The Role of Pregnancy-Specific Glycoprotein 1a (PSG1a) in Regulating the Innate and Adaptive Immune Response

Fernando F. Martinez, Laura Cervi, Carolina P. Knubel, Graciela M. Panzetta-Dutari, Claudia C. Motran

Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina

Keywords
Dendritic cells, macrophages, Th1/Th2 cells, Th17 cells, Tolerance, Treg cells

Correspondence
Claudia C. Motrán, Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba 5000, Argentina.
E-mail: cmotran@fcq.unc.edu.ar

Submission January 7, 2013;
accepted January 10, 2013.

Citation
Martinez FF, Cervi L, Knubel CP, Panzetta-Dutari GM, Motran CC. The role of pregnancy-specific glycoprotein 1a (PSG1a) in regulating the innate and adaptive immune response. Am J Reprod Immunol 2013; 69: 383–394

doi:10.1111/aji.12089

Among several explanations for the acceptance of the fetus, the one that suggests that the maternal immune system is suppressed or modified has been the subject of many studies. Thus, it has been proposed that the cells of innate immune system might be able to distinguish the pregnant from the non-pregnant state producing a signal, the so-called signal P. We have previously proposed that pregnancy-specific glycoprotein 1a (PSG1a), a representative member of the main glycoprotein family secreted by placental trophoblast, may modulate the activation of antigen-presenting cells promoting the T-cell shift of the maternal cell immunity toward a less harmful phenotype. In this review, we summarize current knowledge concerning the contribution of pregnancy-specific glycoprotein 1a (PSG1a) to modulate the maternal innate and adaptive immune response in order to assure a successful pregnancy.

Introduction

The effective defense against infections requires the ability to mount an appropriate and controlled specific immune response able to eradicate the invading pathogen while limiting the collateral damage to self tissues that can result from an exacerbated immune response. The development of an effective type of immune response to eradicate the infection depends primarily on the type of pathogen, and therefore, the pathogen is the main factor skewing the adaptive immune response in a particular direction.[1] The key players that transmit this information are antigen-presenting cells (APCs) such as macrophages (MΦ) and dendritic cells (DCs) that through the innate immune receptors sense the pathogen-associated molecular patterns (PAMPs) present in the microbes and produce surface and secreted proteins that are required together with the antigen to activate naïve T lymphocytes to proliferate and differentiate into effector cells.[2] In addition to the PAMPs that mediate MΦ and DC activation, tissue-derived environmental factors can influence these cells activation and thereby skew the type of adaptive immune response that is initiated by APCs. Depending on the signals transmitted by activated APCs, naïve $CD4^+$ T cells can differentiate into different types of Th cells, which can be distinguished by their cytokine secretion pattern. Th1 cells produce interferon IFN-γ and TNF and are involved in cellular immunity, while Th2 cells, involved in humoral immunity, are characterized by the production of interleukin (IL)-4, IL-5, and IL-10.[3] More

recently, a subset of T cells that produce the pro-inflammatory cytokine IL-17 and plays a critical role in the pathogenesis of autoimmune diseases and rejection have been identified.[4–6] In addition, Th17 cells participate in the host defense against bacteria, fungi, and viruses.[7] The function of effector T cells (Th1, Th2, and Th17 cells) is regulated by $CD4^+$ $CD25^+$ regulatory T (Treg) cells, an heterogeneous population important for the maintenance of peripheral tolerance that have the capacity to suppress the T-cell response through the production of immunoregulatory cytokines such as transforming growth factor (TGF)-β and IL-10 or by cell-to-cell interaction.[8,9]

The key to a healthy immune system is its remarkable ability to distinguish between the body's own cells, recognized as 'self', and foreign cells, or 'non-self'. Consequently, mammalian pregnancy constitutes a distinctive situation in which sophisticated immunoregulatory mechanisms are required to provide tolerance toward a genetically foreign conceptus, while at the same time allowing effective immunity to protect the mother from infections.[10] Among several explanations for the acceptance of the fetus, the one that suggests that the maternal immune system is suppressed or modified has been the subject of many studies. In agreement, several reports have demonstrated that during the normal pregnancy, a state of systemic suppression of the maternal immune system seems to be present.[11–13] However, except for certain pathogens with defined predilection for prenatal infection, in general pregnant women are not significantly more susceptible to infections than non-pregnant.[10,14,15]

Some years ago, Wegmann et al.[16] proposed that normal pregnancy is characterized by a lack of strong maternal, cell-mediated, antifetal immunity (Th1-type reactivity) and that instead, a dominant humoral immune response is prevalent (Th2-type reactivity). In agreement, several reports led to define the pregnancy as a Th2 or anti-inflammatory condition in which a poor pregnancy outcome may be associated with an increase in Th1-type response and a concomitant decrease in Th2-type response. Accordingly, predominant Th1-type immunity has been observed in recurrent spontaneous abortion and in pre-eclampsia.[17–19] However, predominant Th2-type immunity is also reported in recurrent pregnancy loss and, in humans, augmented Th1-type immunity or suppressed Th1-type immunity in the endometrium is observed in repeated implantation failure.[20–23] Therefore, an adequate balance for Th1/Th2 immunity with slight shift to Th2-type immunity may be suitable for the maintenance of pregnancy.

Recently, the Th1/Th2 paradigm in pregnancy has been redefined to include Th17 and Treg cells in view of the facts that these two populations are present in the decidua. Th17 response is known to play a pathogenic role in autoimmune diseases and in tissue rejection and thus may be harmful to the maintenance of pregnancy.[5,6] Th17 cells and the expression of Th17-related factors (IL-17, IL-23, and RORγc) have been found to be increased in deciduas from unexplained inevitable recurrent spontaneous abortion patients; however, because Th17 increase was not observed in the decidua of abortion cases that did not show vaginal bleeding or cervical deletion, it is unclear whether the increased Th17 cells are a primary cause of merely a downstream consequence of fetal loss.[24,25] On the other hand, Saito et al.[26] have reported that the frequency of Th17 in the decidua is significantly higher compared to that in peripheral blood and they suggest that decidual Th17 cells might be playing a role in the protective immune response against extracellular microbes, considering that the uterine cavity is not completely sterile.

Inflammation is necessary for successful implantation, but exacerbated inflammation is harmful to the pregnancy. Treg cells resident in decidua, peripheral blood, and lymph nodes are increased in mice and pregnant women, and there are evidences that fetus-specific Treg cells migrate from peripheral blood to the decidua in human pregnancy.[27–31] In addition, antibody-mediated depletion of $CD25^+$ cells on the day or 2.5 days after mating of allogeneic, but not syngeneic, mice results in severe impairment of implantation.[30] These results suggest that Treg cells are essential for inducing immunological tolerance, and therefore, it can be speculated that decreased Treg cells might induce implantation failure, resulting in unexplained infertility or miscarriage. In agreement, it was observed that infertility, spontaneous abortion, pre-eclampsia, and recurrent spontaneous miscarriage cases are associated with decreased number of peripheral and decidual Treg cells and reduced immunosuppressive activity of Treg.[32–36] Also, it was recently demonstrated that during allogeneic pregnancy, the extrathymic generation of inducible or peripheral Treg (iTreg or pTreg) cells and their accumulation in the placenta play an important

role by preventing embryo resorption and associated defective spiral artery remodeling.[37] Recent data show the reciprocal development pathways between Th17/Th1 subset and Th17/Treg subsets.[38] Thus, it is possible that placental cytokine milieu in addition to hormones and placental-derived factors be responsible for the development and maintenance of a non-pathogenic T-cell response compatible with a successful pregnancy.[36,39–43]

Some years ago, Sacks et al.[44] proposed that soluble placental products released directly into the maternal circulation can generate specific pregnancy signals through interaction with the innate immune system. Thus, the innate immunity might be able to distinguish the pregnant from the non-pregnant state producing a signal, the so-called signal P, that modulates the lymphocyte response to alloantigen stimulation. In line with this hypothesis, several soluble factors and cells of placental origin have been demonstrated to be important to modulate the mothers' T-cell response and thereby would contribute to marshaling the maternal immune system to support pregnancy.[40,43,45–47] In this review, we summarize current knowledge concerning the contribution of pregnancy-specific glycoprotein 1a (PSG1a), a representative member of the main glycoprotein family secreted by placental trophoblast, to modulate the maternal innate and adaptive immune response in order to assure a successful pregnancy.

