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Adoptive Transfer of Paternal Antigen-Hyporesponsive T Cells Induces Maternal Tolerance to the Allogeneic Fetus in Abortion-Prone Matings

Li-Ping Jin,* Da-Jin Li,2* Jin-Ping Zhang,† Ming-Yan Wang,* Xiao-Yong Zhu,* Ying Zhu,* Yi Meng,* and Min-Min Yuan*"}


The embryo expresses paternal Ags foreign to the mother and therefore has been viewed as an allograft. It has been shown that anergic T cells generated by blocking of the CD28/B7 costimulatory pathway with anti B7-1 and anti B7-2 mAbs can be transferred as suppressor cells to prevent allograft rejection. Little is known, however, about the in vivo function of anti-B7-treated T cells after their transfer into abortion-prone mice in the maintenance of materno-fetal tolerance. In the present study, abortion-prone CBA/J females mated with DBA/2 males were administered anti-B7-1 and anti-B7-2 mAbs on day 4 of gestation (murine implantation window). The anti-B7-treated T cells subsequently were adoptively transferred into abortion-prone CBA/J mice. We demonstrated that costimulation blockade with anti-B7 mAbs at the time of implantation resulted in altered allogeneic T cell response and overcome increased maternal rejection to the fetus in the CBA/J×DBA/2 system. The transferred anti-B7-treated T cells appeared to be regulatory, decreasing responsiveness and generating clonal deviation in maternal recipient T cells. The transferred CFSE-labeled T cells were found to reside in the spleen and uterine draining lymph nodes, and a few were localized to the materno-fetal interface of the maternal recipient. Our findings suggest that the anti-B7-treated T cells not only function as potent suppressor cells, but also exert an immunoregulatory effect on the maternal recipient T cells, which suppresses maternal rejection to the fetus. This procedure might be considered potentially useful for fetal survival when used as an immunotherapy for human recurrent spontaneous abortion. The Journal of Immunology, 2004, 173: 3612–3619.
has been the murine (CBA/J×DBA/2) mating combination, in which between 20 and 50% of the fetus is resorbed by gestational day 13 (15). The critical importance of this precise parental genetic combination and the ability to suppress fetal loss by preimmunizing females with BALB/c male lymphocytes or by prior mating to a BALB/c male (13, 16) point to an immunological component that contributes to this fetal loss syndrome. NK cells and macrophages have been implicated as cellular mediators of the syndrome, and excessive NO and TNF-α release by decidual mononuclear cells have been suggested as effector mechanisms that become dysregulated in this strain combination (17, 18). An indirect role for GM-CSF and, significantly, maternal CD8+ T cells in preventing NK cell-mediated fetal loss in this system has also been suggested (19). Recent studies reinforce the connection between TNF-α release by decidual macrophages, which precedes fetal loss (20). These findings send clear messages that cytokine imbalances associated with inappropriate activation of macrophages and NK cells of the innate immune system are detrimental to fetal survival in this experimental model. Furthermore, in this system protection from abortion can be adoptively transferred by CBA/J anti-BALB/c serum or spleen cells from CBA/J females immunized by BALB/c lymphocytes (21). These results suggest that the mechanism triggered by preimmunization with BALB/c strain lymphoid cells that carry the same MHC Ags (H-2k) as DBA/2 may be equivalent to the tolerance signal elicited by a successful pregnancy. In our clinical investigation we also found that recurrent spontaneous abortion could be prevented by third-party and paternal lymphocyte immunization (22). In the present study we investigated the changes in the fetal resorption rates of abortion-prone mice treated with anti-B7-1/B7-2 mAbs in this experimental model and the in vivo maintenance of materno-fetal tolerance by anti-B7-treated T cells transplanted at the same dosage.

