Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases

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Human pregnancy represents a situation of semiallograft to maternal host. Therefore, it has been reported that tolerance to the fetal allograft represents a mechanism for maintaining a pregnancy. CD4+CD25bright regulatory T cells are known to play an important role in the development and maintenance of tolerance in peripheral tissues. However, the potential role of CD4+CD25bright T cells in maintaining human pregnancy has not been reported. In this study, we show that early human pregnancy decidua contains an abundance of CD4+CD25bright T cells, which express CD152(CTLA-4) at a high level. CD4+CD25bright T cells mediate potent inhibition of autologous T-cell proliferation by anti-CD3 stimulation. Furthermore, these cells inhibit the proliferation of autologous CD4+CD25+ T cells in a dose-dependent fashion. This suppressive function of decidual CD4+CD25+ T cells required cell-to-cell contact. The proportion of decidual CD4+CD25bright T cells was significantly lower in specimens from spontaneous abortion compared to those from specimens induced from abortions. These results suggest that decidual CD4+CD25bright T cells contribute to the mechanisms mediating maternal immune tolerance of conceptus antigens and therefore might contribute to the maintenance of pregnancy.

Key words: abortion/decidua/pregnancy/regulatory T cells/tolerance

Introduction
Sir Peter Medawar introduced the concept that human pregnancy represents a condition of semiallograft to maternal host because of the presence of paternally inherited gene products and the production of unique differentiation antigens (Medawar, 1953). Therefore, the process of implantation may include mechanisms preventing allograft rejection (Medawar, 1953; Thellin et al., 2000).

T-cell-mediated immunoregulation is one of the main mechanisms responsible for maintaining antigen-specific operational tolerance in vivo (Graca et al., 2002; Karim et al., 2002; Bach, 2003; Bluestone and Abbans, 2003; Word and Sakaguchi, 2003). Accumulating evidence from both experimental and clinical studies indicates that the balance between regulation and deletion of responder T cells controls immune responsiveness after organ or cell transplantation (Graca et al., 2002; Karim et al., 2002; Bach, 2003; Bluestone and Abbans, 2003; Word and Sakaguchi, 2003). Recently it has been demonstrated that regulatory T (Treg) cells have a role in the induction of transplantation tolerance (Graca et al., 2002; Karim et al., 2002; Bach, 2003; Bluestone and Abbans, 2003; Word and Sakaguchi, 2003). Of these Treg cells, CD4+CD25+ Treg cells play a role in the induction of tolerance (Sakaguchi et al., 1995; Taylor et al., 2001; Beacher-Allan et al., 2001; Jonuleit et al., 2001; Levings et al., 2001; Ng et al., 2001; Stephens et al., 2001; Hoffman et al., 2002; Word and Sakaguchi, 2003). CD4+CD25+ Treg are shown to have potent regulatory properties in both the induction and maintenance phases of in vivo tolerance to alloantigens in mice (Sakaguchi et al., 1995; Taylor et al., 2001; Cohen et al., 2002; Hoffmann et al., 2002) and humans (Beacher-Allan et al., 2001; Jonuleit et al., 2001; Levings et al., 2001; Ng et al., 2001; Stephens et al., 2001; Hoffman et al., 2002). Mouse and human CD4+ T cells that have the most potent regulatory properties express high and sustained levels of CD25, in contrast to recently activated T cells, which express CD25 only transiently and at a lower level. Cytotoxic T-lymphocyte antigen 4 (CTLA4;CD152) is expressed constitutively by CD4+CD25+ Treg cells, and CTLA-4 blockade prevents regulation by CD4+CD25+ Treg cells in vitro and in vivo transplantation models (Read et al., 2000; Takahashi et al., 2000).

In the present study, we compared the proportion of decidual and peripheral blood CD4+CD25bright Treg in the setting of normal early pregnancy and spontaneous abortion. In addition, we performed functional analysis on decidual CD4+CD25+ Treg, to evaluate their antiproliferative effects on activated CD4+CD25- autologous T cells.

