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Mechanisms involved in the expansion of Tregs during pregnancy: role of IL-2/STAT5 signalling

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ABSTRACT

Several studies have reported that the fine-tuning of regulatory T cells (Tregs) is required for a successful pregnancy and for the control of autoimmune diseases. Here, we review the mechanisms that control the expansion of Tregs, based on insights obtained from the study of women suffering unsuccessful pregnancies and on recent data from patients with autoimmune diseases or with chronic viral infections such as HCV. In particular, we review the role of endocrine factors and IL-2/STAT5 signalling in the impaired expansion of Tregs.

1. Introduction

Several types of T regulatory cells (Tregs) have been described in both mice and humans (Fig. 1), including natural or professional Tregs ($CD4^+$ $CD25^+$ T cells) and the adaptive Th3 and Tr1 Treg subsets, which develop as a consequence of the activation of mature T cells under conditions of sub-optimal antigen exposure and/or co-stimulation.

Natural Tregs (nTregs) are produced in the thymus as a functionally mature subpopulation of T cells and comprise a population of cells enriched in $CD4^+$ $CD25^+$ T cells that express the transcription factor forkhead box P3 (FOXP3). FOXP3 expression is necessary and sufficient for Treg development and function and has become a reliable marker for the Treg lineage. These thymus-derived natural Treg cells retain a stable phenotype following emigration to the periphery. Outside the thymus, natural Treg cells can become activated by specific antigens and acquire some of the phenotypic properties of effector or memory T cells, such as the capacity to migrate to inflamed peripheral tissues, while maintaining FOXP3 expression and suppressive function.

In 1980, we were the first to show the generation of human regulatory or suppressor T cells expressing HLA-DR, which were induced by long-term T-cell activation (Fainboim et al., 1980). We can now explain those results, hypothesising that activated Tregs and those originating from $CD4^+$ $CD25^-$ T-cells that become suppressor Tregs can survive preferentially over conventional T cells. More recently, it was shown that inducible Tregs (iTregs) can dif-

Abbreviations: Treg, regulatory T cell; nTreg, natural regulatory T cell; iTreg, inducible regulatory T cell; rTreg, resting regulatory T cell; aTreg, activated regulatory T cell; FOXP3, forkhead box P3; RSA, recurrent spontaneous abortion; E2, 17-β-estradiol; STAT-5, signal transducer and activator of transcription 5.

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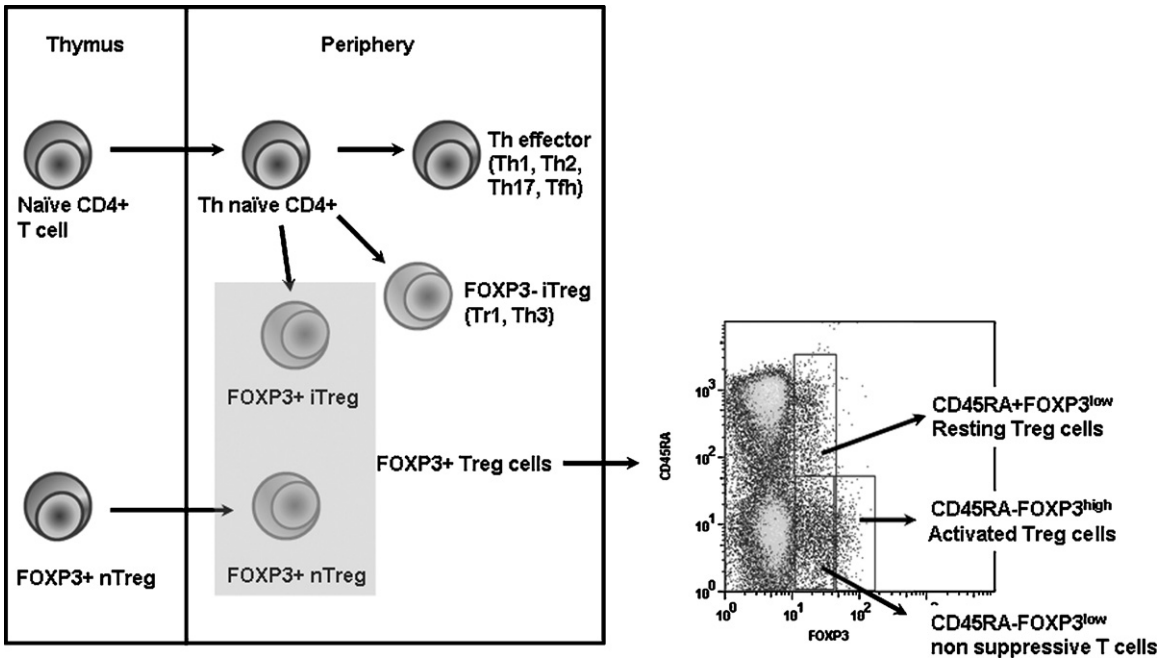