Preparation of spleen cells

The spleen was aseptically removed, and cells were mechanically teased out of the stroma in 10 ml of PBS. The cell suspensions were filtered through 110-μm pore size nylon mesh and then treated with NH4Cl/Tris buffer to remove RBC. Thereafter, the cells were washed three times and prepared for in vitro culture in complete medium of RPMI 1640 containing 10% FCS, 1 mM l-glutamine, 0.05 mM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Proliferation assays

Splenocytes from pregnant CBA/J mice on day 9 of gestation were used as responder cells, and paternal splenocytes were used as stimulator cells. Responder cells (2 × 10^7/well) and mitomycin C-treated stimulator cells (2 × 10^5/well) were aliquoted into 96-well, round-bottom microtiter plates (Nunc, Roskilde, Denmark) in a final volume of 200 μl of complete medium. Responder cells cultured with the complete medium alone in 96-well, round-bottom microtiter plates were used as the control. After a 4-day incubation at 37°C, [3H]thymidine (0.5 μCi/well) incorporation was measured for an additional 6 h. The cells were harvested onto a glass-fiber paper using a semiautomatic cell harvester, and thymidine incorporation was measured in a liquid scintillation counter. Proliferative capacity is shown as stimulatory index (SI) calculated according to the following equation: SI = (cpm of stimulated cultures – cpm of control cultures)/cpm of control cultures.

Measurement of IL-2 production

For measurement of IL-2 production, supernatants of cultured splenocytes were collected after 48 h and stored at −20°C. IL-2 production was analyzed by ELISA (R&D Systems, Minneapolis, MN).

Purification of cells

T and B lymphocytes from the spleens of pregnant CBA/J mice were enriched using a magnetic isolation kit (negative selection; Miltenyi Biotech, Bergish Gladbach, Germany). Briefly, the spleen was harvested from pregnant CBA/J mice on day 9 of gestation, and preparation of spleen cells was performed using the method described above. Then cells were suspended in PBS/EDTA/BSA buffer containing 0.5% BSA and 2 mM EDTA. The cells were first labeled with mixture of biotin-conjugated mAbs against CD11b, CD45R, DX5, and Ter-119 (for isolation of T cells) or against CD43, CD4, and Ter-119 (for isolation of B cells), followed by conjugate binding with anti-biotin microbeads. After incubation for 15 min at 4°C, the cells were resuspended in PBS/EDTA/BSA buffer after washing them twice and were passed through an LS column (Miltenyi Biotec). Finally, the effluent was collected as a fraction with unlabeled cells, representing the enriched T or B cell fraction. The purity of the enriched T or B cells was evaluated by flow cytometry (BD Biosciences, Mountain View, CA). The purity of the isolated cells was >95% (data not shown).

CFSE labeling

The cells purified using the method described above were adjusted to 1 × 10^7/ml in PBS containing 5% FBS. These cells (1 ml) were mixed with 110 μl of 50 μM CFSE solution (Fluka, Buchs, Switzerland). After 5 min at room temperature, the cells were washed three times with PBS containing 5% FBS. The labeled cells were assessed by flow cytometry (BD Biosciences), and the purity of CFSE staining of cells was >99% (data not shown).

Adoptive transfer

CFSE-labeled T or B cells were resuspended in PBS. The T or B cells (1 × 10^7) were injected via tail vein into pregnant CBA/J females mated with DBA/2 males on day 4 of gestation. Recipient mice were divided into the following groups based on the cells injected: T or B cells from anti-B7 mAbs-treated CBA/J mice, T or B cells from isotype IgG-treated CBA/J mice, and T or B cells from CBA/J mice mated with BALB/c males.

Flow cytometry for intracellular cytokine analysis

The spleen was removed from recipient CBA/J mice on day 9 of gestation and processed to single-cell suspensions. The recipient splenocytes (2 × 10^6/well) were cocultured with mitomycin C-treated DBA/2 splenocytes (2 × 10^5/well) as stimulator cells for 4 days in 24-well, flat-bottom plates (Nunc). To inhibit cytokine secretion, brefeldin A (Sigma-Aldrich) was added for the last 12 h of culture. The cells were harvested and treated by density gradient centrifugation to remove dead cells. The viable cells were resuspended in PBS at a density of 1 × 10^6/ml, then distributed (100...


