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Uterine Regulatory T cells, IL-10 and Hypertension

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Abstract

Problem—Regulatory T cells (T_{reg}) are a vital immune cellular population at the maternal-fetal interface. They are likely to aid in immune tolerance by dampening the harmful effects of other immune cellular populations through cell-cell mediated interactions as well as by producing IL-10 and TGF-β. In addition to the anti-inflammatory properties, IL-10 has emerged as an important vascular cytokine choreographing endovascular interactions, angiogenesis and regulates hypertension.

Method of study—Review of innovative concepts to understand the temporal role of Tregs in both mouse and human pregnancy, particularly whether uterine T_{reg} play a potential role in regulating vascular homeostasis and blood flow during pregnancy.

Results—T_{reg} guard immune tolerance, getting cytotoxically activated under certain conditions, leading to adverse pregnancy outcome.

Conclusions—Despite increasing evidence of T_{reg} tissue-specific expansion and functional plasticity, their role in vascular activity, preeclampsia or gestational diabetes is obscure and needs closer investigation to delineate its role later during pregnancy.

Keywords

Pregnancy; preeclampsia; regulatory T cells; interleukin-10; vascular activity

Introduction

Pregnancy is a unique immune phenomenon because the fetus and the placenta can develop in the womb without being attacked by the maternal immunesystem and despite admixing of maternal and fetal cells [1]. One key reason may be the presence of uterine regulatory T cells (Tregs) that play an important role in protecting the fetus by inhibiting harmful immune responses that would otherwise be detrimental to the fetus [2]. In addition to their immuno-suppressive role during pregnancy, accumulating body of evidence now positions Tregs as modulators of vascular homeostasis and blood flow [3].

Regulatory T cells in pregnancy

Regulatory T cells (Tregs) are a specialized population of T lymphocytes known for their properties as potent suppressors of inflammatory immune responses and their ability to mediate immune homeostasis. Their unique properties, particularly their ability to suppress cytotoxically activated T cells and NK cells make them an integral part of immune tolerance during pregnancy. These cells are characterized by the surface expression of CD4, CD25,

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and the intracellular forkhead box transcription factor, Foxp3 [4,5]. In humans, regulatory T cells increase very early in pregnancy, peak during the second trimester and then begin to decrease to pre-pregnancy levels [3, 6]. Mice exhibit a similar trend where Tregs begin to increase as early as day 2.5, peak during mid-gestation, and reach non-pregnant levels around gestational day (GD)17 [7]. Incidentally, the peak of Tregs coincides with the phase of pregnancy that entails intense vascular activity, trophoblast invasion, spiral artery remodeling and peak populace of uterine NK cells at the maternal-fetal interface. The importance of Treg cells for pregnancy was elegantly demonstrated by Aluvihare et al, where they adoptively transferred T lymphocytes depleted of CD4+CD25+ into pregnant T cell deficient mice [6]. Here, the allogeneic fetal units were rejected in the absence of T_{regs}. Importantly, depletion of T_{regs} between GD2.5 and GD4.5 in mice resulted in implantation failure and significantly increased fetal resorption rates, whereas depletion of T_{regs} on GD10 did not induce discernable adverse pregnancies [8]. These findings suggest that regulatory T cells are more important in the implantation phase and the early stages of pregnancies (Fig 1). The importance of Treg cells for successful pregnancy was further verified in another study where depletion of T_{regs} in pregnant mice resulted in fewer fetuses surviving at term [9]. Furthermore, reduced levels of $CD4^{+}CD25^{+}$ cells were found in the decidual tissues of abortion prone DBA/J-mated CBA/J female mice. Adoptive transfer of Tregs isolated from normal pregnant mice but not non-pregnant mice prevented fetal demise [10]. This implies that the hormonal changes associated with pregnancy uniquely influence Treg cell functions. Infertility has been proposed to be associated with reduced Treg cells in endometrial tissue [11]. Spontaneous abortion cases and patients with recurrent miscarriage are also associated with lower systemic T_{regs} compared to that seen in normal pregnancies [12,13]. It is now well appreciated that apart from cell-cell mediated immunoregulation, T_{regs} produce two immunosuppressive cytokines TGF-β and IL-10 that contribute to their anti-inflammatory effects. Since these cytokines also influence vascular activity, it is tempting to speculate that regulatory T cells may play a part in regulation of blood pressure. Conversely, dysregulated T_{regs} may contribute to the onset of preeclampsia (Fig 2).