Pregnancy-specific glycoproteins

PSG1a belongs to the human PSG family, which is part of the immunoglobulin gene superfamily. PSGs are glycoproteins mainly synthesized by the placenta. Mature PSG proteins have molecular weights of 72, 64, and 54 kDa with about 30% of carbohydrates incorporated by N-glycosylation.[48] They represent early biochemical marker of syncytiotrophoblast formation and are the major group of secreted proteins found in maternal serum at the end of normal gestation.[49,50]

The clinical relevance of this group of proteins has been suggested many years ago. Indeed, spontaneous abortion, pre-eclampsia, intrauterine growth retardation, small-for-gestational-age fetuses, and preterm delivery have been linked to low PSG levels in maternal circulation.[51–54]

PSG genes have been identified in non-human primates, rats, and mice; however, no unequivocal orthologous genes can be assigned.[55] In the human genome, there are 11 PSG genes with about 90% nucleotide sequence identity clustered within 700 kilobases on chromosome 19q13.2.[56] All PSG family members possess a similar gene organization. They code for a leader peptide (L) and an N-terminal domain homologous to the immunoglobulin variable domain. Depending on the gene, this domain is followed by up to three immunoglobulin C2-like domains (A and B) and a short variable carboxy-terminal tail (C). Alternative splicing may lead to different PSG transcript (Fig. 1). PSG1a, also named PSG1-4C1 splice variant, has the most frequent (LN-A1-A2-B2-C) arrangement with a C1-terminal tail.[57] PSG transcripts isolated from human placenta cDNA libraries support that all PSG genes are expressed. In addition, transcript-specific PCR analysis suggests that the whole PSG locus is activated in cytotrophoblast that differentiates into the syncytium pathway, although reaching different mRNA abundance levels.[49] It has been reported that in humans, PSGs are produced by the syncytiotrophoblast starting as early as about the time of implantation.[58] Their biosynthesis is regulated mainly at the transcriptional level and markedly increased during syncytiotrophoblast differentiation.[49,59]

Although their functions have not been fully established, several lines of evidence strongly suggest they are essential for the maintenance of a normal pregnancy. A few years after their identification, they were suspected of having a crucial role in modulating the maternal immune system although little is currently known about whether all members of this family of proteins have the same function and bind to the same receptor.[60,61] Thus, several authors reported that placental-derived PSG proteins, which contained a mixture of the PSG family members, suppress mixed lymphocyte reaction and T-cell activation by mitogens.[62,63] In contrast, Arnold et al.[51] found no effects on T-cell proliferation using recombinant human PSG1 or PSG11 molecules obtained in insect cells (which produce N-glycosylated proteins that may not be identical to those obtained using mammalian cells).[64] However, PSG protein participation in immune modulation and T-cell function is reinforced by the fact that high PSG levels are correlated with improved symptoms of rheumatoid arthritis and multiple sclerosis.[65,66]

Years ago, it was reported that a peptide derived from human PSG 11 binds to a human monocyte receptor and to the cell surface of promonocyte lineage, but not to T or B cells.[67] In that report, it was

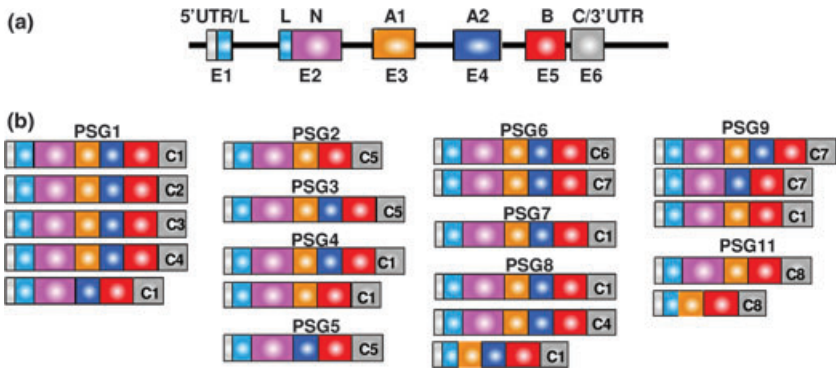

Fig. 1 Schematic representation of pregnancy-specific glycoprotein genes and transcripts. (a) Exon arrangements represented by boxes shaded: cyan for leader (L) peptides; pink for N-terminal IgV-like domain (N); orange for IgC-like domain, A1 subset (A1); blue for IgC-like domain, A2 subset (A2); red for IgC-like domain, B subset (B); gray for hydrophilic tail, carboxy-terminal domain (C). (b) Known transcripts for each gene.

demonstrated that the RGD motif present in the N-terminal domain of PSG11 is important for the binding of purified placental PSG to receptors present on activated human mononuclear cells.[67] More recently, it has been described that murine PSG17 and 19 bind to the tetraspanin CD9 on murine Mo and that the addition of carbohydrates is essential for PSG–receptor interaction.[68,69] In contrast, human PSG1 and murine PSG22 and 23 did not bind to human or murine CD9 and bind to cell surface proteoglycans, while murine PSG17 is able to bind both proteoglycans and CD9.[68,70,71] Together, the results indicate that some members of PSG family have receptors on professional APCs and would have the ability to modulate the activity and function of these cells as will be described below.

Recently, another functions beyond to modulate the maternal immune system have been reported for these proteins. For instance, murine PSG22 and PSG23 are involved in placental angiogenesis because of their capacity to induce the secretion of TGF-β, an anti-inflammatory cytokine that also works as pro-angiogenic factor, in addition to induce VEGF by MΦ, DC, and endothelial cells.[72–74] In addition, we have observed that human PSG1a promotes IL-6, TGF-β, and VEGF secretion by JEG-3 cells (CC Motran and FF Martinez, unpublished data) and Dveksler's group has recently demonstrated that PSG1 induces endothelial tubulogenesis through interaction with cell surface proteoglycans.[70] Therefore, together these reports indicate that members of human and rodent PSG families have conserved functions that include their ability to modulate the immune system and to participate in the trophoblast-mediated vascular remodeling, both conditions that contribute to the establishment of a successful pregnancy.