Materials and methods

Blood and tissue samples
Heparinized venous blood was obtained from non-pregnant healthy women (n = 10), and women in early pregnancy (n = 19). Similarly, heparinized venous blood was also obtained from patients with early abortion (n = 9). Decidual samples were obtained from patients with induced abortion, representing early pregnant deciduas, and from patients with spontaneous abortion, representing abortion samples. All samples were from women in the fifth to ninth gestational week. Decidual mononuclear cells (leukocytes) were purified by the Ficoll-Hypaque method after homogenization and filtration through a 32 μm nylon mesh as previously reported (Saito et al., 1992; Tsuda et al., 2002). Decidual tissues were not enzymatically digested, to prevent the possibility that enzymatic treatment might affect the fluorescence intensity of surface antigens.
Peripheral blood mononuclear cells were isolated by the standard Ficoll-
Hypaque method. Informed consent was obtained from all patients. All of the
sampling and use of the tissues for this study were approved by Toyama
Medical and Pharmaceutical University Ethics Committee.

Flow cytometry
In this study, we used mouse monoclonal antibodies (mAb) including anti-CD4
mAb (fluorescein isothiocyanate; Becton Dickinson, USA), anti-CD25 mAb
(PE, Becton Dickinson) and anti-CD152 (CTLA-4) mAb (Biotin-PCS; Becton
Dickinson). To stain cells for intracellular markers, isolated mononuclear cells
were first stained with anti-CD4 (2 μg/ml) and CD25 mAb (2 μg/ml), then
permeabilized by incubating for 10 min with permeabilizing solution buffer
(Becton Dickinson, dilute 10X solution 1:10 in deionized water) and then
stained with anti-CD152 mAb (2 μg/ml). Flow cytometry analysis was
performed on a FACS Calibur using CellQuest software (Becton Dickinson).
For the analysis, a real-time gate was set around the viable lymphocytes based
on their forward scatter/side scatter profile, avoiding monocyte and granulocyte
populations. The proportions of CD4+CD25bright, CD4+CD25dim, CD4+
CD25bright and CD4+CD25dim cells in lymphocytes were calculated. The
proportions of intracellular CD152+ and extracellular CD152+ cells were also
calculated.

Purification of CD4+ T-cell subsets
Isolated mononuclear cells were purified using magnetic goat anti-mouse IgG
beads and MACS-negative and -positive selection columns (Miltenyi Biotic,
Isolated mononuclear cells were incubated with mouse monoclonal antibodies, anti-CD8 (2 μg/ml), anti-CD25 (2 μg/ml), anti-CD69 (2 μg/ml) and
anti-CD95 (2 μg/ml) (all from Becton Dickinson), and then incubated with
magnetic goat anti-mouse IgG beads. CD4+ T cells were purified using MACS
columns for negative selection. The purity of CD4+ T cells purified by this
method was up to 98%, confirmed by flow cytometric analysis. Purified CD4+ T
cells were then incubated with anti-CD25 mouse monoclonal antibodies and
CD4+CD25+ T cells were separated from CD4+CD25− T cells using MACS
columns. The purity of CD4+CD25+ T cells was >95%.

Proliferation assays
Flat-bottom 96-well plates (Becton Dickinson) were pre-coated with 1 μg/ml of
anti-CD3 mAb (NU-T3; Nichirei, Japan) for 4 h at 37°C. Purified T-cell subsets
were cultured in triplicate wells at a density of 5×10⁵ T cells/well in 200 μl of
culture medium [Roswell Park Memorial Institute Medium 1640 (Sigma-
Aldrich, Japan), supplemented with penicillin/streptomycin, and 10% heat-
inactivated fetal calf serum]. For co-stimulation assays, irradiated (20 Gy)
autologous peripheral blood mononuclear cells were added as antigen-
presenting cells (APC). Cells were cultured for 96 h at 37°C in 5% CO₂, and
during the last 18 h [3H]thymidine (0.5 Ci/well, Amersham Pharmacia
Biotech, Japan) and [3H]thymidine incorporation was measured. Proliferative
responses were expressed as the mean ± SD [3H]thymidine incorporation (cpm)
of triplicate samples.

