concentration of $1x10^6$ cells. PBMCs from another donor, without treatment, were cocultured with the pretreated PBMCs, (density, 500 μl/well; concentration, $1x10^6$ cells). The supernatant and control medium pretreated with anti-MCP-1 mAb (10 μg/ml) or rhMCP-1 (5 μg/ml) for 30 min were added to the 24-well plates (density, 1 ml/well). A mixed lymphocyte reaction (MLR) was conducted for 8 days, cells were collected, and the medium was changed every 3 days.

Statistical analysis. Statistical analysis was performed using SPSS version 11.5. Normality of the data was tested using the Shapiro-Wilk test. Data were normally distributed, and the results are presented as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) test was used for statistical comparisons between groups (where number of groups ≥3) and a Fisher's least significant difference test was used for post hoc analysis of the significant ANOVA results.

Table I. Characteristics of the study groups.

Variable	Secretory phase of menstrual cycle	Pregnant
No. of subjects	21	17
Age (years)	27.7±2.2	29.5±4.4
Height (cm)	163.9±3.5	162.5±3.1
BMI (kg/m^2)	21.8±1.7	20.7±2.5
Gestational age (weeks)		6.6±0.8

All values represent the mean ± SD, unless otherwise indicated. BMI, body mass index.

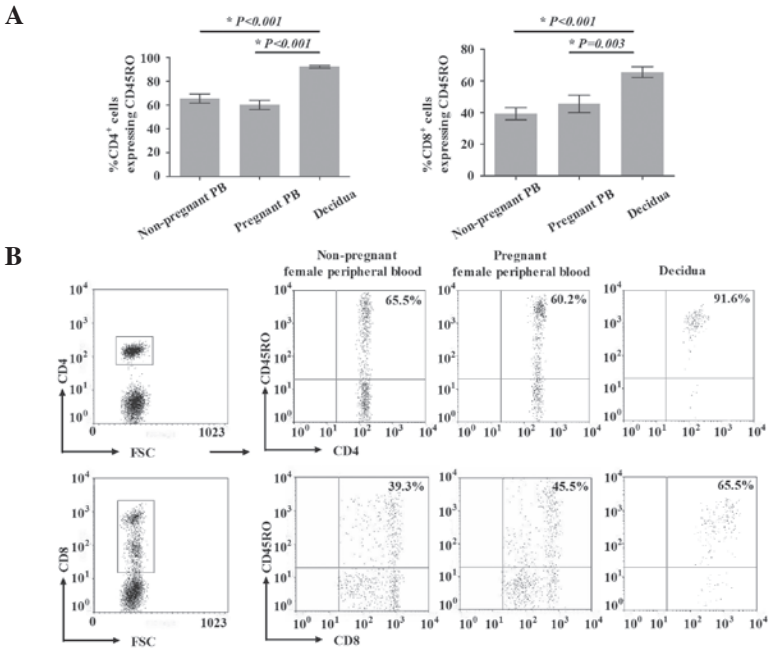

Figure 1. Percentages of CD45RO$^+$ T cells in first trimester human decidua and in the PB of healthy non-pregnant and pregnant females.(A) The percentages of CD4$^+$CD45RO$^+$ T cells among CD4$^+$ T cells and the percentages of CD8$^+$CD45RO$^+$ among CD8$^+$ T cells in the PB from non-pregnant and pregnant females, and in the decidua from pregnant females. The percentage of CD4$^+$CD45RO$^+$ T cells among CD4$^+$ T cells in first trimester human decidua was significantly increased compared with that in the PB of non-pregnant females (P<0.001) and pregnant females (P<0.001). The percentage of decidual CD8$^+$CD45RO$^+$ T cells among CD8$^+$ T cells was also significantly increased compared with that in the PB of non-pregnant females (P<0.001) and pregnant females (P=0.003). Data are presented as the mean ± SD. *P<0.05. (B) The isolated lymphocytes were analyzed for phenotypic frequency by flow cytometry. CD4$^+$ and CD8$^+$ T cells were individually gated and further analysis was conducted. Markers were set according to the CD45RO-isotype IgG. PB, peripheral blood; FSC, forward scatter channel; SSC, side scatter channel.

A paired student's t-test was used for the statistical analysis of the differences in the percentages of CD45RO$^+$ T cells in the model, *in vitro,* on days 0 and 8. P<0.05 was considered to indicate a statistically significant difference.

