Regulatory T cells and the immune pathogenesis of prenatal infection

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Abstract

Pregnancy in placental mammals offers exceptional comprehensive benefits of *in utero* protection, nutrition, and metabolic waste elimination for the developing fetus. However, these benefits also require durable strategies to mitigate maternal rejection of fetal tissues expressing foreign paternal antigens. Since the initial postulate of expanded maternal immune tolerance by Sir Peter Medawar 60 years ago, an amazingly elaborate assortment of molecular and cellular modifications acting both locally at the maternal–placental interface and systemically have been shown to silence potentially detrimental maternal immune responses. In turn, simultaneously maintaining host defense against the infinite array of potential pathogens during pregnancy is equally important. Fortunately, resistance against most infections is preserved seamlessly throughout gestation. On the other hand, recent studies on pathogens with unique predisposition for prenatal infections have uncovered distinctive holes in host defense associated with the reproductive process. Using these infections to probe the response during pregnancy, the immune suppressive regulatory subset of maternal CD4 T cells has been increasingly shown to dictate the inter-workings between prenatal infection susceptibility and pathogenesis of ensuing pregnancy complications. Herein, the recent literature suggesting a necessity for maternal regulatory T cells (Tregs) in pregnancy-induced immunological shifts that sustain fetal tolerance is reviewed. Additional discussion is focused on how expansion of maternal Treg suppression may become exploited by pathogens that cause prenatal infections and the perilous potential of infection-induced immune activation that may mitigate fetal tolerance and inadvertently inject hostility into the protective *in utero* environment.

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Introduction

Pregnancy in eutherian placental mammals requires expanded maternal tolerance to encompass paternal antigens expressed by the developing fetus. This vital process initiated at the earliest stages of pregnancy with the invasion of fetal trophoblast cells into the uterine lining prevents recognition and rejection of foreign fetal cells. Given the essential nature of reproduction for species survival, it is not surprising that numerous non-overlapping immune evasion strategies that together reinforce protection for the developing fetus have been identified. These include sharply reduced or extinguished expression of immune recognition major histocompatibility (MHC) antigens on trophoblast cells (Sunderland *et al.* 1981, Ozato *et al.* 1985, Zuckermann & Head 1986, Hunt *et al.* 1987, Mattsson 1998), tryptophan catabolism that prevents maternal T-cell activation (Munn *et al.* 1998, Mellor & Munn 2004), selective expression of galectin-1 that moderates T-cell differentiation or Crry that prevents complement deposition (Xu *et al.* 2000, Blois *et al.* 2007), entrapment of antigen-presenting cells within the uterus (Collins

et al. 2009), and blunted chemokine expression by decidual stromal cells (Nancy *et al.* 2012). By coalescing these potent immune suppressive features where they are most needed at the maternal–fetal interface, responsiveness that maintains host defense against most pathogens systemically and within non-reproductive tissues would be predicted to be preserved.

On the other hand, since low-level dissemination of fetal cells into maternal blood and non-reproductive tissues also occurs during pregnancy (Liegeois *et al.* 1981, Guetta *et al.* 2003, Khosrotehrani *et al.* 2004), systemic immune modifications may also be needed to reinforce fetal tolerance. Here, multiple non-overlapping strategies are likely to be simultaneously at work. One is the selective elimination of maternal T cells with high affinity to fetal antigens through apoptotic death in early gestation (Erlebacher *et al.* 2007). However, this process is incomplete both temporally and for maternal T cells that recognize fetal antigens with lower affinity, suggesting that other ways to prevent the activation of maternal immune cells with fetal specificity also exist. In this regard, the selective silencing of immune effector cells with specificity to

non-self paternal antigens during pregnancy can be viewed as an example of peripheral immune tolerance. By contrast, even with substantial overlap between maternal and fetal antigens, central tolerance that eliminates developing T cells with self-specificity within the thymus is less operational since maternal thymectomy does not diminish fertility and, in cases of autoimmunity, may improve the outcomes of pregnancy (Visser *et al.* 2004, Hoff *et al.* 2007, Griesemer *et al.* 2010, Stritesky *et al.* 2012). Thus, immune components that sustain peripheral tolerance in other contexts (e.g. commensal microbes in tissues with direct contact with the external environment or self-antigens for immune cells that escape central tolerance) are likely to play expanded roles in the maintenance of fetal tolerance during pregnancy. Since the pregnancy-associated immune modifications that sustain fetal tolerance have recently been summarized in a very comprehensive fashion both in general terms and from more distinctive perspectives including the maternal–fetal interface, lymphoid organs that drain this compartment, antigen-presenting dendritic cells, and how immunological shifts affect local susceptibility to viral pathogens (Moffett & Loke 2006, Bizargity & Bonney 2009, Mor & Cardenas 2010, Taglauer *et al.* 2010, Munoz-Suano *et al.* 2011, Mold & McCune 2012, Erlebacher 2013a, 2013b), we use this opportunity to focus more specifically on evidence for systemic immune modifications and how the dynamic cross-regulation between immunological shifts required for sustaining fetal tolerance dictates susceptibility to prenatal infection and the potential immune pathogenesis of ensuing pregnancy complications.