Fig. 1. Different types of Tregs: (1) natural or thymic-derived FOXP3$^+$ Tregs; and (2) three types of Tregs generated during an adaptive immune response: two are FOXP3-, Tr1 and Th3, and a third is FOXP3$^+$. In addition, according to the expression of CD45RA and FOXP3, three different FOXP3$^+$ Treg subsets have been characterised (Miyara et al., 2009): CD45RA$^+$ FOXP3low resting Treg cells, CD45RA$^-$ FOXP3high activated Tregs and CD45RA$^-$ FOXP3low non-suppressive T cells.

ferentiate from CD4$^+$ CD25$^-$ cells in response to IL-2 and TGF-β (Bluestone and Abbas, 2003). These Tregs share a similar phenotype with nTregs, and IL-2 and TGF-β seem to play a non-redundant role in the maintenance and survival of both types of Tregs (Marie et al., 2005; Davidson et al., 2007; Zheng et al., 2007).

The possibility of dissecting FOXP3$^+$ Treg cells into subsets enables us to analyse Treg cell differentiation dynamics and interactions in normal and disease states and to control immune responses through the manipulation of particular Treg subpopulations.

2. Tregs and successful pregnancy

Human reproduction entails a fundamental paradox: although it is critical to the survival of the species, the process is relatively inefficient. Maximal fecundity (the probability of conception during one menstrual cycle) is approximately 30%. Only 50–60% of all conceptions advance beyond 20 weeks of gestation. Of the pregnancies that are lost, 75% represent a failure of implantation and are therefore not clinically recognized as pregnancies (Wilcox et al., 1988; Zinaman et al., 1996; Rai and Regan, 2006).

Successful implantation is the end result of complex molecular interactions between the hormonally primed uterus and a mature blastocyst. Pregnancy constitutes a major challenge to the maternal immune system, as it has to tolerate the persistence of paternal alloantigen. Although localised mechanisms contribute to foetal evasion of immune attack, maternal alloreactive lymphocytes persist.

Regulatory T cells appear to play an essential role in controlling maternal immunity against paternally inherited foetal alloantigens. In their pioneering work, Aluvihare et al. (2004) first reported the systemic expansion of maternal CD4$^+$ CD25$^+$ T cells that have dominant regulatory T cell activity during mouse pregnancy. They also demonstrated that depletion of peripheral blood CD4$^+$ CD25$^+$ cells leads to gestation failure in allogeneic, but not in syngeneic, pregnancy. Subsequent studies confirmed the expansion of CD4$^+$ CD25$^+$ cells during mouse pregnancy (Zenclussen et al., 2005; Zhao et al., 2007).

Almost simultaneously with these murine reports, human studies also demonstrated an increase in circulating Treg cells during pregnancy. An initial report demonstrated an increase in CD4$^+$ CD25$^+$ T cells during early pregnancy, which peaked during the second trimester and then declined postpartum (Somerset et al., 2004). Also in 2004, two studies demonstrated that early human pregnancy decidua contained an abundance of CD4$^+$ CD25^{high} T cells expressing a high level of CTLA-4, which was significantly reduced in specimens from spontaneous abortion compared with those specimens from induced abortions (Heikkinen et al., 2004; Sasaki et al., 2004). One of these studies also showed that the increased number of Tregs isolated from the human deciduae (Heikkinen et al., 2004) was associated with an increase in this population in the peripheral blood during the first and second trimesters of gestation. The increase in Tregs at the foetal–maternal interface was later confirmed in several human studies (Mjosberg et al., 2010; Tilburgs et al., 2008; Jin et al., 2009). The unusually high frequency of CD4$^+$ CD25$^+$ cells in the uterus has recently been related to a preferential recruit-