Results

Percentage of CD45RO$^+$ T cells in first trimester human decidua. The percentages of CD45RO$^+$ T cells in first trimester human decidua and in the peripheral blood of healthy nonpregnant and pregnant females are presented in Fig. 1. For healthy nonpregnant and pregnant females, the percentages of CD4$^+$CD45RO$^+$ T cells among CD4$^+$ T cells in the peripheral blood were 65.5±12.6 and 60.2±12.3%, respectively. The percentage of CD4$^+$CD45RO$^+$ T cells among CD4$^+$ T cells in the decidua was significantly higher (91.6±4.8%) compared with that in the peripheral blood from nonpregnant (P<0.001) and pregnant (P<0.001) females. Similarly, the percentage of CD8$^+$CD45RO$^+$ T cells among CD8$^+$ T cells in the decidua (65.5±10.7%) was also significantly higher, compared with that in the peripheral blood of nonpregnant (39.3±12.2%) and pregnant (45.5±17.2%) females (P<0.001 and P=0.003, respectively). However, the percentages of CD4$^+$CD45RO$^+$ T cells among CD4$^+$ and CD8$^+$CD45RO$^+$ T cells among CD8$^+$ T cells in the peripheral blood were not significantly different between nonpregnant and pregnant females (P=0.265 and P=0.317, respectively).

Expression of CEACAM1 on the surface of peripheral and decidual T cells. A number of studies have demonstrated that CEACAM1, an activation-induced cell surface molecule of T cells, inhibits the cytokine production, proliferation and cytotoxic activity of activated T cells by homophilic (CEACAM1-CEACAM1) and heterophilic (CEACAM1-