Direct suppression assays
For direct suppression assays, 5×10⁴ CD4+CD25− T cells were stimulated with
anti-CD3 mAb and APC, either in the absence or presence of increasing
numbers (0.5×10⁴, 2.5×10⁴, 5×10⁴) of CD4+CD25+ T cells, resulting in
response-suppressor ratios of 1:0, 1:0.5, 1:0.5 and 1:1. Cells were cultured in
the same way as described for the proliferation assay. The direct suppressive
effect of CD4+CD25+ T cells was evaluated by measuring [3H]thymidine
incorporation.

Transwell experiments
CD4+CD25− T cells were co-cultured with CD4+CD25+ T cells using a semi-
permeable transwell membrane (Nunc, Denmark). Both the lower and upper
chambers of the transwell plate were coated with anti-CD3 mAb for 4 h. To the
lower chamber, 5×10⁴ CD4+CD25− T cells and 5×10⁴ irradiated APC were
added. To the upper chamber, 5×10⁴ irradiated APC were added in the
presence of medium alone, or together with 5×10⁴ CD4+CD25+ T cells or
5×10⁴ CD4+CD25− T cells. At the same time, 5×10⁴ CD4+CD25− T cells and
5×10⁴ irradiated APC were cultured with 5×10⁴ CD4+CD25− T cells or 5×10⁴
CD4+CD25+ T cells without a semi-permeable transwell membrane. The
proliferative responses were assessed as described for the proliferation assays.

Statistical analysis
Data were analysed using the Mann–Whitney U-test and one-way ANOVA. P
< 0.05 was considered significant.

Results
CD25 expression in peripheral and decidual lymphocytes
The subsets of lymphocytes in the decidua were different from those in
peripheral blood, as previously reported (Nishikawa et al., 1991). The
CD4+ T cell population was markedly lower in the decidua because
CD16CD56bright NK cells were the dominant subset (60–70% of lymphocytes in
the decidua during early pregnancy).

CD25 expression was observed in a subset of CD4+ T cells (Figure 1). We subdivided CD25+CD4+ T cells into bright and dim populations (CD25bright and CD25dim, Figure 1). This is in keeping with previous reports that regulatory T cells preferentially reside
within the CD4+CD25bright T cell population, and activated T cells express lower levels of CD25 than that expressed by regulatory T cells
(Kuniyasu et al., 2000; Beacher-Allan et al., 2001; Cao et al., 2003). The
percentages of CD25-expressing cells in the CD4+ T cell population were compared between decidual lymphocytes and peripheral blood lymphocytes. The percentages of CD4+ cells and CD25+ cells in the CD4+ T cell population of peripheral blood lymphocytes did not differ significantly between non-pregnant individuals, normal early pregnant women and patients with spontaneous
abortion (Table I). However, the percentage of peripheral blood CD4+CD25+ cells (activated CD4+ T cells) in lymphocytes was significantly lower in the setting of early pregnancy than in non-
pregnant women and women with spontaneous abortion (P < 0.01 and
P < 0.05 respectively; Table II).

The percentage of CD25bright cells in peripheral CD4+ cells in women with early pregnancy was significantly higher than in non-
pregnant women, while the percentage of CD25+ cells in peripheral
CD4+ T cells from patients with spontaneous abortion was the same as
non-pregnant women (Table I).

In the setting of normal pregnancy, the percentage of CD25bright cells in the CD4+ T cell population was significantly higher in the
decidua than in the peripheral blood (Figure 1 and Table I). However, a higher frequency of CD25bright cells in the CD4+ T cell population
was not seen in patients with spontaneous abortion (Table I). The
percentage of CD4+CD25− T cells in the peripheral blood lymphocytes,
including CD8+ T cells, CD16CD56bright NK cells and CD16CD56dim NK cells was significantly higher in women with spontaneous
abortion than in non-pregnant women or women during early pregnancy (P = 0.013, P = 0.0004 respectively) (Table II). Similarly, the percentage of CD4+CD25+ cells in the decidua lymphocytes in women with spontaneous abortion tended to be higher than in women during early pregnancy, but the difference was not significant (P = 0.06).

