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Severe preeclampsia is characterized by increased placental expression of galectin-1

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Abstract

Objective—Galectin-1 is a major anti-inflammatory protein expressed by the placenta and immune cells that can bias the character of inflammatory responses toward the Th2 type. Galectin-1 is expressed in immune privileged sites, it can facilitate immune tolerance and tumor immune escape, and it has been successfully used for the suppression of experimental autoimmune diseases as well as graft versus host disease in murine models. We propose that an abnormal immune response in some pregnancy complications may be associated with changes in placental expression of galectin-1. To test this hypothesis, we studied placental galectin-1 mRNA and protein expression and localization in women with preeclampsia (PE) and in those who delivered a small-for-gestational age (SGA) neonate.

Study design—This cross-sectional study included pregnant women matched for gestational age at delivery in the following groups: 1) severe PE (n=10); 2) severe PE complicated with SGA (n=10); 3) SGA without PE (n=10); and 4) controls (n=10). Galectin-1 mRNA and protein were localized in placentas by *in situ* hybridization and immunofluorescence microscopy. Galectin-1 mRNA expression was determined by quantitative real-time RT-PCR, and galectin-1 protein content by Western blot. Non-parametric statistics were used for analysis.

Results—1) In normal term placentas, galectin-1 mRNA or immunofluorescence signals were detected in the trophoblasts, villous stromal cells, Hofbauer cells, endothelial cells of the villous blood vessels, and the villous stroma. 2) Placental galectin-1 mRNA expression was significantly higher in severe PE (with or without SGA) than in controls (1.47 fold, p=0.004; 1.44 fold, p=0.003; respectively) and in SGA (1.68 fold, p=0.001; 1.64 fold, p=0.001; respectively). 3) Trophoblasts in placentas of patients with severe PE had the most intense galectin-1 immunostaining.

Conclusion—1) We report for the first time the placental expression and localization of galectin-1 mRNA and demonstrate that the protein is abundantly present in third trimester human placentas. 2) Placental galectin-1 expression is higher in severe PE than in normal pregnancy regardless of the presence of SGA. 3) However, it is not altered in SGA without PE. We propose that the increased placental expression of galectin-1 in patients with severe PE may represent a fetal response to an

exaggerated systemic maternal inflammation; thus, galectin-1 may be implicated in maternal-fetal immune tolerance in humans.

Keywords

glycocode; inflammation; lectin; pregnancy; semi-allograft; tolerance; trophoblast

INTRODUCTION

Preeclampsia (PE) is considered a maternal disease which is associated with fetal growth restriction in 10–25% of the cases, while pregnancies complicated with growth restricted (IUGR) fetuses or small-for-gestational age (SGA) neonates without PE usually have no appreciable clinical impact on the mother[1,2]. These ‘great obstetrical syndromes’[3] share similar pathophysiologic mechanisms[2], such as generalized endothelial cell dysfunction[4–8], abnormal placentation[9–16], anti-angiogenic state[17–35], chronic uteroplacental ischemia[36–40], and an increased maternal systemic inflammatory response[41–46]. The latter is highly exaggerated in PE and involves the activation of the innate immune system [42,47–49].

Galectins are cytokine-like immunoregulatory proteins, members of an evolutionarily-conserved protein family that share similar structures, carbohydrate recognition domains (CRDs) and affinity for beta-galactosides present on cell surface glycoconjugates[50–56]. Galectins exhibit preferential binding to a subset of ligands[57–59] and are implicated in deciphering the high-density “glycocode” stored in glycoproteins, proteoglycans and glycolipids[60–62]. Their versatile functions include the regulation of cell-cell/matrix interactions, cell cycle, apoptosis, cell migration, and the recognition of microbial glycosignatures[50,53,55,56]. Galectins can affect both the innate and the adaptive arms of the immune system, inhibiting (e.g. galectin-1)[56,63] or augmenting (e.g. galectin-3)[56] the inflammatory response.

Galectin-1 was the first human galectin discovered, purified[64] and cloned[65,66] from the placenta[63]. Subsequent studies presented the immunolocalization of galectin-1 in normal first and third trimester placentas; however, the described expression patterns lacked consistency[67–71]. Galectin-1 is expressed in immune-privileged sites (e.g. testis and brain) [72,73], and upregulated in tumors (e.g. melanoma), presumably to facilitate the escape of immunosurveillance[63,74]. Galectin-1 has pleiotropic binding activity [57,59,63] and mediates a wide variety of immune cell interactions (Figure 1)[63], mainly promoting immune tolerance[75] and down-regulating the innate and adaptive immune responses[76]. Moreover, galectin-1 has potent anti-inflammatory effects including: 1) inhibition of acute inflammation [76]; 2) suppression of T cell-mediated autoimmune diseases[77–80]; 3) amelioration of graft versus host disease[81]; and 4) biasing the character of the immune response to the Th2 type [63].