ment of foetus-specific regulatory T cells from maternal peripheral blood to the foetal–maternal interface. This pool of Tregs that expands during pregnancy is enriched in $CD4^+$ $CD25^+$ $FOXP3^+$ cells, has suppressive function in vitro, and has a higher frequency of FOXP3-positive cells when compared with peripheral blood $CD4^+$ $CD25^+$ cells (Tilburgs et al., 2008).Thus, an increase in the number of Tregs at the human maternal-foetal interface seems clear. However, most of the original human studies that showed a systemic increase in the number of Tregs were based on the expression of the CD25 marker. Expression of CD25 is not exclusive to Tregs, and the expression of this marker on activated effector cells might explain the contradictory results obtained in different studies. In fact, studies using additional markers to characterize Tregs fail to demonstrate a systemic increase in phenotypically well-defined Tregs (Mjosberg et al., 2009). An additional source of controversy is the recent data indicating that the so-called $FOXP3^+$ Treg cells are not a homogeneous population of cells (see Fig. 1). According to Sakaguchi group's findings, human $FOXP3^+$ $CD4^+$ T cells are composed of three phenotypically and functionally distinct subpopulations: two with suppressive activity in vitro, the $CD45RA^+$ $FOXP3^{low}$ resting Treg cells (rTreg) and $CD45RA^-$ $FOXP3^{high}$ activated Treg cells (aTreg), and a third with the phenotype $CD45RA^-$ $FOXP3^{low}$, which secretes cytokines and lacks suppressive activity (Miyara et al., 2009). The proportion of the three subpopulations differs between cord blood samples, samples from aged individuals, and samples from patients with immunological diseases. According to this study, aTregs originate from the differentiation of rTregs. In vivo, after their stimulation, rTreg cells upregulate FoxP3 expression, differentiate to aTreg cells, and proliferate. Considering that aTreg cells rapidly die in vitro and that the immune system is constantly challenged by exogenous and endogenous antigens, it is likely that the maintenance of the pool of aTreg cells is the consequence of a tight balance between the constant development of aTreg cells from activated and proliferating rTreg cells and their death after exerting suppression (Miyara et al., 2009). Because aTreg cells appear to be terminally differentiated and rapidly die, attempts to expand Treg cells ex vivo for cell therapy should be focused on the high capacity of rTreg cells to proliferate (Hoffmann et al., 2006). It needs to be determined whether rTreg cells are constantly produced by the thymus, whether they have a high renewal capacity in the periphery, or both.Mjosberg et al. showed that the frequency of human second-trimester circulating $FOXP3^+$ Tregs was reduced, but that these cells still displayed the same suppressive capacity (Mjosberg et al., 2009). Another study found that the total Treg cell numbers did not increase during early pregnancy. However, when Treg subsets were dissected according to their level of expression of FOXP3, into $CD4^+$ $CD25^+$ $FOXP3^{high+}$ Treg cells or $CD4^+$ $CD25^{high+}$ $FOXP3^+$ Tregs, a strong increase in the percentage of the $CD4^+$ $CD25^+$ $FOXP3^{high+}$ Treg cell population was observed during the first and second trimester, whereas in the third trimester, this Treg cell population decreased considerably until term, and the proportion of $CD4^+$ $CD25^+$ $FOXP3^{high+}$ cells correlated with their suppressive capacity (Steinborn et al., 2008).

3. How are Tregs expanded during pregnancy?

Maternal Tregs are critical in responses to the paternal alloantigens expressed by the foetus. In their report, Aluvihare et al. (2004) showed that Treg expansion occurs in both syngeneic and allogeneic mating and suggested that this increase was alloantigen-independent, implying that maternal Tregs do not need to be primed by paternal alloantigens to protect the foetus. More recent studies, which characterise Tregs as $FOXP3^+$, provide a different point of view (Schumacher et al., 2007; Thuere et al., 2007; Zhao et al., 2007; Robertson et al., 2009). For instance, Tai et al. (2008) showed a greater expansion of Tregs in allogeneically compared with syngeneically pregnant mice, suggesting reduced generation of alloreactivity against paternal antigens. Similarly, virgin inbred mice undergoing a first syngeneic pregnancy, in which only the male foetuses were antigenic, showed a restricted maternal proliferative response to the male foetal antigen (H-Y), where a portion of the maternal immune response to foetal antigens was Treg in nature (Kahn and Baltimore, 2010). The influence of foetal alloantigen in stimulating human Tregs was also reported (Tilburgs et al., 2009) to be associated with HLA-C, the only polymorphic classical histocompatibility antigen expressed by the foetal trophoblast at the foetal–maternal interface. Notably, these authors found that pregnancies containing an HLA-C mismatched child induced increased expansion of functional $CD4^+$ $CD25^{high}$ regulatory Tregs in decidual tissue; whereas HLA-C-matched pregnancies did not.

A new factor with the capability to induce Treg expansion during pregnancy was recently described. This protein is a glycoprotein of the lipocalin superfamily known as PP14 or glycodelin, which is involved in the transport of small hydrophobic ligands. Amniotic PP14 is synthesised in secretory endometrial glands and by the gestational deciduae and is regulated by progesterone. Maternal serum levels of PP14 rise in early pregnancy, with the highest concentrations in the deciduae between the sixth and twelfth weeks of gestation. This glycoprotein is known to promote the generation of Treg cells and could be, at least partially, responsible for an alloantigen-independent expansion of Tregs (Ochanuna et al., 2010).