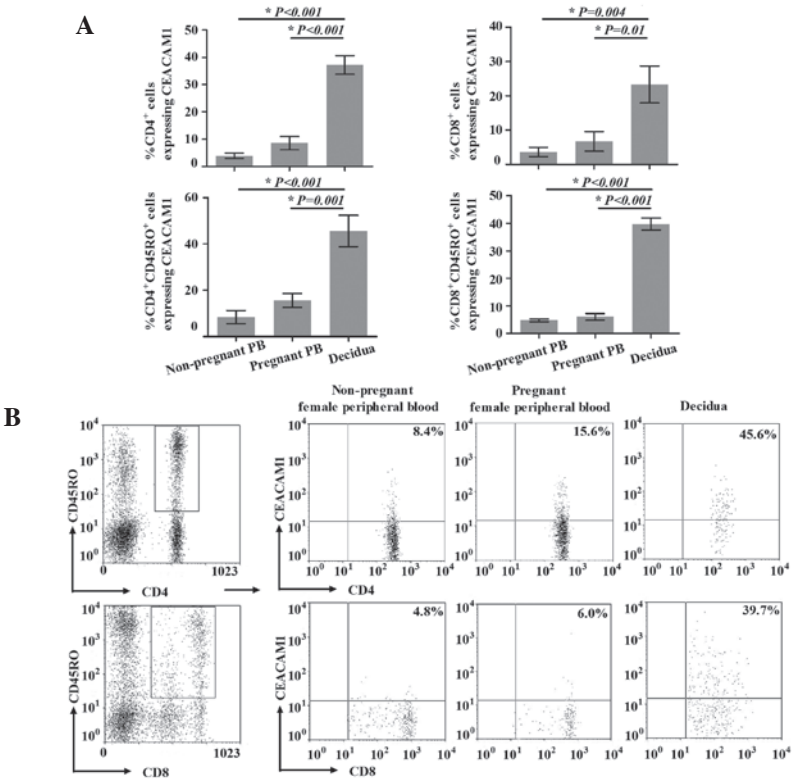

Figure 2. Expression of CEACAM1 on the surface of T cells in first trimester human decidua and in the PB of healthy non-pregnant and pregnant females. (A) The percentages of CEACAM1+ cells among CD4+ T cells, CD8+ T cells, CD4+CD45RO+ T cells and CD8+CD45RO+ T cells are shown. The percentage of CD4+CEACAM1+ T cells in the decidua was significantly increased compared with that in the PB from non-pregnant females (P<0.001) or pregnant females (P<0.001). The percentage of CD8+CEACAM1+ T cells in the decidua was significantly increased compared with that in the PB from non-pregnant females (P=0.004) or pregnant females (P=0.01). The percentage of CD4+CD45RO+CEACAM1+ T cells in the decidua was significantly increased compared with that in the PB from non-pregnant females (P<0.001) and pregnant females (P=0.001). The percentage of CD8+CD45RO+CEACAM1+ T cells in the decidua was significantly increased compared with that in the PB from non-pregnant females (P<0.001) and pregnant females (P<0.001). Data are presented as the mean ± SD. *P<0.05. (B) The isolated lymphocytes were analyzed for phenotypic frequency by flow cytometry. CD4+CD45RO+ and CD8+CD45RO+ T cells were individually gated and further analysis was conducted. Markers were set according to CEACAM1. PB, peripheral blood; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1.

CEACAM5 and CEACAM1-Opa) interactions (26-29). As numerous studies have demonstrated that decidual T cells are regionally activated (25,30,31), we detected the expression of CEACAM1 on the surface of decidual T cells. As shown in Fig. 2A, for healthy nonpregnant and pregnant females, the percentages of CD4+CEACAM1+ T cells in the peripheral blood (3.9±2.7 and 8.5±6.8%, respectively) were not significantly different (P=0.204). However, the percentage of CD4+CEACAM1+ T cells in the decidua (37.2±9.8%) was significantly higher compared with that in the peripheral blood (P<0.001 vs. peripheral blood from nonpregnant females and P<0.001 vs. peripheral blood from pregnant females). The percentages of CD8+CEACAM1+ T cells showed the same trends as those of the CD4+CEACAM1+ T cells, and those in the peripheral blood of nonpregnant and pregnant females, and in the decidua were 3.6±2.6, 6.7±5.8 and 23.3±10.7%, respectively (P=0.004, peripheral blood from nonpregnant females vs. decidua; P=0.01, peripheral blood from pregnant females vs. decidua; P=0.56, peripheral blood from nonpregnant females vs. pregnant females).

Further, we identified the expression of CEACAM1 on the surface of decidual and peripheral CD45RO+ T cells (Fig. 2). The expression of CEACAM1 on the surface of peripheral CD4+CD45RO+T cells from healthy nonpregnant females was identified in 8.4±7.1% of cells, in contrast to 15.6±7.4% of such cells from healthy pregnant females; however, this difference was not significant (P=0.436). Notably, CEACAM1-expressing cells were present at a significantly higher level (45.6±21.4%) in the decidua compared with the peripheral blood from nonpregnant (P<0.001) and pregnant (P=0.001) females. The expression of CEACAM1 on the surface of decidual and peripheral CD8+CD45RO+ T cells demonstrated the same trends, with expression rates of 4.8±1.0 and 6.0±2.0% in healthy nonpregnant and pregnant females, respectively (P=0.586, peripheral blood from nonpregnant females vs. pregnant females). In addition, the expression of CEACAM1 on the surface of decidual CD8+CD45RO+ T cells (39.7±3.8%) was significantly higher than that in the peripheral blood (P<0.001 vs. peripheral blood from nonpregnant females and P<0.001 vs. peripheral blood from pregnant females).

Expansion of CD45RO+ T cells by MHM is MCP-1 dependent. To investigate the potential expansion mechanisms of CD45RO+ T cells, the model to generate CD45RO+ T cells was established *in vitro,* as described in Materials and methods. Compared with the percentages on day 1, the percentages of CD4+CD45RO+ T cells among CD4+ T cells and of CD8+CD45RO+ T cells among CD8+ T cells were significantly

