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Immune Surveillance during Pregnancy

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Introduction

In 1953 Peter Medawar proposed that human pregnancy represents a semiallograft to the maternal host, therefore the process of implantation should include mechanisms preventing allograft rejection. Since then, many researchers have tried to resolve this mechanism. The aim of this study is to discuss immune surveillance during pregnancy.

Role of dendritic cells (DCs) in implantation

Among antigen presenting cells, the most potent inducers of primary immune responses are dendritic cells (DCs). DCs initiate and coordinate the innate and adaptive immune responses. DCs increase their numbers in the uterus during the peri-implantation period (1). Krey et al. firstly reported that DC depletion dramatically impaired implantation using a transgenic mouse system (DTRtg) that allows transient depletion of $CD11c^+$ cells in vivo through administration of diphtheria toxin (2). The same data have also been reported in other studies (3). These findings suggest that fetal-antigen recognition by DCs is necessary for implantation. But Plaks et al. reported that depletion of DCs also causes embryo resorption in syngeneic and T cell-deficient pregnancy, suggesting that DCs appear to govern uterine receptivity by regulating tissue remodeling and angiogenesis, independent of the immunological tolerance. They showed DCs produced sFlt 1 and TGF-β1 that promote coordinated blood vessel maturation. These findings suggest that uterine DCs are crucial for decidual formation during embryo implantation in mice. Furthermore, the maturity of uterine natural killer (uNK) cells was impaired at DC knockout implantation sites (2), and DCKO mice exhibited substantial anomalies in placental development (2). Human decidua contains potent immunostimulatory $CD83^+$ DCs and these DCs contact uNK cells (4). DC-$SIGN^+$-DCs are absent in the non-pregnant uterus in Rhesus Macaque, but uterine DC-$SIGN^+$ DCs increased in number within 1 week of implantation, and these cells are found only adjacent to the implantation site (5). These findings suggest that DCs play an important role in immunology and reproduction, especially in implantation.

Role of $CD4^+$ $CD25^+$ regulatory T cells in allogeneic pregnancy

$CD4^+$ $CD25^+$ regulatory T (Treg) cells play central roles for immune regulation (6). They express high levels of CD25 (IL-2Rα), as well as the cytotoxic T-lymphocyte antigen 4 (CTLA-4) and the transcription factor, Foxp3. Treg cells have potent regulatory properties in both the induction and maintenance phase of *in vivo* tolerance in mice and humans.

Aluvihare et al. reported interesting findings that suggest Treg cells might regulate maternal tolerance to the fetus (7). BALB/c derived–total lymphocytes were injected into T-cell deficient BALB/c nu/nu female mice (Fig. 1). These mice were mated to C57BL/6 male mice, resulting in normal pregnancy. When BALB/c $CD25^+$-cells

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with deleted lymphocytes ($CD25^-$ lymphocytes) were injected into BALB/c nu/nu mice, they did not have $CD4^+CD25^+$ Treg cells (Fig. 1). These Treg cell deficient BALB/c mice were mated with allogeneic C57BL/6 male mice, and abortion occurred (Fig. 1). However, when these Treg cell deficient BALB/c mice were mated to syngeneic BALB/c male mice, they showed normal pregnancy, suggesting that allogeneic fetuses were rejected when $CD4^+CD25^+$ Treg cells were absent. Zenclussen et al. reported that anti-CD25 monoclonal antibody treatment on day 2.5 of gestation induced implantation failure in allogeneic pregnancy, but not in syngeneic pregnancy (8). These data suggest that $CD25^+$ cells play an important role for maintenance of allogeneic pregnancy. $CD4^+CD25^+$ Treg cells might mediate maternal tolerance, but $DX5^+$ $CD25^+$ NKreg cells might also play some roles in successful pregnancy (see following section). We should clarify which cells are important for maintenance of pregnancy. In humans, our group firstly reported that $CD4^+CD25^{bright}$ Treg cells dramatically increased in early pregnancy decidua. And these increased decidual Treg cell ratios were decreased in spontaneous abortion or habitual abortion (9). These findings suggest that increased Treg cells at the fetomaternal interface might play an important role for the maintenance of allogeneic pregnancy.

As a mechanism of immunoregulation, immunostimulation of Treg cells is important. As a first step, Treg cells recognize some antigens via T cell receptors/CD3 complex. At the same time, CD28 on Treg cells bind B7 complex on DCs. T cell receptors and CD28-mediated costimulation are required for Treg cells to exert suppression. These activated Treg cells express CTLA-4 on their surface, and can suppress both $CD4^+$ T cells and $CD8^+$ T cells by cell-to-cell interaction. Therefore, surface CTLA-4 expression on Treg cells is a marker for activated and functional Treg cells. Interestingly, surface CTLA-4 expression on Treg cells increases in decidual Tregs but not in peripheral blood Treg cells of early pregnancy subjects (9). This increased surface CTLA-4 expression decreases in miscarriage cases (9), suggesting that functional Treg cells might induce alloantigen-specific tolerance, resulting in maintenance of pregnancy. Jasper et al. reported that primary unexplained infertility is associated with reduced expression of the Treg cell transcription factor Foxp3 in endometrial tissue (10). Recent data demonstrate that expansion of the Treg cells during pregnancy induces tolerance to paternal alloantigens in mice (11). Expansion of Treg cells in para-aortic lymph modes draining the uterus is observed on day 3.5. This increase in Treg cells is