Maternal regulatory T cells and fetal tolerance

The FOXP3⁺ subset of CD4 T cells called regulatory T cells (Tregs) has potent immune suppressive properties and plays essential roles in the sustainability of peripheral immune tolerance (Littman & Rudensky 2010, Wing & Sakaguchi 2010, Josefowicz *et al.* 2012). Spontaneous FOXP3 defects result in fatal systemic and organ-specific autoimmunity within the first 6 months of life described as the immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome (Bennett *et al.* 2001, Wildin *et al.* 2001). In turn, similar mortal symptoms arise in mice with naturally occurring or targeted disruptions in *Foxp3* (Fontenot *et al.* 2003, Khattri *et al.* 2003). The importance of maternal Tregs in fetal tolerance was first suggested by their progressive expansion in healthy human pregnancy and blunted expansion in cases of spontaneous abortion compared with induced abortion (Sasaki *et al.* 2004, Somerset *et al.* 2004). At the same time, pioneering studies in mice have shown paralleled levels of maternal Treg accumulation throughout gestation, whereas the selective elimination of maternal

Tregs caused fetal wastage and resorption (Aluvihare *et al.* 2004).

Within the next few years after these seminal findings, the critical necessity of maternal Tregs in sustaining fetal tolerance has been reinforced by numerous other studies characterizing these cells in human and animal pregnancies. For example, significantly reduced levels of maternal Treg expansion have been reported repeatedly for women with preeclampsia or recurrent spontaneous abortion (Sasaki *et al.* 2007, Toldi *et al.* 2008, Yang *et al.* 2008, Prins *et al.* 2009, Santner-Nanan *et al.* 2009, Winger & Reed 2011). Along with these quantitative reductions, qualitative diminutions in suppressive function among maternal Tregs on a per cell basis have also recently been described for preterm human pregnancies compared with term human pregnancies (Gomez-Lopez & Laresgoiti-Servitje 2012, Schober *et al.* 2012). Here, it is important to point out blunted maternal Treg expansion may not be universally associated with all cases of preeclampsia since normal Treg accumulation has also been reported (Paeschke *et al.* 2005, Hu *et al.* 2008). However, the interpretation of these isolated negative findings is somewhat moderated by the wide variation in peripheral lymphocyte numbers and Treg percentages among individuals. Nevertheless, consistent reductions in maternal Treg suppression among multiple seemingly unrelated clinical pregnancy complications underscore the potential importance of preserving fetal tolerance by these cells in healthy pregnancy.

The necessity of maternal Tregs in maintaining pregnancy has been more definitively addressed in animal pregnancy models in which experimental manipulation allows the cause and effect relationship of Treg manipulation with regard to pregnancy outcomes to be investigated. During mouse allogeneic pregnancy, maternal Treg depletion or reconstitution of T-cell-deficient mice exclusively with non-Treg effector cells each triggers significantly increased rates of fetal resorption with a reciprocal loss of live pups (Aluvihare *et al.* 2004, Shima *et al.* 2010, Rowe *et al.* 2011, Samstein *et al.* 2012). Furthermore, the accumulation of activated effector T cells within the decidua and systemic expansion of maternal effector T cells with fetal specificity with ablation of maternal Tregs each reinforce a critical protective role for these cells in sustaining fetal tolerance (Rowe *et al.* 2011, Samstein *et al.* 2012). Along with these findings illustrating the necessity for maternal Tregs after the near-complete ablation of these cells, the specific requirement for expanded maternal Foxp3⁺ cells has also been described. The latter using mice in which the high-affinity human diphtheria toxin receptor is co-expressed with Foxp3 and exploiting the X-linked inheritance of *Foxp3* whereby female mice heterozygous for the diphtheria toxin receptor transgene contain an equal ratio of Tregs that are either susceptible or resistant to ablation found that even partial transient ablation to pre-pregnancy levels triggers fetal resorption

and fractures fetal tolerance (Rowe *et al.* 2011). Therefore, despite considerable variation in the overall magnitude of fetal resorption between individual studies that probably reflect differences in the timing and efficiency of Treg depletion using unique experimental approaches (Aluvihare *et al.* 2004, Shima *et al.* 2010, Rowe *et al.* 2011, Samstein *et al.* 2012), these studies in mice collectively illustrate the importance of maternal Tregs and the sustained expansion of these cells in maintaining pregnancy.