Embryo resorption

For macroscopic examination of pregnancy failure, mice were killed on day 14 of gestation, and the uteri were examined for the number of healthy and resorbing embryos. At this stage of gestation, resorbing embryos are subject to ischemia, hemorrhage, and necrosis, making them smaller and darker than the larger, pink, viable embryos. The percentage of embryos undergoing resorption was calculated by the formula: \( \%R = \frac{Re}{Re + F} \times 100 \), where \( R \) represents the percentage of resorptions relative to the total number of effective implantation sites, \( Re \) represents the number of resorbed embryos, and \( F \) represents the number of viable embryos, which was described previously (16).

### Results

Combined administration of anti-B7-1 and anti-B7-2 mAbs suppressed maternal rejection to the fetus

To test whether blockade of the B7/CD28 costimulatory pathway in vivo can decrease the rate of fetal loss, the combination of anti-B7-1 and anti-B7-2 mAbs was injected i.p. into the abortion-prone CBA/J females mated with DBA/2 males on day 4 of gestation during implantation. The embryo resorption rate was counted on day 14 of gestation. The results, shown in Table I, clearly demonstrated that treatment of CBA/J females with anti-B7 mAbs significantly reduced the resorption rate of (CBA/J x DBA/2)F1 fetuses compared with the isotype IgG control treatment. The result resembled that of the normal pregnancy model (CBA/J x BALB/c matings). In summary, these findings indicated that the combined treatment of anti-B7-1 and anti-B7-2 mAbs was effective in preventing maternal rejection of the allogeneic fetus.

Combined administration of anti-B7-1 and anti-B7-2 mAbs induced maternal hyporesponsiveness to paternal Ag

To better understand the in vivo inhibitory effects of anti-B7-1 and anti-B7-2 mAbs on maternal responses to paternal Ags, splenocytes from pregnant CBA/J mice on day 9 of gestation were stimulated for 4 days with mitomycin C-treated paternal

![Figure A](image1.png)

**Figure A.** Effect of anti-B7 mAbs treatment on the proliferation and IL-2 production by pregnant CBA/J splenocytes in response to paternal stimulator cells. Anti-B7 mAbs and isotype IgG were injected i.p. into the abortion-prone CBA/J females mated with DBA/2 males on day 4 of gestation, respectively. CBA/J females mated with BALB/c males received no treatment. On day 9 of gestation, CBA/J splenocytes from pregnant mice were cocultured with mitomycin C-treated paternal splenocytes for 4 days. Proliferation (A) was measured by [3H]Tdr incorporation during the last 6 h. Culture supernatants were collected after 48 h to measure IL-2 production (B). Results are expressed as the mean \( \pm \) SD of triplicate measurements.

![Figure B](image2.png)
spleenocytes. One-way MLR demonstrated that the combination of both mAbs strongly inhibited the proliferation of CBA/J spleenocytes in response to DBA/2 stimulator cells. Moreover, a seriously impaired IL-2 response was observed compared with the isotype IgG control treatment. The loss of MLR reactivity was similar to that observed for CBA/J spleenocytes in response to BALB/c stimulator cells (Fig. 1). These results indicated that the combined administration of anti-B7-1 and anti-B7-2 mAbs successfully induced maternal hyporesponsiveness to paternal Ags, suggesting that blockade of the CD28/B7 pathway inhibited maternal T cell activation in this in vivo system.