In order to investigate the role of human PSG1a in the modulation of the immune system, we have used recombinant PSG1a obtained in mammalian cells using a vaccinia (Vac)-based expression vector harboring the complete open reading frame (ORF) of PSG1a cDNA. Thus, like the placental PSG, the recombinant molecule is synthesized and processed by human or mouse cells in culture to be secreted as a glycoprotein containing roughly 30% of its molecular mass of carbohydrates as was already reported.[48,75,76] In addition, recombinant PSG1a interacted with the Con A lectin, and the binding was inhibited by mannose analogs (α-methyl d-mannoside), a feature that is shared with the placental PSG.[59,76]

Effect of *in vivo* expression of PSG1a on the adaptive T-cell response

Some years ago, we have reported that *in vivo* expression of PSG1a before immunization with OVA in complete Freund's adjuvant favors the Th2-type response against OVA.[77] In this regard, it is important to remark the major role of circulating PSG1a in the decision process to mount a Th2-type immune response, because both adjuvants, CFA and vaccinia virus, used in that experimental system are strong Th1-type response inductors.[78,79]

Recently, in order to investigate whether *in vivo* circulating PSG1a is able to modulate the Ag-specific T-cell response during an infection, the infection with *Listeria monocytogenes*, a facultative intracellular bacterium able to induce a strong protective Th1 response, was used as a model.[80] DO11.10 transgenic mice that were injected with a Vac-PSG1a were infected with *L. monocytogenes* expressing soluble OVA (*rLm-OVA*) to allow the

detection of Ag-specific T-cell responses. In addition, to determine whether the PSG1a-induced T-cell response compromises the protective immunity to *L. monocytogenes* infection, mice that had been injected with Vac-PSG1a were primed with *rLm-OVA* and then challenged with higher *Lm-OVA* doses.

We observed that the infection with *rLm-OVA* in the context of circulating PSG1a (*in vivo* expression of PSG1a) promotes the expansion of Ag-specific IL-17-, IL-4-, and IL-10-secreting cells that are as able as the known effective Th1 response to protect against *L. monocytogenes* infection.[81] Interestingly, although it has been clearly demonstrated that during the acute phase of *L. monocytogenes* infection occurs the priming and expansion of Ag-specific effector T cells without changes in the number of Ag-specific Treg cells,[82] *in vivo* expression of PSG1a induced a similar growth of OVA-specific T cells, but concomitantly significantly expanded the percentage and absolute number of OVA-specific $Foxp3^+$ $CD25^+$ Treg cells.[81]

In those experiments, the Ag-specific $CD4^+$ $IL\text{-}17^+$ cells are in spleen from Vac-PSG1a-treated mice that were infected with *L. monocytogenes*. However, it is possible that during pregnancy, the Th17-mediated response might be restricted to the decidua, as suggested by the report showing that decidual formation prevents the DC stationed at the maternal/fetal interface from migrating to the lymphatic vessels of the uterus, and the fact that the frequency of Th17 cells in $CD4^+$ T cells during all stages of pregnancy period is similar to that in non-pregnant women while being significantly higher in the decidua compared to that in peripheral blood.[26,83] The recent reports showing that IL-17 increases progesterone secretion by the JEG-3 human choriocarcinoma cell line and induces the invasive capacity of these cells, together with those showing the ability of IL-17 to promote angiogenesis and tumor growth, suggest a role of IL-17 in the decidualization process.[84–86] Accordingly, we have observed that human IL-17 promotes VEGF secretion by JEG-3 cells through IL-6 signaling pathway (CC Motran and FF Martinez, unpublished data). Therefore, a population of Th17 cells in the decidua, tightly regulated by the presence of Treg cells and a milieu of cytokines containing IL-10, TGF-β, and/or IL-6 (that abrogate the Th17 pathogenic phenotype),[87,88] but not IL-23, would have a beneficial rather than detrimental role during pregnancy.

PSGs modulate the immune system through their interaction with professional antigen-presenting cells

During the last years, several groups have studied the effects of some variants of PSGs on immune system cells. To this end, placental-derived PSG, which contained a mixture of the PSG family members and recombinant PSGs expressed in insect or mammalian cells, were used obtaining similar results: PSGs are able to induce the secretion of immunomodulatory products by different types of cells such as monocytes, MΦ, DCs, endothelial cells, and trophoblast-derived cells.[51,69,72,77,81,89–91] In addition, it was observed that murine PSGs mimic the biological effects of human PSGs inducing cytokine expression in human MΦ, and human PSGs induce the secretion of anti-inflammatory cytokines by human and murine MΦ.[90,91] In contrast, none of the groups have reported direct effect of any of these glycoproteins on T or B cells, suggesting that PSGs might modulate the adaptive immune response through its interaction with cells other than T or B cells.[67,75]

PSG1a modulates the metabolism and cytokine secretion by monocyte/macrophage (MΦ) populations

MΦ populations, in addition to having a role in innate immunity, participate as effector cells in adaptive immune responses. Two different subsets of MΦ had been described. Classically activated MΦ occur in a type I cytokine environment (IFN-γ and TNF) and are inhibited by type II cytokines (IL-4, IL-10, and IL-13). They have cytotoxic and antimicrobial function, mainly based on their ability to secrete nitric oxide (NO).[92] In contrast, anti-inflammatory agents, such as IL-4, IL-10, IL-13, TGF-β, and glucocorticoids or Treg cells, induce alternative activation in MΦ, resulting in increased arginase activity (that competes with inducible NO synthase (iNOS) for its substrate, L-arginine) and enhanced expression of innate immunity receptors with broad specificity for foreign antigens (e.g., MΦ mannose receptor, scavenger receptor, and CD163).[92–94] IL-4/IL-13- and IL-10-induced MΦ are called alternatively activated MΦ (AAM), or more precisely wound-healing and regulatory MΦ, respectively.[95,96]

AAM secrete anti-inflammatory molecules such as IL-10 and TGF-β and seem to be the first defense line cell that is not dependent on a strong Th1-mediated

immune response to perform their function. In healthy organisms, these AAM are preferentially found in normal placenta and lung.[97] In addition, it has been proposed that AAM are able to induce differentiation of naïve T cells into antigen-specific Th2 cells.[98,99] Moreover, although the contribution of Th17 cell-associated cytokines to MΦ biology is unclear, AAM have been recently associated with increased percentages of IL-17-producing cells in patients with sepsis, and the mannose receptor has been shown to be crucial for the induction of IL-17 by *Candida albicans*.[100,101]

The cytokine milieu generated during pregnancy suggests the presence of AAM, whereas the presence of Th1 cytokines and classically activated MΦ is frequently associated with fetal loss.[17] Accordingly, we demonstrated that human PSG1a induces alternative activation in human peripheral blood monocytes and in human and murine monocyte cell lines because it up-regulates the arginase activity and inhibits the NO production in monocytes activated by lipopolysaccharides (LPS).[75] Moreover, *in vivo* expression of PSG1a by the treatment of mice with Vac-PSG1a induces alternative activation of spleen (SpMΦ) and peritoneal MΦ (PMΦ).[77]

It has been reported that human (PSG1, PSG6, the N-terminal domain of PSG6, PSG11, and its N-terminal domain containing the RGD peptide) as well as murine PSGs (PSG17 and PSG18) induce the secretion of IL-10, IL-6, and TGF-β1 in MΦ.[51,89–91] In addition, *in vivo* expression of PSG1a induces PMΦ that produce, spontaneously in culture or after stimulus with LPS, high levels of TGF-β.[77] In addition, both PMΦ and SpMΦ from Vac-PSG1a-treated mice produce higher levels of IL-10 after LPS activation.[77] Together, these findings agree with the ability of different PSGs to induce an alternative phenotype in MΦ characterized by increased arginase activity and the ability to secrete the anti-inflammatory cytokines IL-10 and TGF-β. In addition, our results showing that PSG1a induces iNOS down-regulation and arginase up-regulation in monocytes stimulated by LPS suggest that the presence of PSG1a at the site of MΦ activation could induce a MΦ metabolic pathway deviation toward the alternatively activated one with the consequent enhancement of its capacity for antigen presentation.[92]