Other complementary data highlighting the importance of Tregs in maternal–fetal tolerance are those on evolutionarily conserved genetic elements required for selective FOXP3 expression unique to humans and other eutherian placental mammals that are conspicuously absent in marsupials and egg-laying monotremes (Andersen *et al.* 2012, Samstein *et al.* 2012). This includes the *Foxp3* enhancer conserved non-coding sequence 1 (CNS1) required for peripheral induced Treg differentiation among non-Treg CD4 T cells (Zheng *et al.* 2010). Pregnancies in mice with targeted defects in *Cns1* show significantly increased rates of fetal resorption with more pronounced decidual inflammation and abnormal spiral artery remodeling consistent with the pathological features of preeclampsia in human pregnancies (Samstein *et al.* 2012). This apparent requirement for induced Treg differentiation parallels the efficiency whereby trophoblast cells and high-level progesterone each induce FOXP3 expression among non-Treg CD4 T cells (Lee *et al.* 2012, Ramhorst *et al.* 2012). On the other hand, using adoptively transferred FOXP3-depleted cells to show that the pre-existing pool of peripheral Foxp3⁺ CD4 T cells also contributes significantly to the overall accumulation of maternal Tregs during pregnancy may explain the considerably reduced magnitude of fetal resorption after the selective elimination of induced Tregs based on CNS1 deficiency compared with bulk Tregs based on Foxp3 expression (Rowe *et al.* 2011, 2012c, Samstein *et al.* 2012).

Additional investigation exclusive to animal pregnancy has uncovered other interesting facets of the fundamental biology whereby maternal Tregs respond to fetal antigen stimulation. For example, using the wealth of defined inbred strains in mice, maternal Tregs have been shown to accumulate to higher levels during allogeneic pregnancy (when MHC-discordant parents are used for breeding) than during syngeneic pregnancy (between MHC-identical parents) where the only potential source of antigen heterogeneity is that encoded by the Y chromosome (Kahn & Baltimore 2010, Rowe *et al.* 2011). Reciprocally, fetal resorption triggered by maternal Treg ablation is consistently reduced in syngeneic pregnancy compared with in allogeneic pregnancy (Aluvihare *et al.* 2004, Shima *et al.* 2010, Rowe *et al.* 2012c, Samstein *et al.* 2012). Accordingly, the degree of mismatch between maternal and paternal–(fetal) MHC alloantigens appears to dictate the necessity

for expanded maternal Tregs during pregnancy. In this regard, the natural heterogeneity between maternal and paternal MHC antigens in human pregnancy is more fully recapitulated in mouse allogeneic pregnancy. However, given the selective loss of male offspring with partial ablation of maternal Tregs during syngeneic pregnancy, these cells also probably mitigate mismatch between minor alloantigens encoded on the Y chromosome (Kahn & Baltimore 2010). Together, these findings suggest a critical role for the differentiation of maternal Tregs and the sustained expansion of these cells in maintaining tolerance to paternal non-self antigens expressed by the developing fetus during pregnancy.

Pregnancy-induced shifts in systemic immune responsiveness and maternal Tregs

In addition to these protective roles in the maintenance of fetal tolerance, the sustained accumulation of immune suppressive maternal Tregs has also been linked with notable shifts in non-fetal responses outside female reproductive tissues throughout gestation. A remarkable example of this is the amelioration of many autoimmune disorders during human pregnancy. For example, significant reductions in disease severity or complete remission occur in women with rheumatoid arthritis during pregnancy (Da Silva & Spector 1992, Barrett *et al.* 1999, Ostensen & Villiger 2007, de Man *et al.* 2009). The protective benefits are most likely conferred by maternal Tregs, given the inverse correlation between disease severity and circulating levels of these cells during pregnancy and after parturition, and the protective capacity of maternal Tregs from mice with pregnancy induced remission from collagen induced arthritis after adoptive transfer into naive recipients (Forger *et al.* 2008, Munoz-Suano *et al.* 2012). Similarly for multiple sclerosis, pregnancy-induced amelioration of weakness and clinical disease exacerbations has been reported to be directly associated with the progressive expansion of maternal Tregs (Confavreux *et al.* 1998, Sanchez-Ramon *et al.* 2005, Iorio *et al.* 2009). In turn, paralleled disease remission also occurs for Graves' disease and autoimmune hepatitis during pregnancy (Colle & Hautekeete 1999, Buchel *et al.* 2002, Weetman 2010). Together, these findings suggest immune tolerance that expands during pregnancy is not restricted only to paternal antigens expressed by the developing fetus, but extends to maternal responsiveness to pathological 'self' antigens that cause autoimmunity as well. Moreover, these unambiguous examples of autoimmunity remission in the joints, thyroid, liver, and central nervous system highlight that although immune modifications at the fetal interface are clearly important for sustaining pregnancy, systemic alterations in immune responsiveness also become engaged during pregnancy.