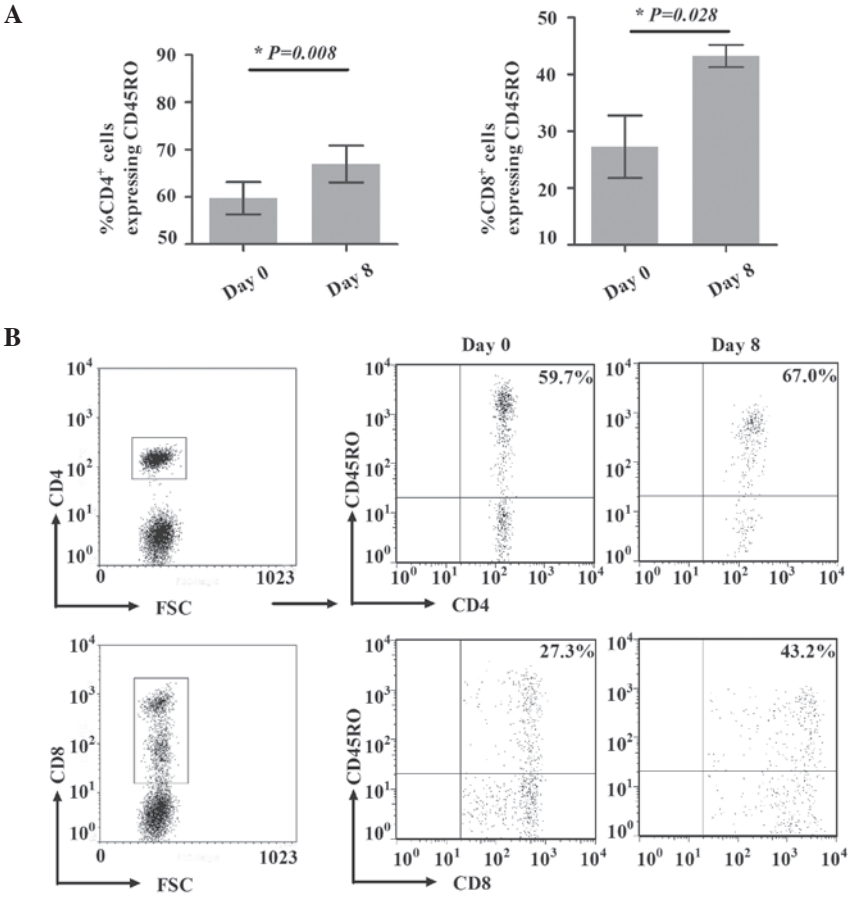

Figure 3. Establishment of the model for the generation of CD45RO+ T cells. (A) The percentage of CD4+CD45RO+ T cells among CD4+ T cells and the percentage of CD8+CD45RO+ T cells among CD8+ T cells in the model on days 0 and 8 are indicated. The experiments were repeated more than three times. The percentages of CD4+CD45RO+ T cells among CD4+ T cells and CD8+CD45RO+ T cells among CD8+ T cells were significantly increased on day 8 compared with those on day 0 (P=0.008 and P=0.028, respectively). Data are presented as the mean ± SD. *P<0.05. (B) CD4+ and CD8+ T cells were individually gated and further analysis was conducted. The percentages of CD45RO+ T cells on days 0 and 8 are indicated.

increased on day 8, from 59.7±6.8 to 67.0±7.7% (P=0.008) and from 27.3±11.1 to 43.2±3.9% (P=0.028; Fig. 3), respectively, indicating that the induced model was successful.

To clarify the effect of the interaction between trophoblast cells and monocytes on the expansion of CD45RO+ T cells, MHM was added to the model, and the percentage of CD4+CD45RO+ T cells among CD4+ T cells on day 8 was significantly increased from 63.6±4.7 to 78.4±3.2% (P=0.002). Similarly, the percentage of CD8+CD45RO+ T cells among CD8+ T cells on day 8 was increased from 41.4±1.6 to 61.6±5.6% (P<0.001; Fig. 4B and C). These data suggested that trophoblasts and monocytes were likely to be involved in the generation of CD45RO+ T cells. The secretion of MCP-1 was determined by ELISA, as it has been demonstrated to be significant in the generation of CD45RO+ T cells (16). Consistent with a previous study (15), we observed that the production of MCP-1 in the MHM was notably higher than that in the monocyte or human trophoblast HTR8/SVneo cell line cultures (Fig. 4A). In order to clarify the effect of MCP-1 from the MHM on the increase of CD45RO+ cells in the model, neutralizing antibody against MCP-1 was added to the MHM; the percentage of CD4+CD45RO+ T cells among CD4+ T cells on day 8 was significantly decreased in the MHM + anti-MCP-1 group compared with that in the MHM group (69.5±5.6 vs. 78.4±3.2, P=0.028; Fig. 4B and C). Compared with the control group, the percentage of CD4+CD45RO+ T cells among CD4+ T cells on day 8 was significantly increased in the rhMCP-1 group (63.6±4.7 vs. 73.0±1.5, respectively, P=0.022). The percentage of CD8+CD45RO+ T cells among CD8+ T cells in the MHM + anti-MCP-1 group was significantly decreased compared with that of the MHM group (46.3±4.8 vs. 61.6±5.6%, P=0.001; Fig. 4B and C). Compared with the control group, the percentage of CD8+CD45RO+ T cells among CD8+ T cells on day 8 was significantly increased in the rhMCP-1 group (41.4±1.6 vs. 51.0±1.0%, P=0.015). These results suggested that MCP-1 was involved in the increase of CD45RO+ T cells through the addition of MHM.