Surface and intracellular expression of CD152 (CTLA-4) on CD4+CD25bright T cells
Recent studies have shown that CD152 is up-regulated on mouse and
human regulatory T cells (Chambers et al., 2001). CD152 is constitutively expressed in the cytoplasm of regulatory T cells, but after
activation by certain antigens, CD152 is expressed on their
surface. Therefore, we analysed the surface and intracellular expression
of CD152 on CD4+CD25bright T cells in peripheral blood and decidua.

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Table I. Population of CD4+ cells in lymphocytes and populations of CD25bright and CD25dim cells in CD4+ cells

<table>
<thead>
<tr>
<th></th>
<th>CD4+/lymphocyte (%)</th>
<th>CD4+CD25bright/CD4+ (%)</th>
<th>CD4+CD25dim/CD4+ (%)</th>
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</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>44.77 ± 7.97</td>
<td>6.50 ± 0.59</td>
<td>23.08 ± 4.97</td>
</tr>
<tr>
<td>Early pregnancy</td>
<td>38.29 ± 6.29</td>
<td>5.98 ± 3.01</td>
<td>21.84 ± 2.92</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>42.28 ± 10.34</td>
<td>5.66 ± 1.58</td>
<td>23.86 ± 5.08</td>
</tr>
</tbody>
</table>

Figure 1. CD4+CD25+ T cells can be classified into CD4+CD25bright T cells and CD4+CD25dim T cells. Representative FACS stainings of peripheral blood mononuclear cells and decidual leukocytes from one of the pregnant women are shown. Markers were set according to the isotype IgG, and lymphocytes were classified into CD4+ cells (right square), CD4- cells (left square), CD25bright cells (upper square), CD25dim cells (middle square) and CD25- (lower square). The percentage of CD4+CD25bright T cells is indicated in the upper right quadrant, and the percentage of CD4+CD25dim T cells is indicated in the middle right quadrant. Upper left quadrant shows CD4-CD25bright cells and middle left quadrant shows CD4-CD25dim cells.

Table II. The population of CD4+CD25bright cells, CD4+CD25dim cells, CD4+CD25bright cells and CD4+CD25dim cells in peripheral blood lymphocytes and decidual lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>CD4+CD25bright/lymphocytes</th>
<th>CD4+CD25dim/lymphocytes</th>
<th>CD4+CD25bright/CD4+ (%)</th>
<th>CD4+CD25dim/CD4+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>2.91 ± 0.27</td>
<td>10.33 ± 2.22</td>
<td>0.49 ± 0.06</td>
<td>3.47 ± 0.71</td>
</tr>
<tr>
<td>Early pregnancy</td>
<td>3.26 ± 0.95*</td>
<td>7.31 ± 2.42***</td>
<td>0.89 ± 0.59</td>
<td>2.58 ± 1.66</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>2.39 ± 0.65</td>
<td>10.09 ± 2.15</td>
<td>0.60 ± 0.20</td>
<td>7.20 ± 2.41†</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. spontaneous abortion (sp-ab) PBL; **P < 0.0001 vs. sp-ab decidua; ***P < 0.01 vs. non-pregnant PBL and early pregnancy PBL; †P < 0.01 vs. non-pregnant PBL or early pregnant PBL; ††P < 0.01 vs. early pregnancy PBL.
Both peripheral blood and decidual CD4+CD25bright T cells expressed intracellular CD152 at >90% (Table III). In early pregnancy decidua, the percentage of surface CD152+ in CD4+CD25bright T cells was significantly higher than in peripheral blood CD4+CD25bright T cells (Table III). In the setting of spontaneous abortion, there was no significant difference in the surface CD152 expression between decidual CD4+CD25bright T cells and peripheral blood CD4+CD25bright T cells (Table III). However, both peripheral blood and decidual CD4+CD25dim T cells expressed intracellular and surface CD152 at a low frequency (Table III).