With these pregnancy-induced protective benefits that clearly extend beyond fetal tolerance, it may be interesting to consider why the physiological set point for Tregs is not more consistently adjusted to the higher levels found during allogeneic pregnancy. Our recent studies have suggested susceptibility to infection offsets the protective benefits of expanded tolerance from pregnancy-induced maternal Treg accumulation (Rowe *et al.* 2011). Intriguingly, however, since resistance against most pathogens is not significantly deteriorated with pregnancy, these holes in host defense are probably limited only to those that share the common theme of intracellular survival and placental cell invasion (Robbins & Bakardjiev 2012). Perhaps the best example of this is the markedly increased susceptibility to disseminated infection with the intracellular bacterium *Listeria monocytogenes* during human pregnancy that extends to mice and other rodents (Redline & Lu 1987, 1988, Schuchat *et al.* 1991, Silver 1998, Bakardjiev *et al.* 2006, Rowe *et al.* 2011). Here, pregnancy-induced Treg expansion dictates innate susceptibility because inducing the accumulation of Foxp3⁺ CD4 T cells in non-pregnant mice to levels comparable to that found during allogeneic pregnancy results in paralleled infection susceptibility (Rowe *et al.* 2011). On the other hand, maternal Treg depletion restores resistance, but at the insufferable cost of fractured fetal tolerance and wastage. Similarly, for *Salmonella typhimurium*, representing another intracellular bacterium that causes more severe disseminated infection during pregnancy, resistance is restored with maternal Treg ablation (Pejcic-Karapetrovic *et al.* 2007, Chattopadhyay *et al.* 2010, Rowe *et al.* 2011). Thus, susceptibility to a relatively small handful of prenatal pathogens that exploit the physiological hole in host defense created by the reproductive process is probably the unfortunate byproduct of the greater good served by expanded maternal Tregs that sustain fetal tolerance (Fig. 1a).

Another interesting example is the increased susceptibility to influenza A virus during pregnancy. Pregnant women have significantly higher rates of hospitalization, morbidity, and mortality after infection with either pandemic or seasonal influenza A strains (Neuzil *et al.* 1998, Siston *et al.* 2010, Pierce *et al.* 2011). Here, the newly recommended use of inactivated vaccine formulations during pregnancy provides an exceptional opportunity to characterize how pregnancy affects systemic immune responsiveness. Consistent with the notion of systemic immune moderation during pregnancy, reductions in vaccine-induced antibody titers among pregnant women compared with non-pregnant controls with both seasonal and pandemic inactivated influenza A vaccines have been described (Schlaudecker *et al.* 2012, Bischoff *et al.* 2013). Similarly, reduced antibody responses have been found among pregnant women after administration of the live attenuated vaccine for yellow fever virus (Nasidi *et al.* 1993).

However, it is also important to highlight that despite reductions in vaccine-induced influenza A antibody titers during pregnancy, these diminished responses nevertheless provide significant protection for both the mother and infant against respiratory infections, especially in resource-limited environments where influenza A virus is highly endemic (Zaman *et al.* 2008). These protective benefits of influenza A vaccination during pregnancy for subsequent infection and possibly fetal death have also recently been confirmed in more developed settings where influenza A virus is less prevalent (Haberg *et al.* 2013). Together, these examples in which immune responsiveness to self-antigens, pathogens, and vaccination each are consistently dampened during pregnancy underscore the exceptional latent systemic immune modulatory properties that become unleashed with gestation. At face value, these findings imply systemic shifts in the balance between immune suppression that averts autoimmunity and immune stimulation required for optimal host defense against infections beyond fetal tolerance become engaged with the accumulation of maternal Tregs during pregnancy.

Along with these quantitative shifts between Tregs and non-Treg effector cells, modifications in Treg suppressive potency on a per cell basis can also occur (Sakaguchi 2003). These qualitative shifts enable Tregs to more efficiently fine-tune the delicate balance between immune activation and suppression by rapidly responding to environmental cues. This feature is probably most critical in the early stages after infection when the race between pathogen replication and immune activation has decisive impacts on the eventual outcome of infection. At the molecular level, this is consistent with drastic shifts in Treg suppressive potency induced by microbial ligands that stimulate cells through conserved pattern recognition receptors such as toll-like receptors. For example, purified lipopolysaccharide (LPS) or flagellin stimulates increased Treg suppressive potency (Caramalho *et al.* 2003, Crellin *et al.* 2005), whereas CpG oligonucleotide or the bacterial lipoprotein Pam3Cys-SK4 primes reductions in suppressive potency (Peng *et al.* 2005, Liu *et al.* 2006). Importantly, these shifts in Treg suppression after stimulation with individual microbial ligands *in vitro* parallel similar changes in suppressive potency after *in vivo* infection with intact pathogens, reflecting the cumulative response to multiple microbial ligands and the ensuing immune responses (Minigo *et al.* 2009, Johans *et al.* 2010, Ertelt *et al.* 2011).