The overexpression of galectin-1 has been observed in cultured human endothelial cells[82], in the synovia of rheumatoid arthritis patients[83] and in activated immune cells[84–87]. Galectin-1 was proposed to regulate the extent of the immune response during inflammation [88]; thus, it might also be involved in the complex inflammatory responses observed in pregnancy complications.

The aims of this study were to determine the: 1) cellular localization of galectin-1 mRNA and protein in normal third trimester placentas; and 2) changes in placental galectin-1 expression in patients with severe PE with and without SGA, as well as in those patients without PE who delivered an SGA neonate.

MATERIAL AND METHODS

Study design and population

This cross-sectional study included pregnant women in the following groups: 1) severe PE (n=10); 2) severe PE with SGA (n=10); 3) SGA without PE (n=10); and 4) pregnant women with preterm and term labor (control group, n=10). Women with severe PE or SGA were matched for gestational age at delivery within two weeks of gestation to women in the control group. Patients with multiple pregnancies, preterm prelabor rupture of membranes, histologic chorioamnionitis, stillbirth or fetal congenital or chromosomal abnormalities were excluded. Samples and data were retrieved from the bank of biological samples and clinical databases of the Perinatology Research Branch. All patients were enrolled at Hutzel Women's Hospital, Detroit, MI, USA, and provided written informed consent prior to the collection of samples. The utilization of samples for research purposes was approved by the Institutional Review Boards of both Wayne State University and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH/DHHS). Many of these samples have been employed to study the biology of inflammation in normal pregnant women and those with pregnancy complications.

Definitions

PE was defined as hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two occasions, 4 hours to 1 week apart) associated with proteinuria (≥ 300 mg in a 24 hour urine collection, or two dipstick measurements of $\geq 1+$ [89], or one dipstick measurement of $\geq 2+$ [90]). Severe PE was defined as systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg and/or proteinuria greater than 5 g in a 24 hour collection or $>3+$ on dipstick [1] and in the presence of multi-organ involvement [1]. SGA was defined as neonatal birthweight below the 10th percentile for gestational age at birth according to the national birthweight distribution [91]. Labor was defined by the presence of regular uterine contractions at a frequency of at least 2 contractions every 10 minutes with cervical changes resulting in delivery <37 (preterm) or ≥ 37 (term) completed weeks of gestation. Control women with preterm or term labor delivered neonates with a birthweight appropriate-for-gestational age ($\geq 10^{\text{th}}$ and $\leq 90^{\text{th}}$ percentile).

mRNA *in situ* hybridization

The 123bp fragment of human galectin-1 cDNA generated by PCR (forward primer: CATCTCTCTCgggTggAgTC, reverse primer: gAAGgCACTCTCCAggTTTg) was subcloned into pGEM-T Easy vector (Promega Corp., Madison, WI, USA) containing SP6 and T7 polymerase promoters. Digoxigenin-labeled anti-sense and sense riboprobes were generated with SP6 and T7 polymerases after linearization of the plasmid with Bam HI and Hind III, respectively. 5 μm sections of paraffin-embedded villous tissues were deparaffinized, hydrated in xylene and graded ethanol and then treated with proteinase K (15 $\mu\text{g}/\text{ml}$) in 0.1 M Tris buffer (pH 8.0) and 50 mM EDTA for 10 minutes at 37°C. Slides were fixed with 4% paraformaldehyde for 20 minutes and acetic anhydride for 10 minutes. Sections were incubated in a hybridization buffer containing digoxigenin-tagged galectin-1 riboprobe (2 $\mu\text{g}/\text{ml}$). Hybridization was carried out in a humidity chamber overnight at 55°C. After repeated post-hybridization washes, sections were incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche Diagnostics, Indianapolis, IN) for 1 hour at room temperature. Nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt were used for detection of the hybridization signal.