Adoptive transfer of paternal Ag-hyporesponsive T cells increased fetal viability in abortion-prone matings

We next determined whether anti-B7-treated lymphocytes could adoptively transfer fetal protection to the abortion-prone mothers. To test this, 1 x 10^7 T or B cells were injected i.v. into other abortion-prone CBA/J females mated with DBA/2 males on day 4 of gestation. The data in Table II showed that adoptive transfer of T cells from anti-B7-treated CBA/J mice increased fetal viability at a level comparable with that of T cells from BALB/c-mated CBA/J mice. In contrast, adoptive transfer of T cells from isotype IgG-treated CBA/J mice or B cells from any source did not significantly reduce the embryo resorption rate. These results indicated that adoptive transfer of paternal Ag-hyporesponsive T cells induced materno-fetal immunotolerance and improved pregnancy outcome, implying that the paternal Ag-hyporesponsive T cells, but not B cells, exerted an immunoregulatory effect in the recipient and suppressed maternal rejection of the allogeneic fetus in vivo.

Adoptive transfer of paternal Ag-hyporesponsive T cells inhibited maternal response to paternal Ag

Based on the findings reported above, to test the in vivo inhibitory effects of the transferred paternal Ag-hyporesponsive T cells, we also examined maternal recipient immune responses to paternal Ags. The proliferation of CBA/J recipient spleenocytes in response to DBA/2 splenocyte stimulator in vitro was performed on day 9 of gestation. The results showed that adoptive transfer of either anti-B7-treated T cells or BALB/c-mated T cells significantly suppressed the proliferation of alloreactive cells in MLR. Adoptive transfer of isotype IgG-treated T cells had no effect (Fig. 2). This indicated that the transferred paternal Ag-hyporesponsive T cells regulated recipient T cell responsiveness, and maternal recipient hyporesponsiveness to paternal Ag was also induced after adoptive transfer of these hyporesponsive T cells.

Effects of the transferred paternal Ag-hyporesponsive T cells on recipient T cells

We next determined the expression of intracellular cytokines, including IL-2, IL-4, IL-10, and IFN-γ, and costimulatory molecules CD28/CTLA-4 in donor T cells as well as recipient T cells. We first injected CFSE-labeled T cells into the abortion-prone CBA/J females on day 4 of gestation. On day 9 of gestation, the recipient spleenocytes containing CFSE− T cells (donor) and CFSE+ T cells (recipient) were stimulated with DBA/2 splenocytes for 4 days in vitro, then stained for IL-2, IL-4, IL-10, IFN-γ, CD28, or CTLA-4 using PE-labeled Abs and flow cytometric analysis. Data summarized from five independent experiments are presented in Fig. 3 and 4. Compared with the isotype IgG-treated T cells, injection of either anti-B7-treated T cells or BALB/c-mated T cells resulted in a lower frequency of cells positive for IL-2 and IFN-γ and a higher frequency of IL-10-producing cells in both the CFSE−CD3+ population and the CFSE−CD3+ population. The frequency of IL-4-producing cells did not appear to be changed. The difference in percentages of cells positive for IL-2, IL-4, IL-10, or IFN-γ between donor and recipient T cells was not statistically significant (p > 0.05; Fig. 3). We also analyzed cell membrane CD28 and CTLA-4 expression in donor and recipient T cells. Compared with the isotype IgG-treated T cells, both CFSE−CD3+ cells and CFSE−CD3− cells showed an increase in CTLA-4 expression (p < 0.01) and a decrease in CD28 expression (p < 0.01) after injection with anti-B7-treated T cells as well as BALB/c-mated T

![FIGURE 2. Effect of adoptive transfer of anti-B7-treated T cells on the proliferation of recipient spleenocytes in response to DBA/2 stimulator cells. T cells from IgG-treated mice, anti-B7 mAbs-treated mice, or BALB/c-mated mice were i.v. injected into abortion-prone CBA/J females mated with DBA/2 males on day 4 of gestation, respectively. On day 9 of gestation, the recipient spleenocytes were cocultured withmitomycin C-treated paternal DBA/2 spleenocytes for 4 days. Proliferation was measured by H3TdR incorporation during the last 6 h. Results are expressed as the mean ± SD of triplicate measurements.](http://www.jimmunol.org/)

Table II. Effect of adoptive transfer of anti-B7-treated T cells on the embryo resorption rate of CBA/J × DBA/2 matings