Proportion of CEACAM1-expressing CD4+CD45RO+ T cells is increased by MHM and rhMCP-1. To investigate the potential involvement of the interaction of trophoblast cells and monocytes in the expression of CEACAM1 on the surface of CD45RO+ T cells in first trimester human decidua, we analyzed the expression of CEACAM1 on the surface of such T cells in the model. As shown in Fig. 5, the proportion of CEACAM1-expressing CD4+CD45RO+ T cells in the control group on day 8 was 8.04±1.31% (Fig. 5). However, when cells in the model were treated with MHM, the propor-

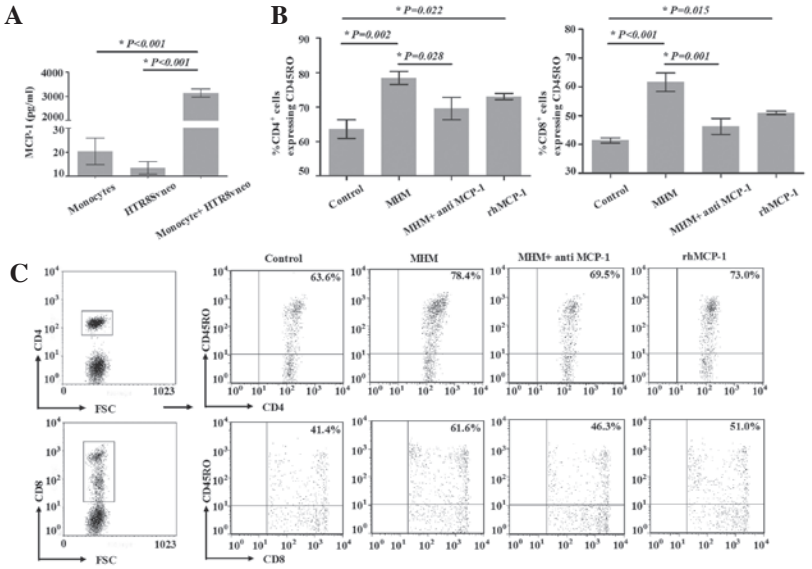

Figure 4. Percentages of CD45RO+ T cells were increased by conditioned medium from the coculture of monocytes and the human trophoblast HTR8/SVneo cell line (MHM) in models in a MCP-1-dependent manner. (A) The concentrations of MCP-1 in differently conditioned media were detected by ELISA. The results are from three cultures performed in parallel. The production of MCP-1 in the MHM was significantly higher than that in monocyte (P<0.001) and human trophoblast HTR8/SVneo cell line (P<0.001) cultures. Data are presented as the mean ± SD. *P<0.05. (B) The effect of different factors on the generation of CD45RO+ T cells *in vitro*. The percentages of CD4+CD45RO+ T cells among CD4+ T cells, and of CD8+CD45RO+ T cells among CD8+ T cells. The experiments were conducted three times. Compared with the control group, the proportion of CD45RO-expressing CD4+ T cells and CD45RO-expressing CD8+ T cells was significantly increased in the MHM group (P=0.002 and P<0.001, respectively). The percentage of CD4+CD45RO+ T cells among CD4+ T cells on day 8 was significantly decreased in the MHM + anti-MCP-1 group compared with the MHM group (P=0.028). The percentage of CD4+CD45RO+ T cells among CD4+ T cells on day 8 was significantly increased in the human recombinant (rhMCP-1) group compared with the control group (P=0.022). The percentage of CD8+CD45RO+ T cells among CD8+ T cells on day 8 was significantly decreased in the MHM + anti-MCP-1 group compared with the MHM group (P=0.001). The percentage of CD8+CD45RO+ T cells among CD8+ T cells on day 8 was significantly increased in the human recombinant MCP-1 (rhMCP-1) group compared with that in the control group (P=0.015). Data are presented as the mean ± SD. *P<0.05. (C) The lymphocytes were analyzed for phenotypic frequency by flow cytometry. CD4+ and CD8+ T cells were individually gated and further analysis was conducted. MCP-1, monocyte chemoattractant protein-1; ELISA, enzyme-linked immunosorbent assay.