### Table III. Surface and intracellular CD152 expression on CD4+CD25bright T cells in early pregnancy and spontaneous abortion

<table>
<thead>
<tr>
<th></th>
<th>CD4+CD25CD152+/CD4+CD25+ (%)</th>
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<tbody>
<tr>
<td></td>
<td>Intracellular</td>
</tr>
<tr>
<td><strong>Non-pregnant (n=10)</strong></td>
<td></td>
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<tr>
<td>Peripheral blood</td>
<td></td>
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<tr>
<td>CD4+CD25bright</td>
<td>92.81 ± 1.72</td>
</tr>
<tr>
<td>CD4+CD25dim</td>
<td>6.78 ± 1.93</td>
</tr>
<tr>
<td><strong>Early pregnancy (n=19)</strong></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
</tr>
<tr>
<td>CD4+CD25bright</td>
<td>96.0 ± 1.01</td>
</tr>
<tr>
<td>CD4+CD25dim</td>
<td>6.83 ± 1.14</td>
</tr>
<tr>
<td>Decidua</td>
<td></td>
</tr>
<tr>
<td>CD4+CD25bright</td>
<td>97.8 ± 3.22</td>
</tr>
<tr>
<td>CD4+CD25dim</td>
<td>5.74 ± 0.75</td>
</tr>
<tr>
<td><strong>Spontaneous abortion (n=9)</strong></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
</tr>
<tr>
<td>CD4+CD25bright</td>
<td>96.0 ± 1.21</td>
</tr>
<tr>
<td>CD4+CD25dim</td>
<td>5.20 ± 1.37</td>
</tr>
<tr>
<td>Decidua</td>
<td></td>
</tr>
<tr>
<td>CD4+CD25bright</td>
<td>91.71 ± 3.82</td>
</tr>
<tr>
<td>CD4+CD25dim</td>
<td>5.83 ± 1.46</td>
</tr>
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*P < 0.05 vs. early pregnant PBL.

### CD4*CD25* regulatory T cells become anergic by anti-CD3 stimulation

Figure 2 shows the proliferative responses of the different isolated CD4+ T-cell subsets with anti-CD3 stimulation and irradiated APC addition. CD4*CD25* T cells were less responsive than either CD4*CD25- T cells or total CD4+ T cells (P = 0.0003 for CD4*CD25+ T cells, P = 0.0008 for total CD4+ T cells in the decidua, P = 0.0002 for CD4*CD25- T cells, P = 0.03 for total CD4+ T cells in peripheral blood). After removing CD4*CD25+ T cells from the total CD4+ T cell population, the proliferative response of the remaining CD4*CD25- T cells was significantly greater (P = 0.0095 for total CD4+ T cells in the decidua, P = 0.02 for total CD4 T cells in peripheral blood). These findings suggest that with the anti-CD3 stimulation, CD4*CD25+ T cells become not only anergic, but also actively suppress the proliferative response of other CD4+ T cells.

### CD4*CD25* regulatory T cells have directly and dose-dependently suppressive potential

To investigate the suppressive potential of CD4*CD25+ T cells, co-culture experiments were performed. The response of CD4*CD25- T cells to anti-CD3 antibodies and APC was significantly suppressed to 50% (P < 0.0001 for the decidua, P = 0.0059 for peripheral blood) by the addition of CD4*CD25+ T cells at a 1:1 ratio of the two cell populations (Figure 3). The suppressive effect of CD4*CD25+ T cells was dose dependent and the use of CD4*CD25+ T cells at a responder:CD4*CD25+ cell ratio of 1:0.1 did not lead to suppression (Figure 3).

### The suppressive effect of CD4*CD25+ T cells requires direct cell–cell contact

When cell–cell contact between peripheral blood CD4*CD25- T cells and decidual CD4*CD25+ T cells (1:1) was prevented by a semi-

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**Figure 2.** Decidual and peripheral blood CD4*CD25+ cells are hyporesponsive to T-cell stimulation. CD4+ cells (——), CD4*CD25+ cells (——) or CD4*CD25- cells (——) were stimulated in the presence of irradiated autologous APC with anti-CD3 antibody for 4 days. Proliferation was measured by [3H]thymidine incorporation. The T-cell responses were increased by removing CD4*CD25+ T cells from the total CD4+ T cell population (——: CD4*CD25+). The proliferative responses of CD4*CD25+ T cells to anti-CD3 were considerably lower than those of CD4+ T cells and CD4*CD25- T cells.
permeable transwell membrane, the suppressive effects of CD4⁺CD25⁺ T cells were abrogated (Figure 4). Similar results were observed in the peripheral blood CD4⁺CD25⁺ T cells (data not shown). These results suggest that cell contact is necessary for the inhibitory effects of CD4⁺CD25⁺.