<table>
<thead>
<tr>
<th>Transferred Cells</th>
<th>No. of Mice</th>
<th>No. of Live Fetuses</th>
<th>No. of Resorbed Fetuses</th>
<th>% Resorption</th>
</tr>
</thead>
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<tr>
<td>Null</td>
<td>10</td>
<td>55</td>
<td>20</td>
<td>26.67</td>
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<td>IgG-treated T cells</td>
<td>10</td>
<td>56</td>
<td>19</td>
<td>25.33</td>
</tr>
<tr>
<td>IgG-treated B cells</td>
<td>7</td>
<td>40</td>
<td>13</td>
<td>24.53</td>
</tr>
<tr>
<td>Anti-B7 mAbs-treated T cells</td>
<td>10</td>
<td>72</td>
<td>9</td>
<td>11.1¹</td>
</tr>
<tr>
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<td>41</td>
<td>13</td>
<td>24.07</td>
</tr>
<tr>
<td>BALB/c-mated T cells</td>
<td>6</td>
<td>44</td>
<td>5</td>
<td>10.20²</td>
</tr>
<tr>
<td>BALB/c-mated B cells</td>
<td>6</td>
<td>34</td>
<td>12</td>
<td>26.29</td>
</tr>
</tbody>
</table>

¹Pregnant CBA/J females mated with DBA/2 males were injected i.v. with 1 x 10^7 T or B cells from IgG-treated mice, anti-B7 mAb-treated mice, or BALB/c-mated mice on day 4 of gestation. Mice were killed on day 14 of gestation, and the embryo resorption rate was counted using the formula given in Materials and Methods.

²By χ² test: p < 0.05 compared with null treatment.
The difference in percentages of cells positive for CD28 or CTLA-4 between donor and recipient T cells was also not statistically significant ($p < 0.05$; Fig. 4). These results suggest that the transferred paternal Ag-hyporesponsive T cells have an immunoregulatory effect on recipient T cells and make recipient T cells express similar intracellular cytokine and costimulatory phenotypes to donor T cells.

**Trafﬁcking and homing of the transferred T cells in the recipient**

We also investigated the homing site of the transferred paternal Ag-hyporesponsive T cells in vivo. To monitor the location of the transferred T cells in the recipient, the intracellular fluorescent dye CFSE was used. We harvested the lymph nodes draining uterus, spleen, and ectoplacental cell cones from the recipient 36 h after adoptive transfer of CFSE-labeled T cells. We searched for the CFSE-labeled T cells within these tissues by two-photon confocal microscopy. The data in Fig. 5A showed that the CFSE-labeled T cells were localized in spleen and uterine draining lymph nodes, but not in the ectoplacental cell cone. Confocal microscopy, however, is not sensitive for detecting low cell numbers, thus we also used FACS to analyze whether CFSE-labeled T cells were present in these tissues. Using the FACS approach, we found CFSE-labeled T cells in the ectoplacental cell cone as well as in the spleen and uterine draining lymph nodes (Fig. 5B).

**Discussion**

Insights into the possible mechanisms by which transplant tolerance occurs have been provided by a variety of experimental models. It is increasingly clear that induction of Ag-speciﬁc unresponsiveness to grafts is responsible for maintaining the tolerant state. Once transplant tolerance is established, it can be transferred to a naive syngeneic animal by T cells (24, 25). Although anergic T cells generated with anti-B7 mAbs have been implicated as pivotal players in prolongation of graft survival, the capacity and possible mechanisms of the anti-B7-treated T cells to suppress maternal immune responses to the allogeneic fetus have received little attention. We thus explored the immunoregulatory roles of the anti-B7-treated T cells in materno-fetal tolerance and its potential application as a novel strategy in the treatment of recurrent spontaneous abortion. Our study reports, for the first time, that paternal Ag-hyporesponsive T cells generated by blockage of the
CD28/B7 pathway could exert an immunoregulatory effect on maternal recipient T cells and suppress maternal rejection to the allogeneic fetus in vivo.