Total RNA extraction

Total RNA was isolated from snap-frozen placental villous tissues using TRIzol reagent (Invitrogen Carlsbad, CA, USA) and then Qiagen RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturers' recommendations. The 28S/18S ratio and the RNA integrity number (RIN) were assessed using a Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE, USA). An $A_{260\text{nm}}/A_{280\text{nm}}$ ratio of 1.8, a 28S/18S ratio of 1.3, and a RIN of 6 were minimum requirements for inclusion in expression analysis.

Quantitative real-time reverse transcription–polymerase chain reaction (qRT–PCR)

Total RNA was reverse transcribed with a TaqMan Reverse Transcription Reagent kit using random hexamers (Applied Biosystems, Foster City, CA, USA). The standard curve was run with the *LGALS1* TaqMan Gene Expression Assay (Hs00169327_m1; Applied Biosystems, Foster City, CA, USA) to determine the quantity of cDNA needed for an approximate cycle threshold (Ct) of 25. The human *RPLPO* (large ribosomal protein) TaqMan Endogenous Control (part number: 4326314E) was used as the housekeeping gene for relative quantitation. The *LGALS1* and *RPLPO* genes were then run in triplicates for each case to allow for the assessment of technical variability.

Immunofluorescence confocal microscopy

Five μm sections of snap-frozen villous tissues were fixed with 4% paraformaldehyde for 1 hour at room temperature and acetone for 1 minute at -20°C . Slides were preincubated with Image-it FX signal enhancer (Molecular Probes, Carlsbad, CA, USA) for 30min and CAS blocking solution (Zymed, San Francisco, CA, USA) for 10 minutes. Sections were incubated with goat anti-human galectin-1 IgG (R&D Systems, Minneapolis, MN, USA) and goat isotype control primary IgG at 1:50 dilutions for 1 hour, and with Alexa Fluor 568 conjugated donkey anti-goat IgG (Invitrogen Co., Carlsbad, CA, USA) at 1:1000 dilution for 1 hour. Sytox Green nuclear counter stain (Cambrex, North Brunswick, NJ, USA) was applied at a 1:100,000 dilution for 3 minutes. Stainings were performed on an autostainer (Dako, Carpinteria, CA, USA). Fluorescent and differential interference contrast (DIC) images were captured with a Zeiss Axiovert 200 Ultra-View ERS Rapid Confocal Imager equipped with an argon laser and a Zeiss Fluor 40x / 1.3 oil objective. Images were evaluated by Perkin Elmer ImageSuite 3.0 version 14 (Perkin Elmer Inc., Waltham, MA, USA).

Western blot analysis

Placental villous tissues were lysed with RIPA buffer (Sigma, St Louis, MO, USA) containing protease inhibitor (Roche Diagnostics, Mannheim, Germany). Thirty μg of the protein lysates and 15 ng of recombinant human galectin-1 (R&D Systems, Minneapolis, MN, USA) were electrophoresed on 15% (w/v) SDS-polyacrylamide gels and electroblotted onto PVDF membranes (Bio-Rad, Hercules, CA, USA). The membranes were probed with a goat anti-human galectin-1 IgG (R&D Systems, Minneapolis, MN, USA) or with a murine monoclonal anti- β -actin antibody (Sigma, St Louis, MO, USA) at 1:2,000 dilutions for 1 hour; then incubated with horse-radish peroxidase conjugated donkey anti-goat IgG (R&D Systems, Minneapolis, MN, USA) or goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) at 1:4,000 dilutions for 1 hour. Protein bands were detected by ECL chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA). The specificity of the anti-human galectin-1 IgG was validated by testing with human recombinant galectins-2,-3,-4,-7, and -8 in the same experimental conditions as galectin-1.

Statistical analysis

Comparisons among groups were performed using Fisher's exact test for proportions and one-way ANOVA test for normally distributed continuous variables, as well as Kruskal-Wallis test

and Mann-Whitney U test for non-normally distributed continuous variables. For the analysis of qRT-PCR data, pair-wise group comparisons were performed using “Generalized estimating equations”[92]. In parallel, the t-test was also applied by averaging over the three technical replicates (Ct values) of each subject. To determine the influence of the gestational age on *LGALS1* gene expression within the groups, a linear model was fitted in which the gestational age was used as a predictor for the Ct values. An adjustment of p-values to account for the six different comparisons among the four groups was performed using the Bonferroni method [93]. An adjusted p-value of <0.05 was considered to be statistically significant. The R statistical software (www.r-project.org) including required libraries and SPSS version 12.0 (SPSS Inc., Chicago, IL) were used for the analyses.