4. Influence of sex hormones in the expansion of Tregs

The role of sex hormones in the regulation of Tregs during pregnancy is also controversial. Because of the changes in these hormones during pregnancy, it has been proposed that they may be involved in Treg expansion. Oestrogen seems to augment Treg numbers, and it was reported that treatment of naive mice with oestrogen (E2) increased both $CD25^+$ cell number and FOXP3 expression level (Polanczyk et al., 2004). However, the mechanism by which hormones participate in the expansion of Tregs during pregnancy has been not completely elucidated. Tai et al. (2008) found that physiological, in vivo doses of E2 expanded $CD4^+$ $CD25^+$ cells. These authors claim that E2 stimulates the conversion of $CD4^+$ $CD25^-$ cells to $CD4^+$ $CD25^+$ cells, which exhibit enhanced FOXP3 and IL-10 expression in vitro and have

regulatory functions similar to natural Tregs. Similarly, it was postulated that both E2 and pregnancy enhance PD-1 expression by DCs and macrophages, which enhances the in vitro expansion and function of Tregs (Polanczyk et al., 2006; Franceschini et al., 2009). In contrast, other reports have excluded hormonal changes during pregnancy as the exclusive driving force in Treg expansion (Schumacher et al., 2007; Thuere et al., 2007). In a previous study, we were able to demonstrate a pre-implantation expansion of Tregs during the normal human menstrual cycle, which tightly correlated with an increase in serum levels of oestrogen. However, this expansion was not detected in patients with recurrent spontaneous abortions, in spite of the presence of normal levels of serum E2 in these patients (Arruvito et al., 2007). As we will discuss in the next section, these findings suggest that additional mechanisms participate in the regulated expansion of Tregs. Similarly, every time a female mouse approaches oestrus, Tregs start to accumulate in the uterus in preparation for a possible implantation event (Kallikourdis and Betz, 2007).

5. Role of the IL-2/STAT-5 signalling pathway in the expansion of Tregs

5.1. Role of IL-2/STAT-5 in pregnancy loss

Human recurrent spontaneous abortions (RSA) have been defined as three or more consecutive clinical pregnancies lost before the 20th week of pregnancy. According to this definition, RSA occurs in ~1 in 100 pregnancies around the world. However, this frequency increases up to 5% when clinicians define RSA as two or more losses of pregnancy. In addition, epidemiological investigations have demonstrated that the frequency of subsequent pregnancy loss is ~24% after two pregnancy losses, 30% after three and 40% after four successive pregnancy losses (Baek et al., 2007). In many cases, the aetiology is unknown, but several hypotheses have been proposed, including chromosomal and uterine anatomical abnormalities, endometrial infections, endocrine abnormalities, anti-phospholipid syndrome, inherited thrombophilias, alloimmune causes, genetic factors, exposure to environmental factors and stress factors.

Women who have had recurrent spontaneous abortions showed similarly low numbers of Tregs at both the follicular and luteal phases, comparable to the numbers observed in postmenopausal women. In addition to their decreased numbers, Tregs from women with RSA were also functionally deficient, as higher numbers were required to exert a similar magnitude of suppression to that induced by Tregs from fertile women (Arruvito et al., 2007). In men, testosterone was shown to play a critical role in maintaining the level of Tregs, without the profound changes in Treg frequencies observed in women under the cyclic influence of female sex hormones (Page et al., 2006). As already discussed, expansion of Tregs in fertile women during the follicular phase is correlated with the levels of serum E2. However, the low expansion of Tregs in RSA patients was observed in patients with similar or even increased levels of E2 (Arruvito et al., 2007). Thus, additional factors must cause the deficit of Tregs observed in RSA patients.

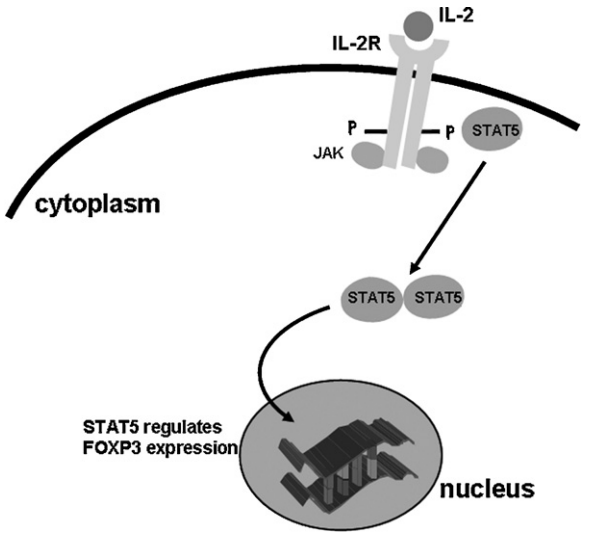

Fig. 2. Upon binding of IL-2 to its receptor (IL-2R), the associated Janus kinases become activated and phosphorylate the cytoplasmic domains of the IL-2R, creating docking sites for intracellular proteins, such as STAT-5. After binding to the receptor complex, STAT-5 is phosphorylated, dimerises and translocates to the nucleus. There, it regulates the transcription of multiple genes including FOXP3.