PSG1a is able to modulate the maternal T-cell immune response, acting as an important accessory cell–dependent T-cell suppressor factor because of its ability to inhibit the anti-CD3- or PHA-induced proliferation of human peripheral blood mononuclear cells.[75] In our experimental model, PMΦ from Vac-PSG1a-treated mice can produce PSG1a and profoundly inhibit the mitogen-induced proliferation of naïve T cells promoting the secretion of Th2-type cytokines (IL-5 and IL-4) and inhibiting the production of IL-2 and IFN-γ by cell-to-cell contact.[77] In contrast, SpMΦ from Vac-PSG1a-treated mice that do not produce PSG1a and are possibly targeted by circulating recPSG1a inhibit the mitogen-induced proliferation of naive T cells and induce Th2-type cytokine secretion by an IL-10-dependent mechanism.[77] Alternatively, activated PMΦ with similar functions have been reported in chronic nematode infections, where they appear to play a role in inducing Th2 and Treg differentiation.[102,103]

The fact that PSG1a inhibits the T-cell proliferative response in accessory cell–dependent T-cell proliferation assays (as allo-mixed lymphocyte reactions, PHA-, and anti-CD3 stimulation) while highly purified T cells keep their ability to respond to MΦ-independent stimulus (such as anti-CD3 plus anti-CD28) suggests that PSG1a has not a direct effect on T lymphocytes.[75]

PSG1a targets DCs to differentiate into a subset with a unique phenotype and function

In virtue of their plasticity and central role in orchestrating immunity and tolerance, DCs appear as candidates, among the decidual APC populations, to promote the appropriate immune response in order to support pregnancy.[104] Recent reports show the important role of uterine DCs for successful implantation.[105,106] Thus, the number of uterine DCs increases at the implantation period, and depletion of DCs impairs uterine NK cell maturation, tissue remodeling, and angiogenesis.[105,106] Moreover, evidence for the regulatory role of uterine DC cells at the fetal–maternal interface comes from studies in mice revealing that DCs present in uterus at the time of implantation belong to the myeloid lineage and exhibit a steady-state phenotype associated with the induction of tolerance.[107,108]

The DC-derived factors that determine the outcome of DC–T-cell interactions are the Ag presentation levels, display of costimulatory molecules, and the presence of immunomodulatory factors such as cytokines. While increased Ag presentation levels and the expression of costimulatory molecules such as CD80, CD86, and CD40 on DC are crucial for

the expansion of Ag-specific T cells, the expression of co-inhibitory molecules, such as programmed cell death-1 (PD-1) ligands, PD-L1 and PD-L2, can act synergistically to inhibit T-cell activation, proliferation, and cytokine production.[109] In addition, stimuli that induce IL-12 promote IFN-γ-producing Th1 cells, stimuli that induce IL-10 and TGF-β favor Treg-cell differentiation, and stimuli that induce TGF-β and IL-6 promote a Th17 response in the mouse.[4,110] In addition, IL-4 and IL-10 are both candidates for a Th2-driving signal from DCs; however, it has been demonstrated that both IL-4- and IL-10-deficient DCs can still drive Th2 responses.[111] Of note, the specific anatomical compartment where the immature DC (iDC) resides and encounters a maturation stimuli profoundly impacts the character of the immune response generated by the DC after it has migrated to the lymph nodes.[112,113] Therefore, DCs can respond to pregnancy-specific signals, thus promoting the appropriate immune response of the mother and consequently the pregnancy outcome.

We used murine bone marrow–derived DCs to investigate the ability of PSG1a to modulate DC cytokine production, the expression of costimulatory molecules, and the T-cell-polarizing function and observed that iDCs treated with PSG1a differentiate into a subset with a unique phenotype and function. PSG1a-treated DCs present a semimature phenotype showing low surface CD40, ICAM-1, and MHC class II expression and the ability to secrete IL-6 and TGF-β. Treatment with PSG1a also affects the maturation of DCs, preventing the up-regulation of some costimulatory molecules and inducing the expression of PD-L1 and the secretion of TGF-β or IL-10 in response to TLR or CD40 ligation.[81]

The PSG-induced secretion of IL-6 and TGF-β has previously been reported in different cell types for human PSG1 and some murine PSGs.[89,90] Taking into account the known roles of TGF-β and IL-6 in implantation, trophoblast differentiation and angiogenesis, and the direct participation of DCs in angiogenic responses, DCs accumulated in the uterus during the peri-implantation period may be targeted by PSG1a to provide DCs with a role in processes such as decidualization and placentation and the associated vascular adaptations.[107,114] In addition, the up-regulation of PD-L1 expression on PSG1a-treated DC after TLR or CD40 ligation is particularly interesting because the syncytiotrophoblast expresses TLR, constitutively expresses PD-L1, and the loss of PD-1:PD-L1 signaling has been reported to impair fetomaternal tolerance.[115–117]

Moreover, in agreement with the reported expression of IL-10 by uterine DC, it also was reported that some PSGs can also induce IL-10 and TGF-β secretion.[89,90,108] In addition, SpMΦ from Vac-PSG1a-treated mice drive Th2 differentiation by an IL-10-dependent mechanism.[77] In our experimental setting, DCs treated with PSG1a secrete IL-10 only after the activation with anti-CD40 (treatment that mimics their interaction with T cells).

Interestingly, the PSG1a-treated DCs are able to activate Ag-specific T cells and to drive the acquisition of mixed phenotypes of Th2-type-/IL-17-secreting cells and Treg cells when they are pulsed with OVA peptide and used both *in vitro* to stimulate OVA-specific naïve T cells (from DO11.10 mice) and *in vivo* to immunize DO11.10 mice.[81] Interestingly, these data provide the first evidence for the existence of placental products able to activate DC to promote IL-17-producing cells.[81]

PSG1a-treated DC primes $CD4^+$ T cells to produce IL-4 by a mechanism that involved IL-6 and IL-10 (as we demonstrated for SpMΦ targeted *in vivo* by PSG1a).[77] Moreover, the priming for Treg cells and IL-17-producing cells involves IL-6, TGF-β, PD-L1 and IL-10 signaling with IL-6, TGF-β, and PD-L1 signaling promoting both cell populations and IL-10 signaling partially suppressing the IL-17 secretion.[81] Recently, it has been demonstrated the key role of PD-L1 in promoting inducible Treg-cell development and function, with PD-L1 and TGF-β having synergistic roles, while PD-L1-PD-1 signaling is able to negatively regulate IL-17 differentiation.[118–120] On the other hand, IL-10 signaling can directly suppress Th17 cells.[87,88]

IL-6, a cytokine produced by endometrial epithelium and stromal cells at the time of implantation,[121] is able to suppress IL-12-mediated T-cell polarization and direct Th2 differentiation of naïve T cells into IL-4-secreting cells.[122] In addition, IL-6 is a key cytokine that in the presence of TGF-β blocks the development of Treg cells and induces the differentiation of Th17 cells.[4,5,110] However, IL-6 neutralization in the PSG1a-treated DC–T cell co-cultures induces a significant decrease in Treg cells as well as IL-17 and IL-4 production, indicating that the ability of PSG1a-treated DCs to promote Treg, IL-17-, and IL-4-secreting cells relies on their capacity to produce IL-6.[81] Thus, IL-6 may be a 'signal P' induced by PSG1a able to modulate the phenotype and consequently