Activation of TCR in the presence of a costimulatory signal results in T cell clonal expansion. Inhibition of the delivery of such a costimulatory signal at the time of TCR-mediated Ag recognition can lead to an Ag-specific T cell inactivation (26). CD28 and its ligands, B7-1 and B7-2, are the primary candidates for transmitting this costimulatory signal (7, 8). Previous studies have demonstrated the induction of Ag-specific tolerance in allograft models by treatment with anti-B7-1 and anti-B7-2 mAbs (9–11). In the present study we evaluated the immunosuppressive effects of anti-B7-1 and anti-B7-2 mAb in vivo treatment. We showed that the embryo resorption rate in abortion-prone matings was significantly reduced by treatment with anti-mouse B7-1/B7-2 mAbs. Consistent with this finding, we observed that combinations of both mAbs strongly inhibited the proliferation of CBA/J splenocytes in response to DBA/2 stimulator cells and that this was accompanied by seriously impaired IL-2 production. We observed that maternal tolerance to the fetus also was associated with maternal hyporesponsiveness to paternal Ag after anti-B7-1/B7-2 mAbs treatment. This also substantiated in vivo inhibitory effects of anti-B7-1 and anti-B7-2 mAbs in abortion-prone mice. In summary, our observations showed that adoptive transfer of anti-B7-treated T cells significantly suppressed the proliferation of recipient splenocytes in response to DBA/2 paternal Ags, and adoptive transfer of isotype IgG-treated mice T cells had no effect, which indicated that paternal Ag hyporesponsiveness was induced in the recipient by transferred anti-B7-treated T cells. Contact to the findings above that the embryo resorption rate of the recipient was significantly reduced after adoptive transfer of anti-B7-treated T cells. These findings indicated that maternal hyporesponsiveness to paternal Ags induced by anti-B7-1/B7-2 mAb treatment was maintained by the transfer of suppressor T cells. The finding that the proliferation of recipient splenocytes in response to DBA/2 paternal Ags was decreased significantly in the recipient by the transfer of anti-B7-treated T cells suggests that the transferred suppressor T cells inhibited the activation of native T cells in these mice, although additional experiments are needed to clarify the issue.

Previous studies demonstrated that the anti-B7-treated T cells could be transferred as suppressor cells to prevent allograft rejection (3). The mechanisms involved have received scant attention. In our study we analyzed the expression of intracellular cytokines (IL-2, IL-4, IL-10, and IFN-γ) and membrane costimulatory molecules (CD28 and CTLA-4) in CFSE−/CFSE+ T cells by flow cytometry. Interestingly, recipient T cells (CFSE−) had the same characteristics in terms of the expression of intracellular cytokines and membrane costimulatory molecules as donor T cells (CFSE+). We found up-regulated expression of IL-10 and CTLA-4, down-regulated expression of IFN-γ, IL-2, and CD28, and variable expression of IL-4. What might explain the relationship between the changes cited above and maternal tolerance to her fetus? The CTLA-4 molecule is important in immune regulation. It competes with CD28 to bind B7 and functions as a counter-regulatory receptor that attenuates T cell responses by down-regulating T cell activation (27–29), facilitating Ag-specific apoptosis (30), and

FIGURE 4. Flow cytometric analysis for cell membrane CD28 and CTLA-4 expression in CFSE− T cells and CFSE+ T cells. Five days after adoptive transfer of CFSE-labeled T cells, the recipient splenocytes were stimulated with mitomycin C-treated DBA/2 splenocytes for 4 days, then labeled for surface expression of CD3 (allophycocyanin), CD28 (PE), and CTLA-4 (PE). Cells were gated according to CFSE and CD3 expression. Cells were analyzed by flow cytometric gating on CFSE−CD3+ cells or CFSE−CD3− cells. The numbers indicate the percentages of positive cells. The results shown are from five separate experiments.
suppressing secretion of cytokines (28, 29, 31). Blockade of CTLA-4 signaling with neutralizing Ab was found to promote the expansion of Ag-specific T cells (27), enhance T cell IL-2 and IFN-γ secretion (31), and augment antitumor immunity (32). In this present study a decrease in the expression of CD28 was accompanied by a parallel increase in the expression of CTLA-4 after adoptive transfer of anti-B7-treated T cells. The higher capacity of the control mice transferred by isotype IgG-treated T cells to up-regulate CD28 on T lymphocytes supported the possibility that in vivo CD28 up-regulation could be responsible for a strong alloresponse, which can lead to maternal rejection of the allogeneic fetus. In contrast, the high CTLA-4 expression by T