Applied to the unique physiological situation of pregnancy, in which immune tolerance to fetal antigens must be sustained, fractured tolerance stemming from disruptions in maternal Treg suppression may explain why fetal resorption occurs after systemic LPS administration, especially in mice lacking the immune regulatory cytokine IL10 (Robertson *et al.* 2006, 2007). Here, a

Figure 1 Shifts in the balance between immune stimulation and suppression during pregnancy or following infection may dictate the immune pathogenesis of pregnancy complications after prenatal infection. (a) The expansion of immune suppressive maternal Tregs essential for sustaining fetal tolerance during pregnancy can also compromise host defense against pathogens that cause prenatal infections. (b) Infection- or inflammation-induced reductions in Treg suppression unleash the activation of immune components required for optimal protection against infections. (c) However, with maternal infection during pregnancy, reductions in Treg suppression that unleash the activation of protective immune components can also fracture fetal tolerance with ensuing immune-mediated pregnancy complications.

specific role for maternal Tregs is supported by the protective properties of purified CD4 T cells that differentiate into Tregs with LPS-induced preterm delivery (Bizargity *et al.* 2009). Interestingly, since mice lacking all T and B cells (*Rag1*^{-/-}) are more susceptible to LPS-induced preterm delivery (Bizargity *et al.* 2009), inflammation-induced activation of innate immune components probably also contributes to pregnancy complications, especially in the absence of Tregs. The extension of Treg suppression in this context is consistent with the increasingly appreciated role that Foxp3⁺ cells play in moderating the activation of more prototypical innate immune cells such as neutrophils and natural killer cells (Murphy *et al.* 2005, D'Alessio *et al.* 2009, Gasteiger *et al.* 2013a, 2013b). Thus, dampening maternal Treg suppression that unleashes the activation of innate or adaptive immune response pathways for optimal protection against infections has the potential to unravel the fine-tuned shifted balance between immune suppression and stimulation that

maintains fetal tolerance during pregnancy. In this regard, infection-induced disruption of maternal Treg suppression that fractures fetal tolerance may represent an underappreciated, but perhaps more unifying, pathway to explain the fundamental biology whereby pregnancy complications occur with clinically apparent or asymptomatic infections.

Infection-induced shifts in Treg suppression and pregnancy complications

Although the cause and effect relationships between maternal infection and unfortunate complications in human pregnancy including spontaneous abortion, stillbirth, and preterm labor have been described (Andrews *et al.* 2000, Goldenberg *et al.* 2008, McClure *et al.* 2010), establishing the mechanistic basis whereby maternal infection triggers these complications has lagged behind. One important limitation has been the lack of representative animal models to specifically

investigate pregnancy outcomes after infection. In particular, while pregnancy outcomes have been extensively characterized using rodent infection models, the discordance in pregnancy kinetics and immune cell development between rodents and humans limits the translational relevance of these findings, especially for complications related to birth timing (Mold & McCune 2012, Bezold *et al.* 2013). These limitations are somewhat bypassed in larger mammals including elegant descriptions of pregnancy in horses, sheep, and non-human primates (Jobe 2005, Barry *et al.* 2006, Noronha & Antczak 2010, Antczak 2012). However, the significantly more prolonged gestation time, relative lack of immunological tools and defined inbred strains, differences in placental architecture, and exponentially higher experimental costs impose other restrictions that are perhaps even more formidable. Furthermore, while human gestational cells and tissues have become more widely used to characterize the pathogenesis of prenatal infections (Robbins *et al.* 2010, Zeldovich *et al.* 2011, Robbins *et al.* 2012), these *in vitro* models recapitulate neither the dynamic crosstalk between maternal and fetal cells nor the immune response to infections that probably play critical roles in the pathogenesis of pregnancy complications. Therefore, we propose combining the most salient aspects of individual models may represent the most efficient way to uncover the fundamental biology whereby pathogens cause prenatal infection and pregnancy complications.