As mentioned above, IL-2 plays a central role in Treg development. In vitro, IL-2 and TGF-β induce FOXP3$^+$ Treg cells from naive CD4$^+$ T cells (Bluestone and Abbas, 2003; Yao et al., 2007), and in vivo, during immune reconstitution of cancer or lymphopaenia patients receiving IL-2 therapy, patients show a homeostatic peripheral expansion of Treg cells (Zhang et al., 2005).

In mice, signal transducer and activator of transcription-5 (STAT-5) are known to be required for the development of FOXP3$^+$ (Fig. 2) Tregs (Burchill et al., 2007). In humans, the activation of the JAK-STAT signalling pathway is required for peripheral expansion and suppressive activity of Tregs (Murray, 2007; Malek, 2008), and individuals with a deficiency of STAT-5b have a decreased frequency of Tregs, which have decreased function (Cohen et al., 2006).

Accordingly, we investigated the participation of the IL-2/STAT-5 signalling in fertile women and showed a physiological expansion of Tregs in comparison with women with reproductive failure (Arruvito et al., 2010). In fertile women, PBMC activation with lectins plus IL-2 expanded the number of FOXP3 cells. In contrast, in RSA patients, the same in vitro activation performed in the absence of E2 resulted in a very poor expansion of Tregs. The impaired response in RSA patients was associated with a low level of secretion of IL-2 and TGF-β. In addition to low-level secretion of IL-2, RSA patients showed a limited expression of CD4$^+$ P-STAT-5 cells in response to the IL-2 stimulus. The decreased expression of P-STAT-5 in RSA patients seemed to be intrinsic because the levels of P-STAT-5 in controls and patients showed an association with the dose of IL-2 used as stimulus. Functional studies also confirmed a more limited suppressive capacity of inducible Tregs from RSA patients. These results are in line with our previous reports, showing not only quantitative but also functional suppressive deficiency in RSA patients.

5.2. Role of IL-2/STAT-5 in autoimmunity

A deficit in the IL-2/STAT signalling axis may also have an impact on the development of tolerance to autoantigens. IL-2 strongly regulates FOXP3 expression in a STAT5-dependent manner (Zorn et al., 2006), and the few reported human cases of deficiency in IL-2Rα resulted in autoimmunity, lymphadenopathy and persistent viral infection (Aoki et al., 2006; Strieder et al., 2006). The aetiology of autoimmune diseases is known to be multi-factorial. It has been estimated that at best 79% of the probability of developing autoimmune thyroid disease can be attributed to genetics (Brix et al., 2000). A study of females with at least one first- or second-degree relative with thyroid autoimmunity (Strieder et al., 2006) revealed reduced serum levels of sIL-2R and reduced percentages of circulating $CD4^+$ $CD25^+$ lymphocytes. These early results suggested that reduced expansion of putative $CD4^+$ $CD25^+$ Tregs was associated with an increased risk of developing thyroid autoimmunity. More recently, a strong relationship was reported between defects in IL-2R signalling and diminished maintenance of FOXP3 expression in $CD4^+$ $CD25^+$ Tregs from type 1 diabetic subjects (Long et al., 2010). In this study, the authors found that after exposure to IL-2, the majority of $CD4^+$ $CD25^+$ T cells isolated from control subjects responded to IL-2 stimulation as measured by P-STAT5. In comparison, significantly fewer T-cells from type 1 diabetic subjects responded to IL-2, indicating that decreased IL-2 responses are linked to defects in IL-2R signalling.

5.3. Role of Tregs in chronic viral infection (HCV)

In addition to suppressing autoimmune responses, $CD4^+$ $CD25^+$ $FOXP3^+$ cells limit T cell responses during chronic infection, thereby minimising T-cell-dependent immunopathology. In livers of patients chronically infected with HCV, a lower number of intra-hepatic Tregs coincided with the upregulation of programmed death-1 (PD-1). Consistent with the possibility that PD-1 controls Tregs, blockade of the interaction between PD-1 and PD-1 ligand (PD-L1) enhances the in vitro expansion and function of Tregs isolated from the livers of patients chronically infected with HCV. Notably, PD-L1 inhibition upregulated STAT-5 phosphorylation of those Tregs, suggesting that PD-L1 negatively regulates Tregs at sites of chronic inflammation by controlling STAT-5 phosphorylation (Franceschini et al., 2009).

6. Role of IL-6 signalling in Tregs development

In addition to its intrinsic defect in IL-2/STAT-5 signalling, IL-6 has been implicated in the pathogenesis of many autoimmune and chronic inflammatory diseases (Kishimoto, 1992; Pasare and Medzhitov, 2003; Wan et al., 2007).