**Figure 3.** The suppressive effect of decidual and peripheral blood CD4⁺CD25⁺ cells is cell ratio dependent. Increasing numbers of CD4⁺CD25⁺ T cells were seeded into a fixed number of autologous CD4⁺CD25⁻ responder T cells. A dose-dependent suppression of DNA synthesis by decidual and peripheral blood CD4⁺CD25⁺ T cells was observed.

**Figure 4.** Cell contact is necessary for suppression. CD4⁺CD25⁻ T cells were stimulated with anti-CD3 and APC in the presence or absence of CD4⁺CD25⁺ T cells, added directly in co-culture or in the upper chamber of a transwell plate together with anti-CD3 and APC. When cell–cell contact between CD4⁺CD25⁺ T cells was prevented by a semi-permeable transwell membrane (right panel), the suppressive effects of CD4⁺CD25⁺ T cells were abrogated.

**Discussion**

This is the first study to demonstrate the presence and abundance of CD4⁺CD25bright Treg in the decidua during early pregnancy. We first reported that the expression of CD25 on decidual CD4⁺ T cells is...
significantly higher compared to that for peripheral blood CD4+ T cells, but the expression rate for CD4+CD25+ T cells in CD4+ T cells is rather low compared to those of CD4+CD69+ and CD4+HLA-DR+ T cells (Saito et al., 1992). In contrast, Chao et al. (2002) reported that the expression of CD25 is decreased on decidual CD4+ and CD8+ T cells in normal pregnancy. However, the mean fluorescence intensity of CD25 on decidual CD4+ T cells was almost twice that on peripheral blood CD4+ T cells (Chao et al., 2002), suggesting that CD4+CD25bright T cells are increased in the decidua, even though the total populations of CD4+CD25dim T cells and CD4+CD25bright T cells are decreased. It has been reported that the regulatory CD4+ T cells in humans preferentially reside within the CD4+CD25bright T-cell population, while the CD4+CD25dim T-cell population holds a majority of normal activated T cells (Kuniyasu et al., 2000; Beacher-Allan et al., 2001; Chao et al., 2002). These reports suggest that CD4+CD25bright T cells are increased in the early human pregnancy decidua.

In this study, the gate for CD25 was deliberately set high to allow for isolation of regulatory T cells. The majority of CD4+CD25bright Treg cells are activated memory T cells expressing CD45RO, CD69, HLA-DR, CD122, CD58, CD71 and CTLA-4 (CD152) (Karimi et al., 2002; Bach, 2003; Bluestone and Abbans, 2003; Word and Sakaguchi, 2003). In this study, decidual CD4+CD25bright T cells expressed CD152 (CTLA-4). Expression of these antigens on decidual CD4+CD25bright T cells are a quite similar to that of CD4+CD25+ Treg, suggesting that decidual CD4+CD25bright T cells have immunoregulatory functions.

CTLA-4 is known to be an important negative regulator of T-cell function (Chambers et al., 2001). CD4+CD25+ Treg cells have been shown to express CTLA-4. Newly translated CTLA-4 emerge from the Golgi apparatus in secretory vesicles, trafficking to the endosomal compartment or directly to the cell surface. Upon T-cell receptor (TCR) stimulation, CTLA-4 is phosphorylated by protein kinases associated with the TCR, resulting in surface stabilization of CTLA-4. Binding of both B7-1 (CD80) and B7-2 (CD86) by CTLA-4 has considerably higher affinity than that of CD28 binding. Surface CTLA-4 can compete with CD28 for B7 binding, resulting in TCR engagement in the absence of B7 expression on antigen-presenting cells (Chambers et al., 2001). This stimulation does not result in T-cell proliferation or induction of T-cell anergy (Chambers et al., 2001). CTLA-4 also delivers inhibitory signals which prevent T-cell activation. In the decidua during early pregnancy, expression of surface CTLA-4 on CD4+CD25bright T cells was increased, indicating that decidual CD4+CD25bright T cells are already stimulated upon TCR, perhaps by fetal antigens.