The additive and potential synergistic value is illustrated by recent complementary studies describing infection and pregnancy outcomes using the intracellular bacterium *L. monocytogenes*. Using this bacterium to investigate the underlying pathogenesis of prenatal infections is clearly important, given the ubiquitous presence of this pathogen in our food supply, colonization within the gastrointestinal tract, propensity for disseminated infection during pregnancy, and alarming rate of morbidity and mortality associated with human prenatal infection (Gellin & Broome 1989, MacGowan *et al.* 1991, Southwick & Purich 1996, Iida *et al.* 1998, Silver 1998, Mylonakis *et al.* 2002). A somewhat surprising degree of resistance for placental cells to *L. monocytogenes* infection has been described using human organ cultures (Abrahams *et al.* 2006, Koga & Mor 2008, Robbins & Bakardjiev 2012). In particular, human syncytiotrophoblasts that line the placental surface where nutrient and gas exchange occurs with direct exposure to maternal blood are highly resistant to bacterial invasion and intercellular spread (Robbins *et al.* 2010). On the other hand, placental invasion primarily occurs through a substantially smaller subset of extravillous trophoblast cells that anchor the placenta in the uterine lining. However, even after invasion into extravillous trophoblast cells, profound defects in *L. monocytogenes* escape from the endocytic vacuole and intracellular replication remain (Robbins *et al.*

2012). Interestingly, these protective properties of trophoblasts are not limited to only *L. monocytogenes*, but have also been shown for a variety of other bacterial, parasitic, and viral pathogens (Abrahams *et al.* 2006, Koga & Mor 2008, Zeldovich *et al.* 2011, Robbins & Bakardjiev 2012). Thus, placental cells provide a protective barrier to fetal infections, at least *in vitro* without the additional constraints imposed by the ensuing inflammatory response and maternal–fetal tolerance.

In light of these innate cellular barriers to infections, it is interesting to reconsider the basic physiology that causes susceptibility to disseminated infections during pregnancy and the underlying mechanism whereby maternal infection triggers pregnancy complications. With regard to maternal susceptibility to disseminated infections, the prior dogma that placental and fetal tissues represent expanded target tissues susceptible to invasion seems less likely, given the finding that placental cells are actually very resistant to infections (Abrahams *et al.* 2006, Koga & Mor 2008, Robbins & Bakardjiev 2012). Similarly, the notion of pregnancy induced dampening of CD4 helper type 1 (Th1) responses required for protection against intracellular pathogens is questionable, given the unimpeded innate and early adaptive immune responses in MHC class II CD4 T-cell-deficient mice (Sun & Bevan 2003, Barber *et al.* 2005). Instead, given the necessity for maintaining fetal tolerance through sustained expansion of immune suppressive maternal Tregs, we propose active suppression by these cells may also play critical roles in causing maternal susceptibility to disseminated infection (Fig. 1a). This notion is supported by the aforementioned epidemiological and experimental data showing increased susceptibility to systemic infections during pregnancy in humans and mice, and non-pregnant mice with expanded Tregs, whereas Treg ablation restores resistance for each group (Redline & Lu 1987, 1988, Schuchat *et al.* 1991, Silver 1998, Rowe *et al.* 2011). Interestingly, however, since immunity against most infections is preserved during pregnancy, these do not appear to be drastic defects in global host defense and are instead more isolated holes that become exploited by pathogens with an established predisposition for infections during pregnancy. Furthermore, given the striking consistency in pathogens that cause prenatal infections in humans and other mammalian species (Givens & Marley 2008, Robbins & Bakardjiev 2012), these host defense defects associated with the reproductive process are apparently widely conserved.

Perhaps, more intriguing are the recent findings using allogeneic pregnancy in mice to investigate the pathogenesis of pregnancy complications triggered by disseminated maternal infection. Here, related studies using *L. monocytogenes* as a model to dissect the basic immunology whereby protective T cells are primed after *in vivo* infection need to be carefully considered in

parallel. Unlike the necessity for distinct cell-intrinsic stimulation signals including the T-cell receptor (signal 1), co-stimulation (signal 2), and inflammatory cytokines (signal 3) for T-cell activation shown using elegantly simplistic *in vitro* models (Curtsinger *et al.* 2005, Curtsinger & Mescher 2010), CD8 T cells responsive to heterologous antigens expressed by recombinant *L. monocytogenes* expand and become activated even when all known inflammatory cytokine third signals have been eliminated (Way *et al.* 2007, Orgun *et al.* 2008, Ertelt *et al.* 2010). Instead, transient reductions in Treg suppression that unleash the activation of protective immune effector cells following infection probably circumvent the need for some, but not all, more classical T-cell-intrinsic activation signals (Ertelt *et al.* 2011, 2013). The importance of overriding Treg suppression for immune activation parallels the robust expansion of protective CD8 effector T cells in mice transiently ablated of Tregs after stimulation with purified peptides (Ertelt *et al.* 2011). Thus, infection- or inflammation-induced reductions in Treg suppression may represent a more fundamental *signal zero* for stimulating the activation of protective T effector cells (Rowe *et al.* 2012a; Fig. 1b).