However, in spite of the role assigned to IL-6 in Tregs, little is known about the mechanism by which IL-6 modulates this cell type. One study of IL-6−/− mice demonstrated that IL-6 can limit the activity of virus-specific Tregs, thereby facilitating the activity of virus-specific memory $CD4^+$ cells (Longhi et al., 2008).

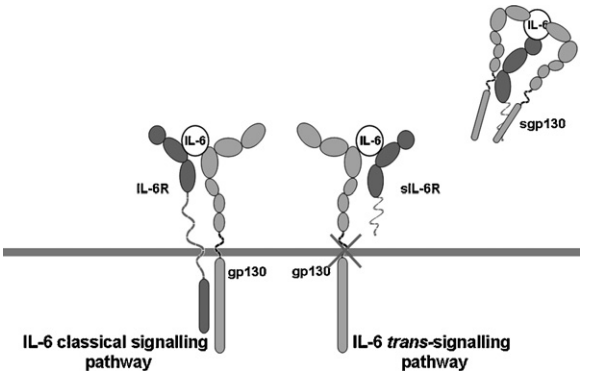

Fig. 3. During the classical pathway of IL-6 signalling, IL-6 first binds to the membrane IL-6 receptor (IL-6R) and the complex of IL-6/IL-6R associates with the signal-transducing membrane protein gp130, promoting its dimerisation and the subsequent initiation of intracellular signals. Alternatively, in the so-called trans-signalling process, soluble forms of the IL-6R (sIL-6R) are capable of forming a complex with IL-6 and of stimulating cells, which only express gp130. Soluble forms of sIL-6R and soluble gp 130 (sgp 130) are naturally present in serum and in several fluids. The process of *trans*-signalling can be inhibited by a molar excess of sgp130.

In mice, IL-6 *trans*-signalling via the soluble IL-6R abrogates both the induction of FOXP3 expression and its suppressive function in naïve mouse $CD4^+$ $CD25^-$ T cells (Fig. 3) (Dominitzki et al., 2007). In line with these data, we found an increase in IL-6 and in the soluble IL-6R in RSA patients, which was associated with a decrease in soluble gp130, a component known to inhibit *trans*-signalling (Arruvito et al., 2009). Notably, the activation of naïve $CD4^+$ T cells or isolated nTregs with IL-2 and TGF-β downregulated IL-6 receptor expression and its signalling pathway and caused them to become resistant to Th17 conversion (Zheng et al., 2008).

Concluding remarks

In this review, we explore mechanisms that may control the expansion of Tregs. We describe the role of Tregs during normal pregnancy. However, new insights into the regulation of Tregs have come from diseases such as recurrent pregnancy loss, autoimmunity and chronic viral infections. From these cases, we have learned that deficiencies in the IL-2/STAT5 signalling pathway play a central role in controlling the expansion of Tregs. However, we caution against analysing the expansion of Tregs solely based on their expression of FOXP3. We have learned that cells expressing this transcription factor represents a heterogeneous population, which contains not only regulatory T cells, but also an important subset of cells without suppressor capacity that are able to secrete inflammatory cytokines. Therefore, further studies examining different subsets of $FOXP3^+$ cells will be required to clarify the role of $FOXP3^+$ Treg cells in many physiological and non-physiological situations.

References

Aluvihare, V.R., Kallikourdis, M., et al., 2004. Regulatory T cells mediate maternal tolerance to the fetus. Nat. Immunol. 5 (3), 266–271.