RESULTS

Demographic, clinical and histopathologic data

Table I displays the demographic and clinical characteristics of the study groups. A larger proportion of women with severe PE complicated by SGA had chronic hypertension than did patients in the other study groups. Placental histopathologic findings consistent with maternal underperfusion[94,95] (e.g. increased syncytial knots, increased intervillous fibrin, distal villous hypoplasia) were more frequent in preterm than in term cases of SGA without PE and in severe PE with or without SGA.

Localization of galectin-1 mRNA and protein in normal term placentas

Galectin-1 mRNA *in situ* hybridization signals were readily detectable in normal term villous placentas, and a similar pattern was observed with galectin-1 immunofluorescence. mRNA hybridization signals were distinct in the trophoblastic layers, especially in cytotrophoblasts, and were also detected in stromal cells (Figure 2A). Immunofluorescence microscopy demonstrated galectin-1 immunopositivity of trophoblasts, Hofbauer cells, stromal cells, and the endothelium of the villous blood vessels (capillaries, arterioles and venules), as well as the syncytiotrophoblast apical membrane and the villous stroma (Figure 2B).

Placental galectin-1 mRNA expression is increased in severe PE

Placental galectin-1 mRNA expression was significantly higher in severe PE (1.44 fold, $p=0.003$) and severe PE complicated by SGA (1.47 fold, $p=0.004$) than in gestational age matched controls. In addition, there was a significantly higher placental galectin-1 mRNA expression in severe PE with or without SGA than in SGA without PE (1.68 fold, $p=0.001$; 1.64 fold, $p=0.001$; respectively). However, there was no difference in galectin-1 mRNA expression between the SGA and control groups and between patients with severe PE and SGA and those with severe PE alone (Figure 3). In these subsets of patients, gene expressions within the groups did not depend on gestational age.

Placental galectin-1 immunoreactivity is increased in severe PE

In control placentas, galectin-1 immunopositivity was detected in trophoblasts, stromal cells, Hofbauer cells, endothelial cells of the villous blood vessels, and the villous stroma (Figure 4A). In patients with SGA without PE, galectin-1 immunofluorescent staining was similar to that in controls (Figure 4B). Severe PE complicated with SGA was characterized by villous galectin-1 immunofluorescence signal present in all cell types, with the strongest signal in trophoblasts, stromal cells and the stroma (Figure 4C). Similarly, in severe PE without SGA, trophoblasts, stromal cells, and stroma had the most intense immunofluorescent staining (Figure 4D). Immunoblots revealed a 15 kDa immunoreactive protein in all placentas, consistent with the size of galectin-1 monomer. Compared to gestational age matched controls, the strongest signal was detected in the severe PE groups both preterm and term as represented

in Figure 5. When testing for cross-reactivity on Western blots, the primary antibody specifically recognized placental and recombinant galectin-1 but not recombinant galectins-2,-3,-4,-7 and -8.

DISCUSSION

Principal findings of this study

1) Galectin-1 mRNA is ubiquitously expressed in the villous placenta, and its expression pattern is similar to that of galectin-1 immunostaining; 2) placental galectin-1 mRNA expression was significantly increased in severe PE with or without SGA compared to gestational age matched controls and to SGA; and 3) increased placental galectin-1 immunofluorescence was detected in trophoblasts, stromal cells and the villous stroma in cases of severe PE with or without SGA when compared to controls or to SGA.

Galectin-1 is abundantly expressed in third trimester villous placenta

This is the first study that localizes galectin-1 in the villous placenta with mRNA *in situ* hybridization along with sensitive immunofluorescence staining. The combination of these methods allowed us to identify galectin-1 in all cell types of the villi, as well as in the syncytiotrophoblast apical membrane at the maternal-fetal interface. Other studies localized galectin-1 with immunohistochemistry in first and third trimester normal human placentas [67–71]; however, these reports gave less comprehensive and inconsistent results. Two studies demonstrated galectin-1 immunopositivity of mesenchymal cells in both trimesters[67,69]. The villous stroma, where galectin-1 had been co-localized with fibronectin and laminin[96], was also immunopositive in the first trimester[67,70] and at term[67]. Endothelial cells, vessel walls[67,68] and the syncytiotrophoblast was shown to be galectin-1 positive in the first[70] and third trimester[68,71]; however, villous cytotrophoblast immunoreactivity was reported only at term[68]. Overall, none of these reports were consistent with others in terms of the described expression patterns[67–71]. This inconsistency might be the consequence of the differences in gestational age of the tissues, the applied methodologies and the type of antibodies used. In our study, the specificity of the immunostaining was supported by the following: 1) there was no immunofluorescent signal (besides the nuclear counterstain) when the isotype control primary antibody was applied; 2) the antibody that recognized placental and recombinant galectin-1 did not cross-react with homologous galectins on Western blot analyses; and 3) *in situ* hybridization confirmed galectin-1 mRNA expression in immunopositive cells.