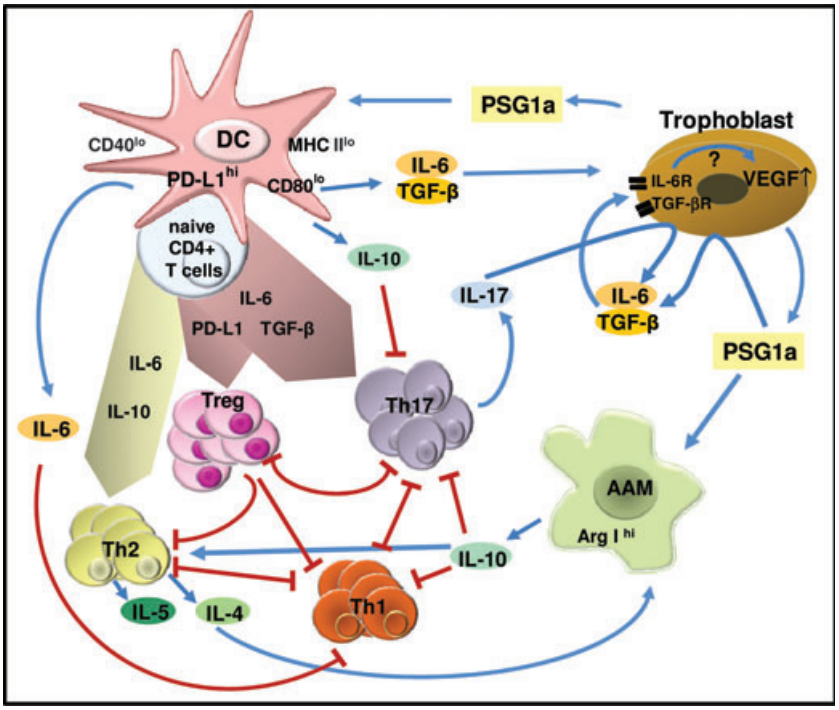

Fig. 2 A model for PSG1a modulation of innate and adaptive immune response and angiogenesis. PSG1a secreted by syncytiotrophoblast cells targets dendritic cells to differentiate into a subset with a unique phenotype and function able to activate Ag-specific T cells and drive the acquisition of mixed phenotypes of Th2-type-/IL-17-secreting cells and Treg cells through the expression of PD-L1 and the secretion of IL-6, TGF-β, and IL-10. PSG1a also induces an alternative phenotype in MΦ characterized by increased arginase activity and the ability to drive Th2-cell differentiation by an IL-10-dependent mechanism. In addition, PSGs as well as the PSG-induced cytokines (IL-6, TGF-β, IL-17) participate in the trophoblast-mediated vascular remodeling.

the function of DCs. In agreement with this IL-6-dependent DC phenotype, a new type of tolerogenic DCs that are induced by activation with TLR2 or TLR4 ligands at low concentrations and including an autocrine/paracrine loop via IL-6 have been recently described.[123] This particular DC phenotype, like PSG1a-treated DC, shows a semimature phenotype and is resistant to TLR ligand-induced maturation.

Conclusions

These findings demonstrated that PSGs may have a 'signal P'-inductor role during pregnancy, thus modulating the activation of the innate immune system and actively contributing to the T-cell shift of the maternal cell immunity toward a less harmful phenotype (Fig. 2).

During the early phase of pregnancy, a successful implantation occurs in a pro-inflammatory microenvironment and a Th1-type response, followed by a shift to a less deleterious response induced in part by some hormones and placental products. Therefore, this process could be seen as a 'controlled inflammatory process' that depends on the development of a Th1 response early modulated by PSG1a-targeted Mo and DC in order to restrict the initial Th1-effector response. Therefore, PSG1a and probably other PSG members may contribute to establishment of a uniquely regulated immune response in order to allow antimicrobial defense and tissue repair, while at the same time preventing damage to developing fetal organs or the triggering of preterm labor. In agreement with this hypothesis, pregnant women are generally not significantly more susceptible to infections than non-pregnant ones, with their adaptive immune system being prepared to mount an effective immune response to invading pathogens.

References

1 Reis e Sousa C: Dendritic cells as sensors of infection. *Immunity* 2001; 14:495–498.
2 Diebold S: Determination of T-cell fate by dendritic cells. *Immunol Cell Biol* 2008; 86:389–397.
3 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136:2348–2357.
4 Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK: Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; 441:235–238.
5 McGeachy M, Cua D: Th17 cell differentiation: the long and winding road. *Immunity* 2008; 28:445–453.
6 Vanaudenaerde BM, Dupont LJ, Wuyts WA, Verbeken EK, Meyts I, Bullens DM, Dilissen E, Luyts L, Van Raemdonck DE, Verleden GM: The role of interleukin-17 during acute rejection after lung transplantation. *Eur Respir J* 2006; 27:779–787.

7 Curtis M, Way S: Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology* 2009; 126:177–185.

8 Sakaguchi S: Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005; 6:345–352.

9 Shevach EM: From vanilla to 28 flavors: multiple varieties of T regulatory cells. *Immunity* 2006; 25:195–201.

10 Mor G, Cardenas I: The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol* 2010; 63:425–433.

11 Matthiesen L, Berg G, Ernerudh J, Hakansson L: Lymphocyte subsets and mitogen stimulation of blood lymphocytes in normal pregnancy. *Am J Reprod Immunol* 1996; 35:70–79.

12 Bermas B, Hill J: Proliferative responses to recall antigens are associated with pregnancy outcome in women with a history of recurrent spontaneous abortion. *J Clin Investig* 1997; 100:1330–1334.

13 Porcu P, Gaddy J, Broxmeyer H: Alloantigen-induced unresponsiveness in cord blood T lymphocytes is associated with defective activation of Ras. *Proc Natl Acad Sci USA* 1998; 95:4538–4543.

14 Mylonakis E, Paliou M, Hohmann E, Calderwood S, Wing E: Listeriosis during pregnancy: a case series and review of 222 cases. *Medicine* 2002; 81:260–269.

15 Gellin B, Broome C, Bibb W, Weaver R, Gaventa S, Mascola L: The epidemiology of listeriosis in the United States–1986. Listeriosis Study Group. *Am J Epidemiol* 1991; 133:392–401.

16 Wegmann TG, Lin H, Guilbert L, Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; 14:353–356.

17 Raghupathy R: Th1-type immunity is incompatible with successful pregnancy. *Immunol Today* 1997; 18:478–482.

18 Shiraishi H, Hayakawa S, Satoh K: Murine experimental abortion by IL-2 administration is caused by activation of cytotoxic T lymphocytes and placental apoptosis. *J Clin Lab Immunol* 1996; 48:93–108.

19 Lin Y, Ren L, Wang W, Di J, Zeng S, Saito S: Effect of TLR3 and TLR7 activation in uterine NK cells from non-obese diabetic (NOD) mice. *J Reprod Immunol* 2009; 82:12–23.

20 Bates M, Quenby S, Takakuwa K, Johnson P, Vince G: Aberrant cytokine production by peripheral blood mononuclear cells in recurrent pregnancy loss? *Hum Reprod* 2002; 17:2439–2444.

21 Saini V, Arora S, Yadav A, Bhattacharjee J: Cytokines in recurrent pregnancy loss. *Clin Chim Acta; Int J Clin Chem* 2011; 412:702–708.

22 van Mourik M, Macklon N, Heijnen C: Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. *J Leukoc Biol* 2009; 85:4–19.

23 Ledee-Bataille N, Dubanchet S: Coulomb-L'hermine A, Durand-Gasselin I, Frydman R, Chaouat G: A new role for natural killer cells, interleukin (IL)-12, and IL-18 in repeated implantation failure after in vitro fertilization. *Fertil Steril* 2004; 81:59–65.

24 Wang W-J, Hao C-F, Yi L, Yin G-J, Bao S-H, Qiu L-H, Lin Q-D: Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients. *J Reprod Immunol* 2010; 84:164–170.