Regulation of hypertension and vascular remodeling by IL-10

Interleukin-10 (IL-10), a key immunosuppressive cytokine, produced by T_{regs}, is increased early in pregnancy and remains elevated until the onset of labour [14]. Various cellular populations are involved in its production at the maternal–fetal interface. Notable, villous cytotrophoblast produce IL-10 whereas extravillous trophoblasts (EVT) are poor producers of IL-10 [15]. IL-10 and its receptor (IL-10R) are expressed on a variety of cell types found at the maternal-fetal interface in both mice and humans namely, placental trophoblasts, decidual stromal cells, macrophages, and uNK cells [16,17]. Mice genetically deficient in IL-10 have been very useful in the investigation into the precise functions of IL-10 during pregnancy. $IL\text{-}10^{-/-}$ mice are highly sensitive to pro-inflammatory stimuli such as lipopolysaccharide, polyinosinic:polycytidylic acid (poly I:C) and CpG, which are known ligands for toll-like receptors,[18–21] as well as environmental toxicants like polychloride biphenyls[22]. Fetal resorption could be observed as early as GD9 in response to early treatment with polyI:C, a ligand for TLR-3 (unpublished data). Although it is known that T_{regs} host a repertoire of toll-like receptors and respond differentially in response to specific TLR ligation[21], what is not clear is if the density and variety of TLR expressed by uterine T_{regs} is different from those cells in circulation or in spleen and if so what are their tissue-specific implications at different stages of gestations. In this context, it has been shown that viral infections sensed by TLR3 are associated with hypertensive disorders of pregnancy. Treatment of pregnant rats with the viral mimic poly I:C on gd 10 resulted in elevated systolic blood pressures, decreased aortic vasodilation, increased urinary protein concentrations, and had more malformed pups [23]. This suggests that perturbations of T_{regs} during different phases of pregnancy may precipitate a diverse spectrum of pregnancy

outcomes. In addition to its known anti-inflammatory functions, recent observations suggest that IL-10 can regulate vascular activity and endovascular interactions at the maternal-fetal interface[23,24]. Administration of recombinant IL-10 reversed hypoxia-induced hypertension, proteinuria and intrauterine growth restriction in IL-$10^{-/-}$ mice [24].Overall, these studies imply that IL-10 is likely to play key protective roles against preeclampsia.

Do Tregs play a role in vascular homeostasis during pregnancy?

Many developmental and immune-regulatory changes occur in pregnancy. Changes that involve the maternal adaptation to blood flow and vascular homeostasis include reduced pressor response to angiotensin II, increase in plasma volume resulting in a decrease in the proportion of red blood cells, peripheral vasodilation associated with significant increase in cardiac output, and transient low blood pressure[25–28]. Thus, understanding the blood flow in the context of changes that occur during normal pregnancy is important because failure to adjust to these changes may result in hypertensive disorders of pregnancies such as preeclampsia. Although not well understood, immune regulation may play a central role in the pathogenesis of hypertensive disorders of pregnancy. The focus has been on uterine NK cells. However, recent studies by Croy and colleagues demonstrate that uterine NK cells do not affect blood pressure regulation during pregnancy [29]. This raises a question whether T_{regs} are involved in regulating blood flow and pressure during pregnancy.

Various reports have demonstrated that T_{regs} can modulate heart fibrosis in hypertension, control coronary arteriolar endothelial dysfunction in hypertensive mice and suppress angiotensin II-induced hypertension and vascular injury[3,30,31]. Angiotensin II is known for its role in the development of hypertension by mechanisms mediated in part by innate and adaptive immunity. Studies in which T_{regs} were adoptively transferred into Ang II-infused hypertensive mice prevented cardiac hypertrophy and reduced blood pressure, vascular damage and prevented Ang II-induced hypertension[30,32]. The concept that T_{regs} can control inflammation in a mouse model of chronic Ang II infusion demonstrated that Ang II does not directly influence T_{regs}. However, T_{reg}-treatment maintained other T cell populations, signifying the mechanistic importance of these cells[32].

The soluble fms-like tyrosine kinase receptor (sFlt1) and soluble endoglin have been implicated in the pathogenesis of preeclampsia[33–36]. Interestingly, soluble endoglin has been shown to precipitate hypertension and endothelial dysfunction by inhibiting TGFβ, [36] which incidentally is a vital cytokine essential to the tolerogenic actions of T_{regs}. Patients with preeclampsia produce high levels of Th1 cytokines (IL-2, IL-6, TNFα and IFN-γ), and reduced secretion of Th 2 cytokines (IL-10 and IL-15). Interestingly, IL-6 and TNF-α have been shown to induce hypertension and proteinuria, hallmark features of preeclampsia[37]. Several studies have reported a reduction in the numbers of T_{regs} in peripheral blood and also the placental bed sections in pre-eclamptic women compared to healthy pregnant controls[38–41]. Recently, our lab has shown that injection of preeclampsia serum in pregnant IL-$10^{-/-}$ mice causes hypertension and proteinuria[42]. Interestingly, injection of human preeclampsia serum resulted in full spectrum of PE-like symptoms and spiral artery remodeling defects and reduced T_{regs} population (unpublished results) in IL-$10^{-/-}$ mice[43,44]. The significance of persistence and peak amplification of T_{regs} around gd 12 in mice and by the end of first trimester of human pregnancy on placental angiogenesis and blood pressure regulation is not clear. Since T_{regs} reach peak on GD 12 in mice, it is tempting to speculate that besides immune reactions, T_{regs} may also play a role in these processes as well which become consequential at around this period during gestation.