**FIGURE 5.** The trafficking of CFSE-labeled T cells within the spleen, lymph nodes draining the uterus, and ectoplacental cell cone. The recipient spleen (SP), lymph nodes draining the uterus (LN), and ectoplacental cell cones (EPC) were harvested and sectioned 36 h after adoptive transfer of CFSE-labeled T cells. Two-photon confocal microscopy was used to image the positioning of CFSE-labeled T cells within these tissues. Two-photon imaging showed the localization of three kinds of T cells within the spleen and uterine draining lymph nodes, but not within the ectoplacental cell cone (A). Using flow cytometry, CFSE-labeled T cells were found in the ectoplacental cell cone as well as in the spleen and uterine draining lymph nodes (B).
lymphocytes in mice that received anti-B7-treated T cells could contribute to the suppression of maternal immune responses to the allogenic fetus in vivo and produce an increase in fetal survival. We proposed that paternal Ag-hyporesponsive T cells as suppressor cells exerted an immunoregulatory effect on recipient T cells and inhibited recipient T cell activation, which induced recipient T cells to express similar intracellular cytokine and costimulatory molecule profiles to donor T cells. The mechanisms by which the transferred suppressor T cells exert suppressive activity on recipient T cells have not been well defined. Our observations suggest that the suppression might partially depend on local IL-10 production, which can inhibit activation of reactive T cells, although additional studies are needed to clarify this possibility.

The migration pattern of the transferred T cells was monitored using the fluorescent dye CFSE. The labeled T cells were detected using confocal microscopy in spleen and uterine draining lymph nodes, but not in the ectoplacental cell cone. However, a more sensitive analysis using flow cytometric analysis indicated that the CFSE-labeled T cells resided mostly in peripheral immune organs, but a few resided at the materno-fetal interface. One might speculate that a direct cell-cell contact between transferred suppressor cells and host T cells might initiate this inhibitory effect.

Certain cytokines produced by T cells and some non-T cells, for example, TGF-β, IFN, IL-1, IL-4, IL-6, and IL-10, favor fetal survival and growth, whereas other cytokines, such as TNFα, IFN-γ, and IL-2, can compromise pregnancy (33, 34). Thus, despite the complexity of the cytokine network, it appears that cytokines favoring the maintenance of fetal survival mainly are of the Th2 type, whereas pregnancy failure is associated with the Th1 type and/or the absence of Th2-type cytokines. In this study we showed that a decrease in the expression of IL-2 and IFN-γ was accompanied by a parallel increase in the expression of IL-10 in both transferred suppressor T cells and recipient T cells. This suggests that the transferred suppressor T cells exert their immunoregulatory effect and induce clonal deviation in recipient T cells. Thus, changes in the expression of these cytokines might contribute to maternal tolerance to the fetus.

In conclusion, maternal tolerance for the fetus induced by anti-B7-1/B7-2 mAbs treatment was maintained by the transfer of suppressor cells. The paternal Ag-hyporesponsive T cells not only functioned as potent suppressor cells, but also modulated the activation of recipient T cells, to cosuppress maternal rejection actions to the allogeneic fetus. This resulted in a decrease in the embryo resorption rate of the abortion-prone matings to mirror that of normal pregnancy. Our data might be helpful in clinical trials for immunotherapy of recurrent spontaneous abortion and increase fetal survival rates.

Acknowledgments

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References