Galectin-1 is expressed in placentas of other mammals[97,98]. Murine galectin-1, which has an 88% amino acid sequence identity to its human ortholog, was found to be ubiquitously expressed in the mouse placenta, including trophoblast cells in the labyrinth region and the spongy layer[97]. Recent publications revealed that cytoplasmic galectin-1 can be translocated to the intra-[63,99] and extracellular side of the cell membrane[63,100], and that recombinant galectin-1 is capable of binding to human syncytiotrophoblast and extravillous trophoblast cell surface[101]. Thus, the abundance and ubiquitous expression of galectin-1 by the villous tissues and its presence at the maternal-fetal interface suggests that this galectin might have several functions in the placenta.

What is the role of galectin-1 in the placenta?

The most important biological processes to which galectin-1 has been linked include connective tissue organization, tumor invasiveness and metastasis, regulation of cell proliferation and differentiation, and local immunomodulation[63]. Currently, there are no functional data on the effect of galectin-1 on normal human placental cells. Based on its

placental expression pattern and functional effects on other cell types, we propose that galectin-1 may participate in the following processes in the placenta:

Extracellular matrix organization—The strong galectin-1 immunopositivity of villous stromal cells and the villous stroma found in this study is in accordance with the abundance of galectin-1 in cells of mesenchymal origin and their ECM, where it was proposed to have a pivotal role in the organization and presentation of connective tissue components and tissue development[63,70]. Galectin-1 co-localizes, binds and cross-links beta-integrins and poly-N-acetyl-lactosamine-rich components of the placental ECM (e.g. laminin and fibronectin)[59, 69,70,96,102], which are important in the control of cell attachment, migration, invasion, as well as the assembly and remodeling of the ECM[63]. Indeed, overexpression of galectin-1 decreases the incorporation of its ligands (vitronectin and chondroitin sulphate) into the ECM of smooth muscle cells[103]. Based on these findings, we propose that the expression of galectin-1 may have importance in the cross-talk between trophoblasts, stromal cells and the stroma during placentation and in the development and maintenance of villous tissues.

Immune regulation by villous endothelial cells—Our data demonstrated that galectin-1 is expressed by the endothelium of villous capillaries, arterioles and veins in the villous placenta. This is consistent with previous studies demonstrating the expression of galectin-1 in endothelial cells of human umbilical vein and aorta, bovine aorta, and microvessels in rat lung and mouse lung and brain[82,104–106]. Microvascular endothelial cells form specialized microcirculatory networks, which regulate coagulation, angiogenesis and the distribution of activated immune cells, thus, innate and adaptive immune responses[107]. Indeed, galectin-1 was demonstrated to inhibit polymorphonuclear cell chemotaxis and *trans*-endothelial migration *in vitro* and interleukin (IL)-1-induced polymorphonuclear cell recruitment into the mouse peritoneal cavity *in vivo*[106]. Galectin-1 inhibited T cell migration across endothelial cells expressing increased amounts of the protein[108], and induced apoptosis of susceptible T cells bound to cultured human aortic endothelial cells expressing high amounts of galectin-1 [105]. These data suggest that galectin-1 expressed by the villous endothelium may also be part of an anti-inflammatory loop, regulating recruitment and transmigration of fetal immune cells in the villi.

Host-pathogen immune response—Hofbauer cells are also regarded as fetal macrophages capable of phagocytosis[109–111] and production of cytokines [e.g., IL-1, IL-8, suppressor of cytokine signaling (SOCS) proteins][112–114], chemokines [e.g. macrophage inhibitory protein (MIP)-1-beta][115] and phagocytosis-related enzymes [acid phosphatase (ACP) and glucose-6-phosphate dehydrogenase (G6PD)][111]. Hofbauer cells exhibiting strong G6PD staining and ACP labeling in the phagosomes are phagocytic cells. The percentage of these activated macrophages is significantly higher in placentas of patients with infectious miscarriages than in gestational age-matched controls[111]. Of interest, there is an up-regulation of galectin-1 expression in macrophages during their activation[84,87] and in infection[85]. In turn, galectin-1 inhibits macrophage microbicidal activity [85] decreases iNOS expression and NO metabolism[116], and regulates constitutive and inducible expression of high affinity FcγRI (CD64) and phagocytosis[117]. Based on these data, galectin-1 may also have a role in the fetal response to pathogenic insults and in the regulation of the extent of the inflammatory response in the villi.