25 Nakashima A, Ito M, Shima T, Bac ND, Hidaka T, Saito S: Accumulation of IL-17-positive cells in decidua of inevitable abortion cases. *Am J Reprod Immunol* 2010; 64:4–11.

26 Saito S, Nakashima A, Shima T, Ito M: Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *Am J Reprod Immunol* 2010; 63:601–610.

27 Zenclussen A, Gerlof K, Zenclussen M, Sollwedel A, Bertoja A, Ritter T, Kotsch K, Leber J, Volk H-D: 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* 2005; 166:811–822.

28 Somerset D, Zheng Y, Kilby M, Sansom DM, Drayson MT: Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. *Immunology* 2004; 112: 38–43.

29 Zhao J-X, Zeng Y-Y, Liu Y: Fetal alloantigen is responsible for the expansion of the CD4(+)CD25(+) regulatory T cell pool during pregnancy. *J Reprod Immunol* 2007; 75:71–81.

30 Aluvihare V, Kallikourdis M, Betz A: Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; 5:266–271.

31 Tilburgs T, Roelen D, van der Mast B, de Groot-Swings G, Kleijburg C, Scherjon S, Claas F: 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* 2008; 180:5737–5745.

32 Jasper M, Tremellen K, Robertson SA: Primary unexplained infertility is associated with reduced expression of the T-regulatory cell transcription factor Foxp3 in endometrial tissue. *Mol Hum Reprod* 2006; 12:301–308.

33 Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S: Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol Hum Reprod* 2004; 10:347–353.

34 Yang H, Qiu L, Chen G, Ye Z, Lu C, Lin Q: Proportional change of CD4+CD25+ regulatory T cells in decidua and peripheral blood in unexplained recurrent spontaneous abortion patients. *Fertil Steril* 2008; 89:656–661.

35 Hsu P, Santner-Nanan B, Dahlstrom J, Fadia M, Chandra A, Peek M, Nanan R: Altered Decidual DC-SIGN(+) Antigen-Presenting Cells and Impaired Regulatory T-Cell Induction in Preeclampsia. *Am J Pathol* 2012; 181:2149–2160.

36 Arruvito L, Sanz M, Banham A, Fainboim L: Expansion of CD4+CD25+ and FOXP3+ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. *J Immunol* 2007; 178:2572–2578.

37 Samstein R, Josefowicz S, Arvey A, Treuting P, Rudensky A: Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* 2012; 150:29–38.

38 Peck A, Mellins E: Plasticity of T-cell phenotype and function: the T helper type 17 example. *Immunology* 2010; 129:147–153.

39 Mao G, Wang J, Kang Y, Tai P, Wen J, Zou Q, Li G, Ouyang H, Xia G, Wang B: Progesterone increases systemic and local uterine proportions of CD4+CD25+ Treg cells during midterm pregnancy in mice. *Endocrinology* 2010; 151:5477–5488.

40 Wan H, Versnel MA, Leijten LME, van Helden-Meeuwsen CG, Fekkes D, Leenen PJM, Khan NA, Benner R, Kiekens RCM: Chorionic gonadotropin induces dendritic cells to express a tolerogenic phenotype. *J Leukoc Biol* 2008; 83:894–901.

41 Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, Oleszczuk J: The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. *J Reprod Immunol* 2012; 93:75–81.

42 Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC: A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* 2007; 13:1450–1457.

43 Ermisch C, Markert U: PIBF – Progesterone-Induced Blocking Factor. *Z Geburtshilfe Neonatol* 2011; 215:93–97.

44 Sacks G, Sargent I, Redman C: An innate view of human pregnancy. *Immunol Today* 1999; 20:114–118.

45 Liang J, Sun L, Wang Q, Hou Y: Progesterone regulates mouse dendritic cells differentiation and maturation. *Int Immunopharmacol* 2006; 6:830–838.

46 Siiteri P, Febres F, Clemens L, Chang R, Gondos B, Stites D: Progesterone and maintenance of pregnancy: is progesterone nature's immunosuppressant? *Ann NY Acad Sci* 1977; 286:384–397.

47 Branch D: Physiologic adaptations of pregnancy. *Am J Reprod Immunol* 1992; 28:120–122.

48 Zhou G, HammarstrÃm S: Pregnancy-specific glycoprotein (PSG) in baboon (*Papio hamadryas*): family size, domain structure, and prediction of a functional region in primate PSGs. *Biol Reprod* 2001; 64:90–99.

49 Camolotto S, Racca A, Rena V, Nores R, Patrito L, Genti-Raimondi S, Panzetta-Dutari G: Expression and transcriptional regulation of individual pregnancy-specific glycoprotein genes in differentiating trophoblast cells. *Placenta* 2010; 31:312–319.

50 Lin T, Halbert S, Spellacy W: Measurement of pregnancy-associated plasma proteins during human gestation. *J Clin Investig* 1974; 54:576–582.

51 Arnold L, Doherty T, Flor A, Simon J, Chou J, Chan W, Mansfield B: Pregnancy-specific glycoprotein gene expression in recurrent aborters: a potential correlation to interleukin-10 expression. *Am J Reprod Immunol* 1999; 41:174–182.

52 Silver RM, Heyborne KD, Leslie KK: Pregnancy specific beta 1 glycoprotein (SP-1) in maternal serum and amniotic fluid; pre-eclampsia, small for gestational age fetus and fetal distress. *Placenta* 1993; 14:583–589.

53 Tamsen L, Johansson S, Axelsson O: Pregnancy-specific beta 1-glycoprotein (SP1) in serum from women with pregnancies complicated by intrauterine growth retardation. *J Perinat Med* 1983; 11:19–25.

54 Pihl K, Larsen T, Laursen I, Krebs L, Christiansen M: First trimester maternal serum pregnancy-specific beta-1-glycoprotein (SP1) as a marker of adverse pregnancy outcome. *Prenat Diagn* 2009; 29:1256–1261.

55 McLellan A, Zimmermann W, Moore T: Conservation of pregnancy-specific glycoprotein (PSG) N domains following independent expansions of the gene families in rodents and primates. *BMC Evol Biol* 2005; 5:39.

56 Teglund S, Olsen A, Khan W: FrÃ¤ngsmyr L, Hammarstrom S: The pregnancy-specific glycoprotein (PSG) gene cluster on human chromosome 19: fine structure of the 11 PSG genes and identification of 6 new genes forming a third subgroup within the carcinoembryonic antigen (CEA) family. *Genomics* 1994; 23:669–684.

57 Beauchemin N, Draber P, Dveksler G, Gold P, Gray-Owen S, Grunert F, HammarstrÃm S, Holmes K, Karlsson A, Kuroki M, Lin S, Lucka L, Najjar S, Neumaier M, Obrink B, Shively J, Skubitz K, Stanners C, Thomas P, Thompson J, Virji M, von Kleist S, Wagener C, Watt S, Zimmermann W: Redefined nomenclature for members of the carcinoembryonic antigen family. *Exp Cell Res* 1999; 252:243–249.

58 Grudzinskas J, Lenton E, Gordon Y, Kelso I, Jeffrey D, Sobowale O, Chard T: Circulating levels of pregnancy-specific beta1-glycoprotein in early pregnancy. *Br J Obstet Gynaecol* 1977; 84:740–742.

59 Bocco J, Panzetta G, Flury A, Patrito L: Expression of pregnancy specific beta 1-glycoprotein gene in human placenta and hydatiform mole. *Biochem Int* 1989; 18:999–1008.