Applied to pregnancy when the sustained expansion of maternal Tregs is essential for maintaining fetal tolerance, infection- or inflammation-induced reductions in Treg suppression have the critical potential to fracture fetal tolerance with ensuing pregnancy complications (Fig. 1c). This has been most definitively shown with a highly informative mating strategy using transgenic male mice that ubiquitously express defined model antigens to establish allogeneic pregnancy with non-transgenic female mice that transform model antigens into surrogate fetal antigens (Erlebacher *et al.* 2007, Moldenhauer *et al.* 2009, Taglauer *et al.* 2009). After *L. monocytogenes* infection, the normally blunted accumulation of maternal T effector cells with fetal specificity is overturned (Rowe *et al.* 2012b). In turn, the robust expansion and activation of maternal T effector cells with fetal specificity with maternal infection recapitulate the quantitative accumulation and qualitative activation of these cells with maternal Treg ablation during pregnancy (Rowe *et al.* 2011). Intriguingly, although fetal resorption with a reciprocal loss of live pups occurs in a dose-dependent fashion that parallels infection-induced reductions in maternal Treg suppression, bacteria could not be recovered from the majority of resorbed placental–fetal units after infection with low or intermediate dosages of virulent *L. monocytogenes* (Rowe *et al.* 2012b). These more recent findings suggesting a threshold inoculum is required for establishing sustained systemic infection are consistent with prior studies illustrating dose-dependent rates of placental invasion after i.v. *L. monocytogenes* inoculation (Redline & Lu 1987). Here, it is important to highlight that even attenuated

L. monocytogenes that cannot invade the placental–fetal unit due to targeted defects in *ActA* required for intercellular spread can also induce fetal wastage with disruptions in fetal tolerance and loss of live pups (Bakardjiev *et al.* 2005, Le Monnier *et al.* 2007, Rowe *et al.* 2012b). Thus, *L. monocytogenes* infection-induced pregnancy complications do not require *in utero* pathogen invasion and may instead be due to reductions in maternal Treg suppression with ensuing disruptions in fetal tolerance (Fig. 2a).

By showing that pregnancy complications can occur without direct bacterial invasion of the placental–fetal unit, the high frequency of pregnancy complications associated with *L. monocytogenes* infection during human pregnancy despite placental cell resistance to direct bacterial invasion can be potentially reconciled (Gellin & Broome 1989, Silver 1998, Mylonakis *et al.* 2002, Robbins & Bakardjiev 2012). On the other hand, since a majority of newborn infants are infected when born to mothers with disseminated infection (Mylonakis *et al.* 2002), there are also presumably ways to bypass the protective placental barrier with infection *in vivo*. We hypothesize inflammation at the maternal–placental interface induced by disrupted fetal tolerance may provide a more direct conduit drawing circulating bacteria into otherwise resistant placental cells. Thereafter, the previously described local immune suppression at the maternal–placental interface and high bacterial concentrations within the infected placenta probably provide a repository for continuous re-infection (Redline & Lu 1987, 1988, Bakardjiev *et al.* 2006). Based on these findings with perinatal *L. monocytogenes* infection, we propose a model whereby i) the expansion of immune suppressive maternal Tregs required for maintaining fetal tolerance compromises innate protection against disseminated infection, ii) the normally protective blunting of Treg suppression that unleashes the activation of protective immune components to eradicate infection disrupts fetal tolerance with fetal wastage caused by attack from maternal immune cells with infection during pregnancy, and iii) inflammation at the maternal–placental interface with fractured fetal tolerance draws circulating pathogens through the normally protected placental cell barrier promoting fetal invasion (Fig. 2b).

The more general applicability of this model now requires analogous studies with other pathogens that cause prenatal infections and pregnancy complications. In this regard, some clues for pathogens known to cause more severe systemic infections during pregnancy are already in place. For example, *S. typhimurium* infection during pregnancy triggers catastrophic fetal wastage with exaggerated immune responses within the placenta and approximately twofold reductions in Treg suppressive potency, especially in the later stages of persistent infection (Pejcic-Karapetrovic *et al.* 2007, Chattopadhyay *et al.* 2010, Johanns *et al.* 2010). Similarly, quantitative