Maternal-fetal immune tolerance—This study confirmed the expression of galectin-1 in the syncytiotrophoblast[68,70,71] and showed its sublocalization onto the apical membrane. The syncytiotrophoblast is a rich source of immunomodulatory molecules[118–120], and those with immunosuppressive properties [e.g. human chorionic gonadotropin, human placental lactogen, indoleamine 2,3-dioxygenase, CD95L/Fas ligand, pregnancy-specific beta-1

glycoproteins, SOCS proteins, TNF-related apoptosis-inducing ligand (TRAIL)[114,121–126] are proposed to attenuate maternal immune responses and, thus, maintain tolerance to the fetus. The evidence supporting that placental galectin-1 may also have an important role in immune tolerance includes the following: 1) Galectin-1 reduces host alloreactivity and ameliorates graft versus host disease in mice[81]; 2) it is capable of triggering the apoptosis of immature thymocytes[105] and activated T cells[75], biasing the immune responses to the Th2 type[63,127,128], and suppressing experimental T cell-mediated autoimmune diseases (e.g. encephalomyelitis, retinal disease, arthritis or hepatitis) in rats and mice[77–80]; 3) galectin-1 plays a role in the immune escape of a wide variety of tumors (e.g. head and neck carcinoma, astrocytoma, glioma, melanoma) by possibly down-regulating tumor resident T cell survival and linking tumor hypoxia and immune privilege [74,129–132]; 4) galectin-1 is up-regulated in mammary adenocarcinoma cells by transforming growth factor (TGF)- β [133], an important molecule in tolerance[125]; 5) it is highly expressed in uterine NK cells[134] and activated CD4+CD25+ regulatory T cells[85] that have been implicated in maternal-fetal tolerance [125,135,136].

Moreover, a recent study reported that *LGALS1*-null mice show higher rates of fetal loss compared to wild-type mice in allogeneic matings, and treatment with recombinant galectin-1 prevents fetal loss and restores tolerance through various mechanisms, such as the induction of tolerogenic dendritic cells and the expansion of CD4+CD25+ IL-10 secreting regulatory T cells[137]. Thus, galectin-1 expressed by uterine tissues (decidua, myometrium) has been suggested to have a pivotal role in conferring maternal-fetal tolerance[137]. These results mainly relate the effects of galectin-1 to the maternal side of the fetal-maternal interface. However, based on the abundance of galectin-1 in the placenta, especially in the syncytiotrophoblast, our study suggests that galectin-1 may also be important in the fetal immune response.

Placental galectin-1 is up-regulated in severe PE but not in SGA

A novel finding described herein is that both galectin-1 mRNA expression and immunopositivity were significantly increased in the placentas of patients with severe PE. The up-regulation of galectin-1 in severe PE was independent of gestational age, placental histopathologic findings and the presence or absence of SGA; thus, it is most likely to be associated with the exaggerated maternal systemic inflammatory response[41–46,138], which is generally found in PE but may be less developed in pregnancies complicated by SGA or IUGR[2]. The semi-quantitative results of our immunostainings are in accord with those described by a recent study[71] however, we also quantitatively verified these results by measuring galectin-1 mRNA expression in the villi.

An analogy between PE and allograft rejection has recently been proposed [139]. It was reported that in rejected human kidney allograft, galectin-1 was up-regulated in endothelial cells of peritubular capillaries and large vessels in inflammatory regions, at the sites of direct contact between host immune cells and the rejected graft[140]. In the light of this finding, it is not surprising that we found up-regulation of galectin-1 expression in the syncytiotrophoblast, which is also in direct contact with activated maternal leukocytes[42]. Moreover, galectin-1 is a pattern-recognition molecule which operates as a “cell stress sensor” under physiological and pathological conditions[141]. It is up-regulated during cell activation and at the time and loci of acute and chronic inflammation and infection[82–87], where it is proposed to regulate the extent of the immune responses[88]. “Danger signals” and pattern-recognition receptors at the maternal-fetal interface have recently been proposed to create an abnormal placental cytokine milieu[142] and link the activation of the innate immune system and PE [48,49]. Hence, the increased expression of galectin-1 in trophoblasts of patients with severe PE may reflect an enlarged “cellular stress” response.