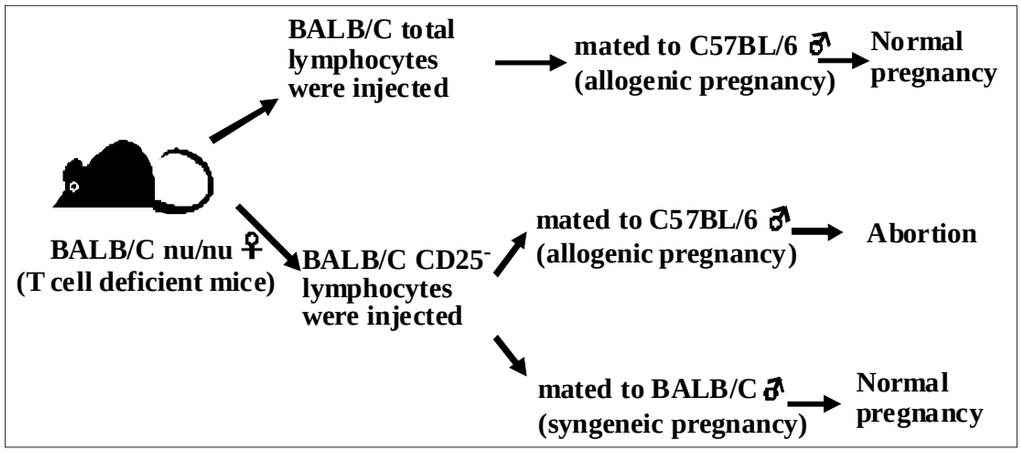

Fig. 1: Regulatory T cells mediate maternal tolerance to the fetus.

abrogated when males are vasectomized or seminal vesicles are excised (11). These findings suggest seminal fluid plays an important role for induction of tolerance to paternal alloantigens in mice.

Role of regulatory NK cells during pregnancy

NK cells are one of the key cell types involved in allograft rejection. However, in certain transplant models, NK cells also express potent immunoregulatory properties such as tolerance induction.

We have reported that leukemic peripheral blood $DX5^+$ $CD25^+$ $Thy1.2^{++}$ $c\text{-}kit^+$ NK cells have immunoregulation ability such as inhibition of allo-T cell stimulatory activity of DCs and autotumor specific CTL induction in mice (12). When the myelomonocytic leukemia cell line, WEH1 3B, is injected into BALB/c mice, $CD3^-$ $DX5^+$ NK cells are rapidly increased in the peripheral blood.

These phenotypes are quite different from those of conventional NK cells (Table 1). These NK cells express CD25, Thy-1.2 and c-kit. Thy 1.2 and c-kit are markers for progenitor cells, suggesting that these leukemic NK cells are an immature type of NK cells. Leukemic NK cells downregulate the expression of MHC class II antigen on DCs mediated by TGF-β production. These NK cells suppress the allo-T cell stimulatory activity of DCs. These NK cells inhibit generation of autotumor-specific CTL, suggesting that these NK cells are regulatory NK cells. Very interestingly, these $CD25^+$ $Thy1^{high}$ $c\text{-}kit^{high}$ NK cells accumulate in the pregnant uterus of BALB/c mice. And most of the decidual lymphocytes in NOD/SCID mice are $CD3^-$, $Ly49^-$, $CD25^+$, $Thy1^{high}$, $c\text{-}kit^{high}$ cells. Most of the uterine NK cells produce IL-10 and 10-20% of the uterine NK cells produce TGF-β in SCID mice.

In BALB/c mice, 30% of uterine lymphocytes are NKreg cells, but in NOD/SCID mice, this

Table I. The phenotypes of regulatory NK cells and conventional NK cells in mice.

	Regulatory NK cells	Conventional NK cells
Surface marker		
CD94	negative ~ low	high and low
Ly49 C/F	low	high and low
acialo GMI	high	high
CD25	high	negative-low
CD122	high	high
Thy-1, 2	very high	medium
c-kit	medium ~ high	negative ~ low
Cytokine production		
IFN-γ	low	high
IL-4	low	low
TGF-β	medium	low
IL-10	high	low
Ctytoxic activity	low	high
TLR3 stimulation	low activation	high activation
TLR4 stimulation	low activation	high activation

population is 85-90%. Poly (1:C) treatment or LPS treatment on gestational day 8.5 and 9.5 induce abortion. But these treatments do not induce abortion in NOD/SCID mice. NK activity in spleen, peripheral blood and the uterus are elevated by the treatment of Poly (1:C) or LPS in BALB/c mice. But in NOD/SCID mice, these treatments do not augment the NK cell activity. These findings suggest that NKreg cells might regulate inflammation, resulting in maintenance of pregnancy (Table I).

Our group already reported that CD25 and c-kit are expressed on decidual $CD56^{bright}$ NK cells in humans (14, 15). And we also reported that IL-10 producing NK cells increase in peripheral blood, and TGF-β producing NK cells increase in decidua of early pregnancy subjects (16). These findings suggest NKreg cells are also present in human pregnancy, and they may play important roles for maintenance of pregnancy.

Conclusion

Many papers support the idea that immune cells play important roles for successful implantation and pregnancy. These data may assist in resolving the limited implantation success of embryos transferred following IVF-ET.

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