Figure 2 Proposed model for how pregnancy complications may occur with or without direct pathogen invasion of the placental–fetal unit. Infection-induced reductions in maternal Treg suppression that unleash the activation of protective immune components fracture fetal tolerance unmasking normally tolerized fetal antigens. In turn, inflammation occurs at the maternal–fetal interface containing the highest concentration of exposed fetal cells. With low-dosage infection (a), the circulating pathogen is eradicated, but pregnancy complications ensue from damage caused by the activated maternal immune effector cells. With high-dosage infection (b), the persistence of circulating pathogen and inflammation at the maternal–fetal interface provides a conduit for invasion overriding the normally protective placental barrier.

reductions in peripheral Treg levels have been described in early stages after systemic murine cytomegalovirus and intestinal *Toxoplasma gondii* infections (Li *et al.* 2008, Oldenhove *et al.* 2009). If similar reductions in maternal Treg suppression were to occur with infection during pregnancy, disruptions in fetal tolerance with ensuing immune-mediated fetal wastage would be predicted.

It is also important to point out a potentially interesting exception for *Plasmodium* infection that results in placental localization and robust inflammatory changes leading to intrauterine fetal demise (Poovassery & Moore 2009, Poovassery *et al.* 2009). Unlike most other acute infections in which Treg suppression is reduced, increased proportions of activated Tregs with higher suppressive potency are found after *Plasmodium* infection, and these shifts are likely to minimize inflammatory sequelae at the expense of parasite replication (Walther *et al.* 2005, Minigo *et al.* 2009). Therefore, for this infection, other molecules such as chondroitin sulfate providing placental adhesion may provide a more direct conduit for fetal invasion without the need for overturning maternal Treg suppression (Fried & Duffy 1996, Duffy & Fried 2003). Nevertheless, given the necessity for sustained maternal Treg expansion in fetal tolerance, infection-induced qualitative or quantitative shifts in Treg suppression highlight a potentially more unifying pathway whereby maternal infection may trigger pregnancy complications.

Summary

Reproduction and averting infections are arguably the two most dominant fundamental driving forces in nature. Adaptations that simultaneously improve both reproductive fitness and host defense are most ideal and should be strongly enriched for through positive selection. On the other hand, adaptations that favor only one must be counterbalanced by concurrent adaptations that promote or at least sustain the other. With regard to host defense against infections, diversity in immune-recognition MHC molecules between individuals is clearly advantageous for species survival. However, in placental mammals that also require the benefits of more prolonged *in utero* protection for the fetus, the same antigenic diversity beneficial for host defense is potentially detrimental for reproductive fitness, unless foreign antigens associated with reproduction can be discriminated against those that cause infections. Fortunately, humans and other mammalian species are endowed with many such discriminatory mechanisms that act at the maternal–fetal interface and act more systemically to ensure protection for the developing fetus. Using animal pregnancy models and investigating complications in human pregnancy, we have uncovered some, but certainly not all, the key players in this meticulously orchestrated process.

Based on what we currently know, an elaborate plethora of molecular and cellular changes occurs at the maternal–placental interface to silence local

activation of potentially detrimental to maternal immune cells. However, these local modifications alone are insufficient as the systemic expansion of immune suppressive maternal regulatory CD4 T cells is also essential. Although Tregs have been shown to impair optimal host defense against a wide variety of potential human pathogens in non-pregnancy models, the expansion of these cells during pregnancy does not lead to increased susceptibility to most. In fact, the detrimental impacts on host defense for expanded maternal Tregs during pregnancy across mammalian species seem to be limited to a remarkably small handful of microbes that share the common features of residence within infected cells and placental colonization. Thus, prenatal pathogens exploit a naturally occurring hole in host defense created by the reproductive process.

As biologists, we can marvel at this apparent intricate regulation that allows protection against the vast array of potential human pathogens and reproduction to occur efficiently enough as we exponentially approach a population of 7 billion individuals. However, as pediatricians and parents, our struggle is to uncover ways to optimize the outcomes of every last pregnancy. It is through the latter perspective that we have focused on why pregnancy leads to susceptibility and the pathogenesis of pregnancy complications after infection with the prototypical prenatal pathogen *L. monocytogenes*. The most critical data to support increased infection susceptibility during pregnancy and ensuing pregnancy complications associated with maternal Tregs have been discussed. Based on these results, we propose critically important next steps are to investigate whether other microbes that cause prenatal infections either systemically or locally by ascending through the birth canal utilize similar pathogenesis pathways. If so, therapeutic strategies that focus on moderating the immune response to infections, as well as pathogen eradication, may help improve the outcomes of pregnancy. Furthermore, given the remarkable heterogeneity among Tregs in terms of both antigen specificity and cell-intrinsic molecules utilized for suppression, establishing and reinforcing the protective maternal Treg features that sustain pregnancy and dissociating them from others that compromise host defense also represent areas with critical implications for improving maternal and infant health.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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