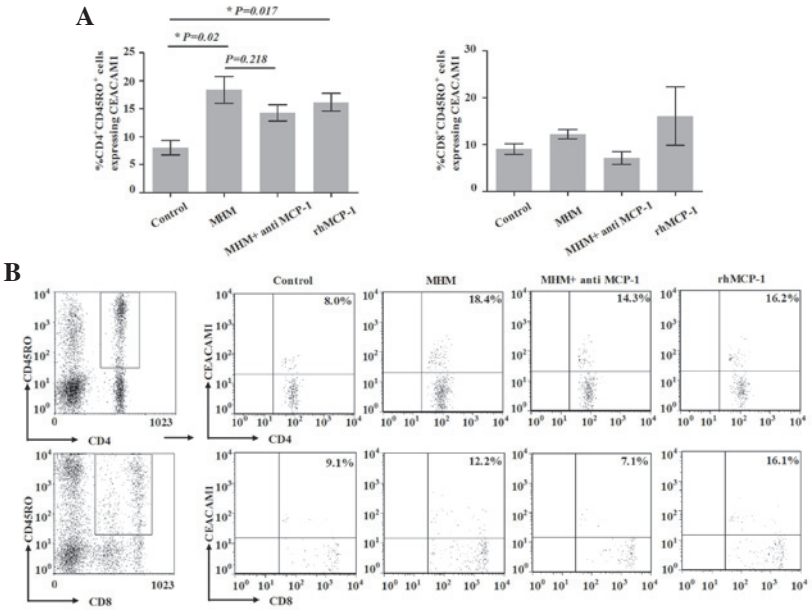

Figure 5. Expression of CEACAM1 on the surface of CD45RO+ T cells in the induced CD45RO+ T cell model. (A) The percentages of CD4+CD45RO+ and CD8+CD45RO+ T cells expressing CEACAM1 in the induced CD45RO+ T cell model are shown. The experiments were performed three times. The proportion of CEACAM1-expressing CD4+CD45RO+ T cells was significantly increased in the group with conditioned medium from the coculture of monocytes and the human trophoblast HTR8/SVneo cell line (the MHM group) compared with the control group (P=0.02). The proportion of CEACAM1-expressing CD4+CD45RO+ T cells was not significantly decreased in the MHM + anti-MCP-1 group, compared with that in the MHM group; however, that proportion was significantly increased by human recombinant MCP-1 (rhMCP-1) compared with the control group (P=0.017). The proportion of CEACAM1-expressing CD8+CD45RO+ T cells did not show a statistically significant difference among the four groups (P=0.303). Data are presented as the mean ± SD. *P<0.05. (B) The lymphocytes were analyzed for phenotypic frequency by flow cytometry. CD4+CD45RO+ and CD8+CD45RO+ T cells were individually gated and further analysis was conducted. Markers were set according to the CEACAM1-isotype IgG. CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; MCP-1, monocyte chemoattractant protein-1.

tion of CEACAM1-expressing $CD4^+CD45RO^+$ T cells on day 8 was significantly increased (18.38±2.4%) compared with that of the control group (P=0.02). Compared with the MHM group, the proportion of CEACAM1-expressing $CD4^+CD45RO^+$ T cells was not significantly decreased in the MHM + anti-MCP-1 group (P=0.218); however, that proportion was significantly increased in the rhMCP-1 group compared with that in the control group (P=0.017). These results implied that MCP-1 was involved in the increase in the proportion of CEACAM1-expressing $CD4^+CD45RO^+$ T cells induced by MHM. The proportion of CEACAM1-expressing $CD8^+CD45RO^+$ T cells did not exhibit a statistically significant difference among the four groups (P=0.303). These data implied that the proportion of CEACAM1-expressing $CD4^+CD45RO^+$ T cells increased with the expansion of $CD4^+CD45RO^+$ T cells, and that the interaction of trophoblast cells and monocytes may be involved in the process.