Conclusions

Hypertension during pregnancy, particularly as related to preeclampsia, predominantly leads to preterm birth, intrauterine growth restriction (IUGR) and placental dysfunction[45]. Additionally, women with pre-existing and gestational diabetes are at an even higher risk for acquiring hypertensive disorders of pregnancies, which in turn, significantly increase the risk for future cardiovascular complications[46]. Thus clinicians and/or health care providers are faced with the challenge of controlling maternal blood pressure and glucose levels so as to improve maternal and fetal outcome. T_{regs} are guardians of immune tolerance, yet are flexible to get cytotoxically activated under certain conditions which may lead to adverse pregnancy outcomes. Increasing evidence is emerging regarding their tissue-specific expansion and functional plasticity; however, their role in vascular activity, preeclampsia or gestational diabetes is obscure and needs closer investigation to delineate its role later during pregnancy.

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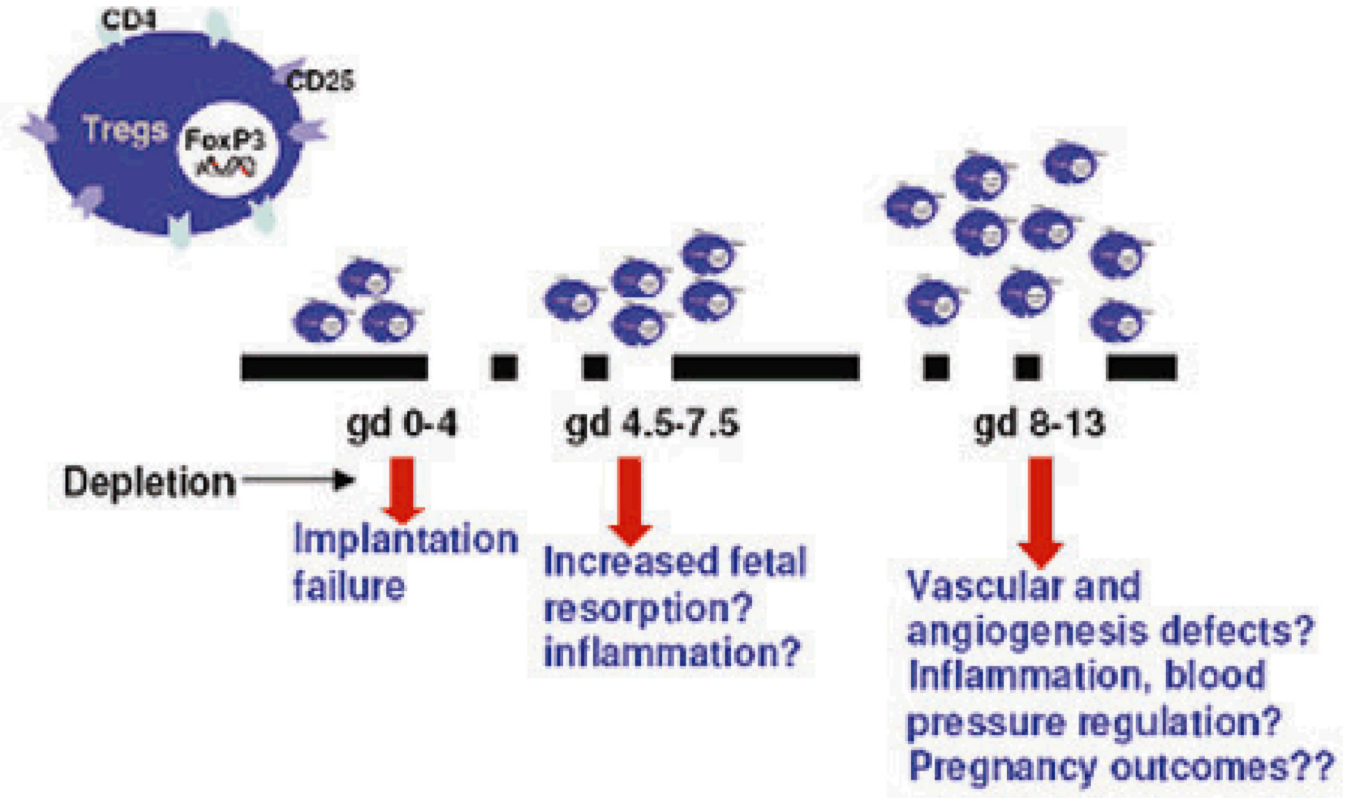

Figure 1. Kinetics of possible functional implications of uterine T_{regs}

T_{regs} begin to increase as early as gestational day (gd) 2.5, peak during mid-gestation (gd 12), then reach non-pregnant levels at term in mice. The importance of T_{regs} during implantation and early stages of pregnancies are supported by depletion studies.