Recently, an analogy between immune responses to infections and transplants, which involves terminating mechanisms that may help to avoid damage to either normal or infected tissues, has been proposed [143]. The newly evolved 'danger model' of immunity suggests that tissues may have control over immune cells and restrict the class and extent of immune reactions [144,145]. The current approach in the immunology of pregnancy that has challenged the traditional transplantation paradigm also focuses on the unique uterine immune response to the placenta and on the local interactions between placental and maternal immune cells[146]. The increased expression of galectin-1 in the placenta of patients with severe PE is in agreement with these concepts, and may represent a mechanism by which the placenta may control exaggerated immune responses, which include both the fine-tuning of host-pathogen interactions and the maintenance of maternal-fetal immune tolerance.

Conclusions

We report for the first time the placental expression and localization of galectin-1 mRNA, and demonstrate that the protein is abundantly present in third trimester placentas. Placental galectin-1 expression is higher in severe PE than in normal pregnancy, regardless of the presence of SGA. This finding was not observed in patients with SGA alone. We propose that the increased placental expression of galectin-1 in patients with severe PE may represent a local response to systemic maternal inflammation, suggesting that galectin-1 may be implicated in maternal-fetal tolerance.

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Reference List

1. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005;785–99. [PubMed: 15733721]
2. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obstet Gynecol* 2006;40–9. [PubMed: 16813742]
3. Romero R. Prenatal medicine: the child is the father of the man. *Prenatal and Neonatal Medicine* 1996;8–11.
4. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol* 1989;1200–4. [PubMed: 2589440]
5. Bretelle F, Sabatier F, Blann A, D'Ercole C, Boutiere B, Mutin M, Boubli L, Sampol J, Dignat-George F. Maternal endothelial soluble cell adhesion molecules with isolated small for gestational age fetuses: comparison with pre-eclampsia. *BJOG* 2001;1277–82. [PubMed: 11843391]
6. Johnson MR, Anim-Nyame N, Johnson P, Sooranna SR, Steer PJ. Does endothelial cell activation occur with intrauterine growth restriction? *BJOG* 2002;836–9. [PubMed: 12135223]
7. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005;1592–4. [PubMed: 15947178]
8. Poston L. Endothelial dysfunction in pre-eclampsia. *Pharmacol Rep* 2006;69–74. [PubMed: 17332674]
9. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of pre-eclampsia. *J Pathol* 1970;vi.
10. De Wolf F, Brosens I, Renaer M. Fetal growth retardation and the maternal arterial supply of the human placenta in the absence of sustained hypertension. *Br J Obstet Gynaecol* 1980;678–85. [PubMed: 7426529]

11. Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, van Assche A. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol* 1991;648–55. [PubMed: 1883787]
12. Myatt L. Role of placenta in preeclampsia. *Endocrine* 2002;103–11. [PubMed: 12583607]
13. Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig* 2004;342–52.
14. Fisher SJ. The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. *Reprod Biol Endocrinol* 2004;53. [PubMed: 15236649]
15. Lyall F. Priming and remodelling of human placental bed spiral arteries during pregnancy--a review. *Placenta* 2005;S31–S36. [PubMed: 15837064]
16. Espinoza J, Romero R, Mee KY, Kusanovic JP, Hassan S, Erez O, Gotsch F, Than NG, Papp Z, Jai KC. Normal and abnormal transformation of the spiral arteries during pregnancy. *J Perinat Med* 2006;447–58. [PubMed: 17140293]
17. Kupferminc MJ, Daniel Y, Englender T, Baram A, Many A, Jaffa AJ, Gull I, Lessing JB. Vascular endothelial growth factor is increased in patients with preeclampsia. *Am J Reprod Immunol* 1997;302–6. [PubMed: 9352019]
18. Torry DS, Wang HS, Wang TH, Caudle MR, Torry RJ. Preeclampsia is associated with reduced serum levels of placenta growth factor. *Am J Obstet Gynecol* 1998;1539–44. [PubMed: 9855593]
19. Tidwell SC, Ho HN, Chiu WH, Torry RJ, Torry DS. Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. *Am J Obstet Gynecol* 2001;1267–72. [PubMed: 11349200]
20. Torry DS, Mukherjea D, Arroyo J, Torry RJ. Expression and function of placenta growth factor: implications for abnormal placentation. *J Soc Gynecol Investig* 2003;178–88.
21. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;649–58. [PubMed: 12618519]
22. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Kim YM, Goncalves LF, Gomez R, Edwin S. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. *Am J Obstet Gynecol* 2004;1541–7. [PubMed: 15284729]
23. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004;672–83. [PubMed: 14764923]
24. Malamitsi-Puchner A, Boutsikou T, Economou E, Sarandakou A, Makrakis E, Hassiakos D, Creatsas G. Vascular endothelial growth factor and placenta growth factor in intrauterine growth-restricted fetuses and neonates. *Mediators Inflamm* 2005;293–7. [PubMed: 16258196]
25. Bujold E, Romero R, Chaiworapongsa T, Kim YM, Kim GJ, Kim MR, Espinoza J, Goncalves LF, Edwin S, Mazor M. Evidence supporting that the excess of the sVEGFR-1 concentration in maternal plasma in preeclampsia has a uterine origin. *J Matern Fetal Neonatal Med* 2005;9–16. [PubMed: 16105786]
26. Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, Bujold E, Goncalves L, Gomez R, Edwin S, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. *J Matern Fetal Neonatal Med* 2005;3–18. [PubMed: 15804781]
27. Padavala S, Pope N, Baker P, Crocker I. An imbalance between vascular endothelial growth factor and its soluble receptor in placental villous explants of intrauterine growth-restricted pregnancies. *J Soc Gynecol Investig* 2006;40–7.
28. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;992–1005. [PubMed: 16957146]
29. Crispi F, Dominguez C, Llurba E, Martin-Gallan P, Cabero L, Gratacos E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in