60 Bohn H, Weinmann E: Antifertility effect of an active immunization of monkeys with human pregnancy-specific beta 1-glycoprotein (SP1) (author's transl). *Archiv für Gynäkologie* 1976; 221:305–312.

61 Horne CH, Towler CM, Pugh-Humphreys RG, Thomson AW, Bohn H: Pregnancy specific beta1-glycoprotein–a product of the syncytiotrophoblast. *Experientia* 1976; 32:1197.

62 Harris S, Anthony F, Jones D, Masson G: Pregnancy-specific-beta 1-glycoprotein: effect on lymphocyte proliferation in vitro. *J Reprod Immunol* 1984; 6:267–270.

63 Cerni C, Tatra G, Bohn H: Immunosuppression by human placenta lactogen (HPL) and the pregnancy-specific beta 1-glycoprotein (SP-1). Inhibition of mitogen-induced lymphocyte transformation. *Archiv für Gynäkologie* 1977; 223:1–7.

64 Marchal I, Jarvis D, Cacan R, Verbert A: Glycoproteins from insect cells: sialylated or not? *Biol Chem* 2001; 382:151–159.

65 Fialova L, Kohoutova B, Pelaskova Z, Malbohan I, Mikulakova L: [Serum levels of trophoblast-specific beta-1-globulin (SP1) and alpha-1-fetoprotein (AFP) in pregnant women with rheumatoid arthritis]. *CeskoslovenskÃ¡ Gynekologie* 1991; 56:166–170.

66 Bebo B, Dveksler G: Evidence that pregnancy specific glycoproteins regulate T-Cell function and inflammatory autoimmune disease during pregnancy. *Curr Drug Targets Inflamm Allergy* 2005; 4:231–237.

67 Rutherfurd K, Chou J, Mansfield B: A motif in PSG11s mediates binding to a receptor on the surface of the promonocyte cell line THP-1. *Mol Endocrinol* 1995; 9:1297–1305.

68 Waterhouse R, Ha C, Dveksler GS: Murine CD9 is the receptor for pregnancy-specific glycoprotein 17. *J Exp Med* 2002; 195:277–282.

69 Ha CT, Waterhouse R, Warren J, Zimmermann W, Dveksler GS: N-glycosylation is required for binding of murine pregnancy-specific glycoproteins 17 and 19 to the receptor CD9. *Am J Reprod Immunol* 2008; 59:251–258.

70 Lisboa FA, Warren J, Sulkowski G, Aparicio M, David G, Zudaire E, Dveksler GS: Pregnancy-specific Glycoprotein 1 Induces Endothelial Tubulogenesis through Interaction with Cell Surface Proteoglycans. *J Biol Chem* 2011; 286:7577–7586.

71 Sulkowski G, Warren J, Ha C, Dveksler G: Characterization of receptors for murine pregnancy specific glycoproteins 17 and 23. *Placenta* 2011; 32:603–610.

72 Wu JA, Johnson BL, Chen Y, Ha CT, Dveksler GS: Murine pregnancy-specific glycoprotein 23 induces the proangiogenic factors transforming-growth factor beta 1 and vascular endothelial growth factor a in cell types involved in vascular remodeling in pregnancy. *Biol Reprod* 2008; 79:1054–1061.

73 Blois S, Tirado-Gonzalez I, Wu J, Barrientos G, Johnson B, Warren J, Freitag N, Klapp B, Irmak S, Ergun S, Dveskler G: Early expression of pregnancy-specific glycoprotein 22 (PSG22) by trophoblast cells modulates angiogenesis in mice. *Biol Reprod* 2012; 86:191.

74 Ha CT, Wu JA, Irmak S, Lisboa FA, Dizon AM, Warren JW, Ergun S, Dveksler GS: Human pregnancy specific beta-1-glycoprotein 1

(PSG1) has a potential role in placental vascular morphogenesis. *Biol Reprod* 2010; 83:27–35.

75 Motran CC, Diaz FL, Gruppi A, Slavin D, Chatton B, Bocco JL: Human pregnancy-specific glycoprotein 1a (PSG1a) induces alternative activation in human and mouse monocytes and suppresses the accessory cell-dependent T cell proliferation. *J Leukoc Biol* 2002; 72:512–521.

76 Watanabe S, Chou JY: Isolation and characterization of complementary DNAs encoding human pregnancy-specific beta 1-glycoprotein. *J Biol Chem* 1988; 263:2049–2054.

77 Motran CC, Diaz FL, Montes CL, Bocco JL, Gruppi A: In vivo expression of recombinant pregnancy-specific glycoprotein 1a induces alternative activation of monocytes and enhances Th2-type immune response. *Eur J Immunol* 2003; 33:3007–3016.

78 Yip HC, Karulin AY, Tary-Lehmann M, Hesse MD, Radeke H, Heeger PS, Trezza RP, Heinzel FP, Forsthuber T, Lehmann PV: Adjuvant-guided type-1 and type-2 immunity: infectious/noninfectious dichotomy defines the class of response. *J Immunol* 1999; 162:3942–3949.

79 Kovarik J, Gaillard M, Martinez X, Bozzotti P, Lambert PH, Wild TF, Siegrist CA: Induction of adult-like antibody, Th1, and CTL responses to measles hemagglutinin by early life murine immunization with an attenuated vaccinia-derived NYVAC(K1L) viral vector. *Virology* 2001; 285:12–20.

80 Geginat G, Lalic M, Kretschmar M, Goebel W, Hof H, Palm D, Bubert A: Th1 Cells Specific for a Secreted Protein of Listeria monocytogenes Are Protective In Vivo. *J Immunol* 1998; 160:6046–6055.

81 Martínez FF, Knubel CP, Sánchez MC, Cervi L, Motrán CC: Pregnancy-specific glycoprotein 1a activates dendritic cells to provide signals for Th17-, Th2-, and Treg-cell polarization. *Eur J Immunol* 2012; 42:1573–1584.

82 Ertelt JM, Rowe JH, Johanns TM, Lai JC, McLachlan JB, Way SS: Selective Priming and Expansion of Antigen-Specific Foxp3-CD4+ T Cells during Listeria monocytogenes Infection. *J Immunol* 2009; 182:3032–3038.

83 Collins M, Tay C, Erlebacher A: Dendritic cell entrapment within the pregnant uterus inhibits immune surveillance of the maternal/fetal interface in mice. *J Clin Invest* 2009; 119:2062–2073.

84 Pongcharoen S, Niumsup P, Sanguansermsri D, Supalap K, Butkhamchot P: The effect of interleukin-17 on the proliferation and invasion of JEG-3 human choriocarcinoma cells. *Am J Reprod Immunol* 2006; 55:291–300.

85 Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT: Interleukin-17 promotes angiogenesis and tumor growth. *Blood* 2003; 101:2620–2627.

86 Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H: IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. *J Exp Med* 2009; 206:1457–1464.

87 Huber S, Gagliani N, Esplugues E, O'Connor W, Huber Francis J, Chaudhry A, Kamanaka M, Kobayashi Y, Booth Carmen J, Rudensky Alexander Y, Roncarolo Maria G, Battaglia M, Flavell Richard A: Th17 Cells Express Interleukin-10 Receptor and Are Controlled by Foxp3 and Foxp3+ Regulatory CD4+ T Cells in an Interleukin-10-Dependent Manner. *Immunity* 2011;34:554–565.

88 Chaudhry A, Samstein Robert M, Treuting P, Liang Y, Pils Marina C, Heinrich J-M, Jack Robert S, Wunderlich FT, Brüning Jens C, Müller W, Rudensky Alexander Y: Interleukin-10 signaling in regulatory T cells is required for suppression of TH17 cell-mediated inflammation. *Immunity* 2011; 34:566–578.