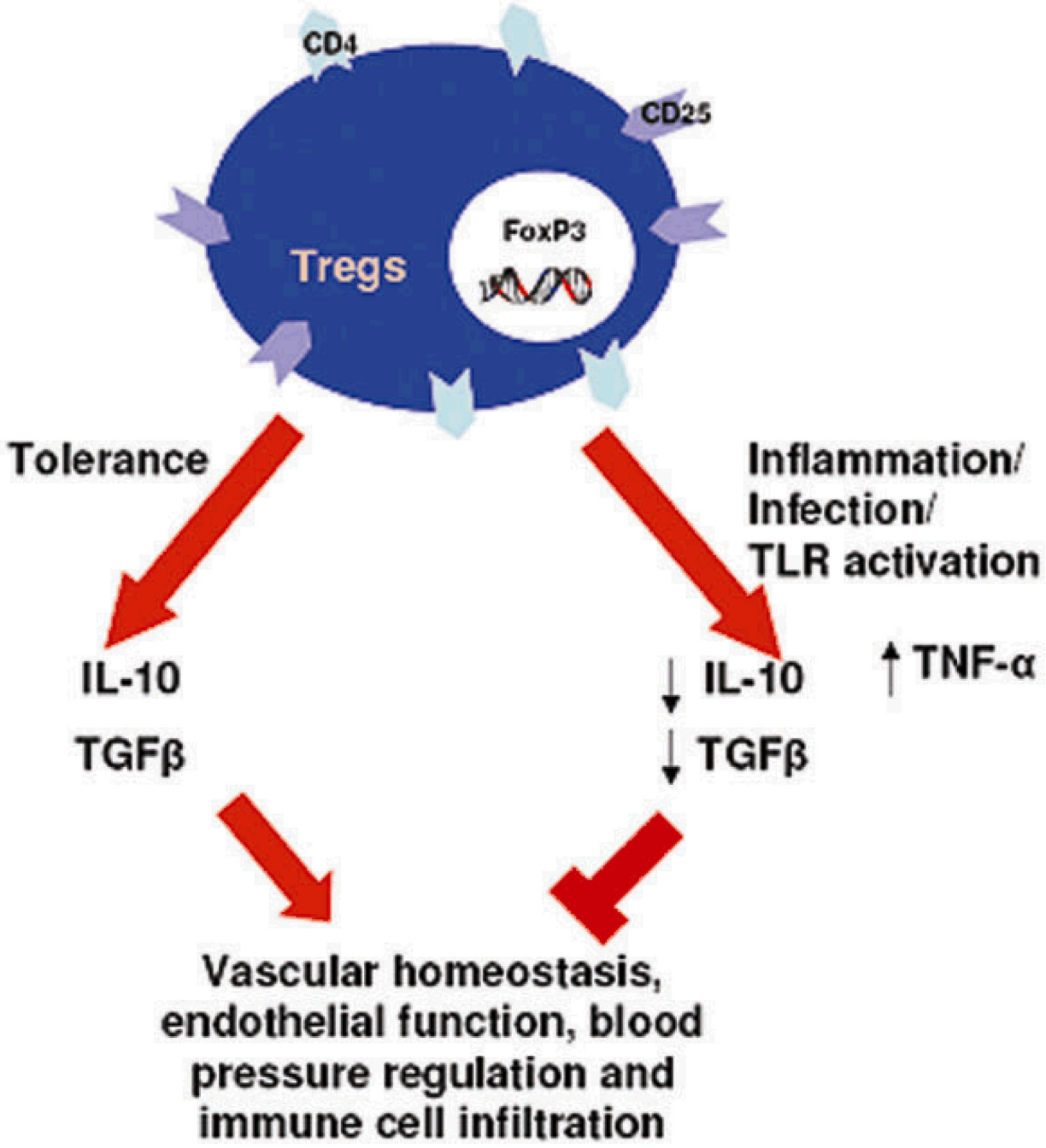

Figure 2. Uterine T_{regs} and Vascular Homeostasis

T_{regs} produce two immunosuppressive cytokines TGF-β and IL-10 that can influence vascular activity and may play a part in regulation of blood pressure. Conversely, dysregulated T_{regs} either due to infection, inflammation or TLR activation may perturb vascular homeostasis by producing TNFα.