Aoki, C.A., Roifman, C.M., et al., 2006. IL-2 receptor alpha deficiency and features of primary biliary cirrhosis. J. Autoimmun. 27 (1), 50–53.
Arruvito, L., Sanz, M., et al., 2007. Expansion of $CD4^+CD25^+$and $FOXP3^+$ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. J. Immunol. 178 (4), 2572–2578.
Arruvito, L., Billordo, A., et al., 2009. IL-6 trans-signaling and the frequency of $CD4^+FOXP3^+$ cells in women with reproductive failure. J. Reprod. Immunol. 82 (2), 158–165.
Arruvito, L., Sotelo, A.I., et al., 2010. A physiological role for inducible FOXP3(+) Treg cells. Lessons from women with reproductive failure. Clin. Immunol. 136 (3), 432–441.
Baek, K.H., Lee, E.J., et al., 2007. Recurrent pregnancy loss: the key potential mechanisms. Trends Mol. Med. 13 (7), 310–317.
Bluestone, J.A., Abbas, A.K., 2003. Natural versus adaptive regulatory T cells. Nat. Rev. Immunol. 3 (3), 253–257.
Brix, T.H., Kyvik, K.O., et al., 2000. A population-based study of chronic autoimmune hypothyroidism in Danish twins. J. Clin. Endocrinol. Metab. 85 (2), 536–539.
Burchill, M.A., Yang, J., et al., 2007. IL-2 receptor beta-dependent STAT5 activation is required for the development of $Foxp3^+$ regulatory T cells. J. Immunol. 178 (1), 280–290.
Cohen, A.C., Nadeau, K.C., et al., 2006. Cutting edge: decreased accumulation and regulatory function of $CD4^+$ CD25(high) T cells in human STAT5b deficiency. J. Immunol. 177 (5), 2770–2774.
Davidson, T.S., DiPaolo, R.J., et al., 2007. Cutting Edge: IL-2 is essential for TGF-beta-mediated induction of $Foxp3^+$ T regulatory cells. J. Immunol. 178 (7), 4022–4026.
Dominitzki, S., Fantini, M.C., et al., 2007. Cutting edge: trans-signaling via the soluble IL-6R abrogates the induction of FoxP3 in naive $CD4^+CD25$ T cells. J. Immunol. 179 (4), 2041–2045.
Fainboim, L., Navarrete, C., et al., 1980. Precursor and effector phenotypes of activated human T lymphocytes. Nature 288 (5789), 391–393.
Franceschini, D., Paroli, M., et al., 2009. PD-L1 negatively regulates $CD4^+CD25^+Foxp3^+$ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. J. Clin. Invest. 119 (3), 551–564.
Heikkinen, J., Mottonen, M., et al., 2004. Phenotypic characterization of regulatory T cells in the human decidua. Clin. Exp. Immunol. 136 (2), 373–378.
Hoffmann, P., Eder, R., et al., 2006. Only the $CD45RA^+$ subpopulation of $CD4^+$CD25high T cells gives rise to homogeneous regulatory T-cell lines upon in vitro expansion. Blood 108 (13), 4260–4267.
Jin, L.P., Chen, Q.Y., et al., 2009. The $CD4^+$CD25 bright regulatory T cells and CTLA-4 expression in peripheral and decidual lymphocytes are down-regulated in human miscarriage. Clin. Immunol. 133 (3), 402–410.
Kahn, D.A., Baltimore, D., 2010. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. Proc. Natl. Acad. Sci. U.S.A. 107 (20), 9299–9304.
Kallikourdis, M., Betz, A.G., 2007. Periodic accumulation of regulatory T cells in the uterus: preparation for the implantation of a semi-allogeneic fetus? PLoS One 2 (4), e382.
Kishimoto, T., 1992. Interleukin-6 and its receptor in autoimmunity. J. Autoimmun. 5 (Suppl. A), 123–132.
Long, S.A., Cerosaletti, K., et al., 2010. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes 59 (2), 407–415.
Longhi, M.P., Wright, K., et al., 2008. Interleukin-6 is crucial for recall of influenza-specific memory CD4 T cells. PLoS Pathog. 4 (2), e1000006.
Malek, T.R., 2008. The biology of interleukin-2. Annu. Rev. Immunol. 26, 453–479.
Marie, J.C., Letterio, J.J., et al., 2005. TGF-beta1 maintains suppressor function and Foxp3 expression in $CD4^+CD25^+$ regulatory T cells. J. Exp. Med. 201 (7), 1061–1067.
Miyara, M., Yoshioka, Y., et al., 2009. Functional delineation and differentiation dynamics of human $CD4^+$ T cells expressing the FoxP3 transcription factor. Immunity 30 (6), 899–911.
Mjosberg, J., Svensson, J., et al., 2009. Systemic reduction of functionally suppressive CD4dimCD25highFoxp3$^+$ Tregs in human second trimester pregnancy is induced by progesterone and 17beta-estradiol. J. Immunol. 183 (1), 759–769.
Mjosberg, J., Berg, G., et al., 2010. $FOXP3^+$ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. Biol. Reprod. 82 (4), 698–705.
Murray, P.J., 2007. The JAK-STAT signaling pathway: input and output integration. J. Immunol. 178 (5), 2623–2629.
Ochanuna, Z., Geiger-Maor, A., et al., 2010. Inhibition of effector function but not T cell activation and increase in FoxP3 expression in T cells differentiated in the presence of PP14. PLoS One 5 (9), e12868.