89 Ha CT, Waterhouse R, Wessells J, Wu JA, Dveksler GS: Binding of pregnancy-specific glycoprotein 17 to CD9 on macrophages induces secretion of IL-10, IL-6, PGE2, and TGF-beta1. *J Leukoc Biol* 2005; 77:948–957.

90 Snyder SK, Wessner DH, Wessells JL, Waterhouse RM, Wahl LM, Zimmermann W, Dveksler GS: Pregnancy-specific glycoproteins function as immunomodulators by inducing secretion of IL-10, IL-6 and TGF-beta1 by human monocytes. *Am J Reprod Immunol* 2001; 45:205–216.

91 Wessells J, Wessner D, Parsells R, White K, Finkenzeller D, Zimmermann W, Dveksler G: Pregnancy specific glycoprotein 18 induces IL-10 expression in murine macrophages. *Eur J Immunol* 2000; 30:1830–1840.

92 Goerdt S, Orfanos CE: Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity* 1999; 10:137–142.

93 Munder M, Eichmann K, Modolell M: Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. *J Immunol* 1998; 160:5347–5354.

94 Tiemessen M, Jagger A, Evans H, van Herwijnen M, John S, Taams L: CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci USA* 2007; 104:19446–19451.

95 Stein M, Keshav S, Harris N, Gordon S: Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 1992; 176:287–292.

96 Mosser D, Edwards J: Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008; 8:958–969.

97 Mues B, Langer D, Zwadlo G, Sorg C: Phenotypic characterization of macrophages in human term placenta. *Immunology* 1989; 67:303–307.

98 Cua DJ, Stohlman SA: In vivo effects of T helper cell type 2 cytokines on macrophage antigen-presenting cell induction of T helper subsets. *J Immunol* 1997; 159:5834–5840.

99 Loke P, MacDonald AS, Robb A, Maizels RM, Allen JE: Alternatively activated macrophages induced by nematode infection inhibit proliferation via cell-to-cell contact. *Eur J Immunol* 2000; 30:2669–2678.

100 Brunialti M, Santos M, Rigato O, Machado F, Silva E, Salomao R: Increased percentages of T helper cells producing IL-17 and monocytes expressing markers of alternative activation in patients with sepsis. *PLoS ONE* 2012; 7:e37393.

101 van de Veerdonk F, Marijnissen R, Kullberg B, Koenen H, Cheng S-C, Joosten I, van den Berg W, Williams D, van der Meer J, Joosten L, Netea M: The macrophage mannose receptor induces IL-17 in response to *Candida albicans*. *Cell Host Microbe* 2009; 5:329–340.

102 Loke P, MacDonald AS, Allen JE: Antigen-presenting cells recruited by Brugia malayi induce Th2 differentiation of naive CD4(+) T cells. *Eur J Immunol* 2000; 30:1127–1135.

103 Taylor MD, Harris A, Babayan SA, Bain O, Culshaw A, Allen JE, Maizels RM: CTLA-4 and CD4+ CD25+ regulatory T cells inhibit protective immunity to filarial parasites in vivo. *J Immunol* 2007; 179:4626–4634.

104 Steinman RM, Hawiger D, Nussenzweig MC: Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; 21:685–711.

105 Krey G, Frank P, Shaikly V, Barrientos G, Cordo-Russo R, Ringel F, Moschansky P, Chernukhin I, Metodiev M, Fernandez N, Klapp B, Arck P, Blois S: In vivo dendritic cell depletion reduces breeding efficiency, affecting implantation and early placental development in mice. *J Mol Med* 2008; 86:999–1011.

106 Nevers T, Kalkunte S, Sharma S: Uterine Regulatory T cells, IL-10 and hypertension. *Am J Reprod Immunol* 2011; 66(Suppl 1): 88–92.

107 Barrientos G, Tirado-Gonzalez I, Klapp BF, Karimi K, Arck PC, Garcia MG, Blois SM: The impact of dendritic cells on angiogenic responses at the fetal-maternal interface. *J Reprod Immunol* 2009; 83:85–94.
108 Blois SM, Alba Soto CD, Tometten M, Klapp BF, Margni RA, Arck PC: Lineage, maturity, and phenotype of uterine murine dendritic cells throughout gestation indicate a protective role in maintaining pregnancy. *Biol Reprod* 2004;70:1018–1023.
109 Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ: The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol* 2007; 8:239–245.
110 Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT: Transforming growth factor-beta induces development of the T(H) 17 lineage. *Nature* 2006; 441:231–234.
111 MacDonald AS, Maizels RM: Alarming dendritic cells for Th2 induction. *J Exp Med* 2008; 205:13–17.
112 Constant SL, Brogdon JL, Piggott DA, Herrick CA, Visintin I, Ruddle NH, Bottomly K: Resident lung antigen-presenting cells have the capacity to promote Th2 T cell differentiation in situ. *J Clin Invest* 2002; 110:1441–1448.
113 Iwasaki A, Kelsall BL: Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 1999; 190:229–239.
114 Conejo-Garcia JR, Benencia F, Courreges MC, Kang E, Mohamed-Hadley A, Buckanovich RJ, Holtz DO, Jenkins A, Na H, Zhang L, Wagner DS, Katsaros D, Caroll R, Coukos G: Tumor-infiltrating dendritic cell precursors recruited by a beta-defensin contribute to vasculogenesis under the influence of Vegf-A. *Nat Med* 2004; 10:950–958.
115 Koga K, Mor G: Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy disorders. *Am J Reprod Immunol* 2010; 63:587–600.
116 Petroff MG, Chen L, Phillips TA, Azzola D, Sedlmayr P, Hunt JS: B7 Family Molecules Are Favorably Positioned at the Human Maternal-Fetal Interface. *Biol Reprod* 2003; 68:1496–1504.
117 Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, Noelle RJ, Coyle A, Mellor AL, Khoury SJ, Sayegh MH: A critical role for the programmed death ligand 1 in fetomaternal tolerance. *J Exp Med* 2005; 202:231–237.
118 Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH: PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med* 2009; 206:3015–3029.
119 Schreiner B, Bailey S, Shin T, Chen L, Miller S: PD-1 ligands expressed on myeloid-derived APC in the CNS regulate T-cell responses in EAE. *Eur J Immunol* 2008; 38:2706–2717.
120 Carter LL, Leach MW, Azoitei ML, Cui J, Pelker JW, Jussif J, Benoit S, Ireland G, Luxenberg D, Askew GR, Milarski KL, Groves C, Brown T, Carito BA, Percival K, Carreno BM, Collins M, Marusic S: PD-1/PD-L1, but not PD-1/PD-L2, interactions regulate the severity of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2007; 182:124–134.
121 Cork B, Tuckerman E, Li T, Laird S: Expression of interleukin (IL)-11 receptor by the human endometrium in vivo and effects of IL-11, IL-6 and LIF on the production of MMP and cytokines by human endometrial cells in vitro. *Mol Hum Reprod* 2002; 8:841–848.
122 Rincon M, Anguita J, Nakamura T, Fikrig E, Flavell RA: Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4+ T cells. *J Exp Med* 1997; 185:461–469.
123 Frick JS, Zahir N, Müller M, Kahl F, Bechtold O, Lutz MB, Kirschning CJ, Reimann J, Jilge B, Bohn E, Autenrieth IB: Colitogenic and non-colitogenic commensal bacteria differentially trigger DC maturation and Th cell polarization: An important role for IL-6. *Eur J Immunol* 2006; 36:1537–1547.