Next, we analysed whether functional regulatory CD4+CD25+ T cells exist in the decidua during early human pregnancy. The proliferative response of decidual CD4+CD25+ T cells to anti-CD3 stimulation in the presence of autologous antigen-presenting cells was considerably lower than those of CD4+CD25+ T cells. When decidual CD4+CD25+ cells were co-cultured with peripheral blood CD4+CD25+ cells, the responses to anti-CD3 antibody were significantly suppressed. To determine whether the suppressive function of decidual CD4+CD25+ cells is mediated by the secretion of soluble factors, a transwell system was used. Based on the findings, the suppressive function of decidual CD4+CD25+ T cells requires cell--cell contact. These characteristics of decidual CD4+CD25+ T cells are quite similar to those of peripheral blood CD4+CD25+ Treg cells. Recently, a unique population of extrathyrmically derived δβTREG-CD4-CD8- B220lowCD25+ T cells with regulatory functions has been described in the mouse female genital tract (Johansson and Lycke, 2003). However, we could not detect these phenotypic cells in the human decidua.

There are several mechanisms that might explain the enrichment of CD4+CD25+ Treg in the decidua. Chemokines have recently been described that attract CD4+CD25+ T cells carrying the chemokine receptors CCR4 and CCR8. Interestingly, the number of CCR4-expressing T cells is increased in the decidua during early human pregnancy, and the chemotactic factor for CCR4, TARC (CCL17), is expressed on trophoblasts, endometrial gland cells, and uterine epithelial cells (Tsuda et al., 2002). CD4+CD25+ Treg cells might be recruited to the materno-fetal interface, at least in part, through a TARC-mediated pathway. Ho et al. (1996) reported that the population of CD3+CD25+ T cells decreases significantly in the endometrium of ectopic pregnancies compared to the endometrium of normal pregnancies. Therefore, a fetus or placenta might be necessary for the accumulation of CD3+CD25+ T cells. The proportion of decidual CD4+CD25bright NK cells expressing surface CTLA-4 in spontaneous abortion cases was decreased in this study. But Vassiliadou et al. (1999) reported that a significantly increased number of CD25+ T cells was detected in the decidua of spontaneous abortions based on immunohistochemical methods. These differences could be explained by the fact that activated CD4+CD25dim T cells were increased in the spontaneous abortion decidua. Indeed, Quack et al. (2001) reported that significantly more activated leukocytes were detected in the decidua of women with unexplained recurrent spontaneous abortion (RSA) who had a normal male karyotype compared to either a control group or a group of patients with RSA and abnormal chromosomes. However, the expression of CD4+CD25dim T cells and CD4+CD25bright cells was not determined in Vassiliadou’s study. In the present study, the proportion of decidual CD4+CD25dim cells in the spontaneous abortion cases was increased (Table 1; F = 0.06), but CD4+CD25dim cells in the decidua of spontaneous abortions were the same as in the decidua of induced abortions. These results suggested that activated decidual CD8+ T cells or NK cells might be increased in spontaneous abortion. Furthermore, the proportions of peripheral blood CD4+CD25dim and CD4+CD25dim cells in the spontaneous abortion cases were significantly increased compared to those in induced abortion subjects. These data suggest that activated CD4+ T cells, CD8+ T cells or NK cells might be increased in peripheral blood of spontaneous abortion cases. It can be speculated that decreased CD4+CD25bright regulatory T cells in abortion cases might induce maternal lymphocyte activation to the fetal allograft. Further studies are needed to identify the proportion of CD4+CD25bright T cells, CD4+CD25dim T cells, CD8+CD25+ T cells and CD56+CD25+ NK cells in those cases.

Our data showed that the proportion of CD4+CD25bright Treg cells is increased in therapeutic abortion decidua and is decreased in spontaneous abortion decidua. These CD4+CD25bright Treg cells might play a role in the maintenance of pregnancy.

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