Page, S.T., Plymate, S.R., et al., 2006. Effect of medical castration on $CD4^+$ $CD25^+$ T cells, $CD8^+$ T cell IFN-gamma expression, and NK cells: a physiological role for testosterone and/or its metabolites. Am. J. Physiol. Endocrinol. Metab. 290 (5), E856–E863.
Pasare, C., Medzhitov, R., 2003. Toll pathway-dependent blockade of $CD4^+CD25^+$ T cell-mediated suppression by dendritic cells. Science 299 (5609), 1033–1036.
Polanczyk, M.J., Carson, B.D., et al., 2004. Cutting edge: estrogen drives expansion of the $CD4^+CD25^+$ regulatory T cell compartment. J. Immunol. 173 (4), 2227–2230.
Polanczyk, M.J., Hopke, C., et al., 2006. Estrogen-mediated immunomodulation involves reduced activation of effector T cells, potentiation of Treg cells, and enhanced expression of the PD-1 costimulatory pathway. J. Neurosci. Res. 84 (2), 370–378.
Rai, R., Regan, L., 2006. Recurrent miscarriage. Lancet 368 (9535), 601–611.
Robertson, S.A., Guerin, L.R., et al., 2009. Seminal fluid drives expansion of the $CD4^+CD25^+$ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol. Reprod. 80 (5), 1036–1045.
Sasaki, Y., Sakai, M., et al., 2004. Decidual and peripheral blood $CD4^+CD25^+$ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol. Hum. Reprod. 10 (5), 347–353.
Schumacher, A., Wafula, P.O., et al., 2007. Mechanisms of action of regulatory T cells specific for paternal antigens during pregnancy. Obstet. Gynecol. 110 (5), 1137–1145.
Somerset, D.A., Zheng, Y., et al., 2004. Normal human pregnancy is associated with an elevation in the immune suppressive $CD25^+$ $CD4^+$ regulatory T-cell subset. Immunology 112 (1), 38–43.
Steinborn, A., Haensch, G.M., et al., 2008. Distinct subsets of regulatory T cells during pregnancy: is the imbalance of these subsets involved in the pathogenesis of preeclampsia? Clin. Immunol. 129 (3), 401–412.
Strieder, T.G., Drexhage, H.A., et al., 2006. A reduced IL2R (CD25) expression level in first and second degree female relatives of autoimmune thyroid disease patients. A sign of a poor capability to preserve tolerance? Autoimmunity 39 (2), 93–98.
Tai, P., Wang, J., et al., 2008. Induction of regulatory T cells by physiological level estrogen. J. Cell Physiol. 214 (2), 456–464.
Thuere, C., Zenclussen, M.L., et al., 2007. Kinetics of regulatory T cells during murine pregnancy. Am. J. Reprod. Immunol. 58 (6), 514–523.
Tilburgs, T., Roelen, D.L., et al., 2008. Evidence for a selective migration of fetus-specific $CD4^+$CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. J. Immunol. 180 (8), 5737–5745.
Tilburgs, T., Scherjon, S.A., et al., 2009. Fetal-maternal HLA-C mismatch is associated with decidual T cell activation and induction of functional T regulatory cells. J. Reprod. Immunol. 82 (2), 148–157.
Wan, S., Xia, C., et al., 2007. IL-6 produced by dendritic cells from lupus-prone mice inhibits $CD4^+CD25^+$ T cell regulatory functions. J. Immunol. 178 (1), 271–279.
Wilcox, A.J., Weinberg, C.R., et al., 1988. Incidence of early loss of pregnancy. N. Engl. J. Med. 319 (4), 189–194.
Yao, Z., Kanno, Y., et al., 2007. Nonredundant roles for Stat5a/b in directly regulating Foxp3. Blood 109 (10), 4368–4375.
Zenclussen, A.C., Gerlof, K., et al., 2005. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced $CD4^+CD25^+$ T regulatory cells prevents fetal rejection in a murine abortion model. Am. J. Pathol. 166 (3), 811–822.
Zhang, H., Chua, K.S., et al., 2005. Lymphopenia and interleukin-2 therapy alter homeostasis of $CD4^+CD25^+$ regulatory T cells. Nat. Med. 11 (11), 1238–1243.
Zhao, J.X., Zeng, Y.Y., et al., 2007. Fetal alloantigen is responsible for the expansion of the CD4(+)CD25(+) regulatory T cell pool during pregnancy. J. Reprod. Immunol. 75 (2), 71–81.
Zheng, S.G., Wang, J., et al., 2007. IL-2 is essential for TGF-beta to convert naive $CD4^+CD25^-$ cells to $CD25^+Foxp3^+$ regulatory T cells and for expansion of these cells. J. Immunol. 178 (4), 2018–2027.
Zheng, S.G., Wang, J., et al., 2008. Cutting edge: $Foxp3^+CD4^+CD25^+$ regulatory T cells induced by IL-2 and TGF-beta are resistant to Th17 conversion by IL-6. J. Immunol. 180 (11), 7112–7116.
Zinaman, M.J., Clegg, E.D., et al., 1996. Estimates of human fertility and pregnancy loss. Fertil. Steril. 65 (3), 503–509.
Zorn, E., Nelson, E.A., et al., 2006. IL-2 regulates FOXP3 expression in human $CD4^+CD25^+$ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood 108 (5), 1571–1579.