Discussion

In the present study, we demonstrated that the percentages of $CD45RO^+$ T cells were significantly higher in first trimester human decidua than in the peripheral blood, which is concordant with previous studies (5,6).

A number of studies have indicated that T cells in first trimester human decidua are regionally activated (5,30,31). As CEACAM1 has been demonstrated to be important in modulating the functions of T cells and is regarded as an activation-induced cell surface molecule of T cells (20,32), we measured the expression of CEACAM1 on the surface of T cells in an early stage of pregnancy. Notably, we identified CEACAM1-expressing cells in significantly higher numbers among freshly isolated $CD4^+$ and $CD8^+$ T cells in first trimester human decidua than in the peripheral blood. The data also suggested that these decidual T cells were activated. We analyzed the expression of CEACAM1 on the surface of $CD45RO^+$ T cell subsets and identified the proportion of cells expressing CEACAM1 among $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$ T cells to be significantly higher in first trimester human decidua than in the peripheral blood. These data implied that a high percentage of decidual $CD45RO^+$ T cells were in an active state, and that CEACAM1 may participate in the regulation of the activation and functions of the decidual $CD45RO^+$ T cells. In further experiments, we determined which factors induce the increase in the percentage of $CD45RO^+$ T cells, and in the percentage of $CD45RO^+$ T cells expressing CEACAM1, in first trimester human decidua.

Previous studies have demonstrated that during the invasion and migration of fetal trophoblast cells into the maternal decidua, the two come into close contact with maternal leukocytes (1). Previous studies have focused on the recruitment of leukocytes by trophoblast cells (8,12,13), and limited data are available on the ability of trophoblast cells to modulate the expansion and activation of decidual T cells (14). It has been demonstrated that the interaction of trophoblast cells and monocytes markedly enhances the expression of cytokines/chemokines, including MCP-1 (15). MCP-1, the main monocyte chemoattractant, has been demonstrated to be associated with the generation and survival of $CD45RO^+$ T cells (16). In the present study, to investigate the possible expansion and activation mechanisms of decidual $CD45RO^+$ T cells, we developed an effective *in vitro* model to generate these cells. We identified that $CD45RO^+$ T cells were greatly expanded when the model was supplemented with MHM, suggesting that the direct interaction between trophoblast cells and monocytes may contribute to the increase in $CD45RO^+$ T cells in first trimester human decidua. Further studies are required to determine whether this increase in $CD45RO^+$ T cells is a result of the proliferation of inherent memory T cells *in vitro*, or the conversion from naïve T cells (or other subsets). Our results also demonstrated that the increase in $CD45RO^+$ T cells was dependent on the increased expression of MCP-1 in the MHM.

In addition, we demonstrated that in the induced model used to generate $CD45RO^+$ T cells, as the percentage of $CD45RO^+$ T cells increased, the expression of CEACAM1-expressing $CD4^+CD45RO^+$ T cells also increased (from 8.0±1.3 to 18.4±2.4%). Notably, we identified that the number of CEACAM1-expressing $CD4^+CD45RO^+$ T cells significantly increased when the model was supplemented with MHM; however, the expression of CEACAM1-expressing $CD8^+CD45RO^+$ T cells did not show a statistically significant difference between the groups. These data further implied that the soluble immune mediators resulting from the direct interaction between trophoblast cells and monocytes may contribute to the increase in the expression of CEACAM1 on the surface of $CD4^+CD45RO^+$ T cells, but not on that of $CD8^+CD45RO^+$ T cells, in first trimester human decidua. CEACAM1 has been detected on the surface of extravillous trophoblast cells, and is hypothesized to promote the invasion of these cells (33-35). In addition, numerous studies have demonstrated that CEACAM1 inhibits the cytokine production, proliferation and cytotoxic activity of activated T cells, by homophilic and heterophilic interactions (26-29). The effect of the homophilic interaction of CEACAM1 on the surface of decidual T cells and extravillous trophoblast cells in the induction of maternal-fetal tolerance remains to be elucidated.

In conclusion, our data indicated that during the invasion and migration of fetal trophoblast cells into the maternal decidua, the soluble immune mediators that are secreted as a result of the interaction between trophoblasts and monocytes may be involved in the increase of decidual $CD45RO^+$ T cells and the expression of CEACAM1 on their surfaces. Our results provide insights into the interaction between maternal immune cells and fetal antigens.

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