- preeclampsia and in isolated intrauterine growth restriction. *Am J Obstet Gynecol* 2006;201–7. [PubMed: 16545329]
30. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;642–9. [PubMed: 16751767]
31. Wallner W, Sengenberger R, Strick R, Strissel PL, Meurer B, Beckmann MW, Schlembach D. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin Sci (Lond)* 2007;51–7. [PubMed: 16928195]
32. Espinoza J, Romero R, Nien JK, Gomez R, Kusanovic JP, Goncalves LF, Medina L, Edwin S, Hassan S, Carstens M, et al. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. *Am J Obstet Gynecol* 2007;326.e1–326.e13. [PubMed: 17403407]
33. Chaiworapongsa T, Espinoza J, Gotsch F, Kim YM, Kim GJ, Goncalves LF, Edwin S, Kusanovic JP, Erez O, Than NG, et al. The maternal plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated in SGA and the magnitude of the increase relates to Doppler abnormalities in the maternal and fetal circulation. *J Matern Fetal Neonatal Med* 2008;25–40. [PubMed: 18175242]
34. Erez O, Romero R, Espinoza J, Fu W, Todem D, Kusanovic JP, Gotsch F, Edwin S, Nien JK, Chaiworapongsa T, et al. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. *J Matern Fetal Neonatal Med* 2008;279–87. [PubMed: 18446652]
35. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazaki-Tovi S, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *J Matern Fetal Neonatal Med* 2008;9–23. [PubMed: 18175241]
36. Robertson WB, Brosens I, Dixon G. Maternal uterine vascular lesions in the hypertensive complications of pregnancy. *Perspect Nephrol Hypertens* 1976;115–27. [PubMed: 1005030]
37. Campbell S, az-Recasens J, Griffin DR, Cohen-Overbeek TE, Pearce JM, Willson K, Teague MJ. New doppler technique for assessing uteroplacental blood flow. *Lancet* 1983;675–7. [PubMed: 6132039]
38. Harrington KF, Campbell S, Bewley S, Bower S. Doppler velocimetry studies of the uterine artery in the early prediction of pre-eclampsia and intra-uterine growth retardation. *Eur J Obstet Gynecol Reprod Biol* 1991;S14–S20. [PubMed: 1809604]
39. Papageorgiou AT, Yu CK, Bindra R, Pandis G, Nicolaides KH. Multicenter screening for pre-eclampsia and fetal growth restriction by transvaginal uterine artery Doppler at 23 weeks of gestation. *Ultrasound Obstet Gynecol* 2001;441–9. [PubMed: 11844162]
40. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of preeclampsia: linking placental ischemia/hypoxia with microvascular dysfunction. *Microcirculation* 2002;147–60. [PubMed: 12080413]
41. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 1998;80–6. [PubMed: 9704769]
42. Gervasi MT, Chaiworapongsa T, Pacora P, Naccasha N, Yoon BH, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in preeclampsia. *Am J Obstet Gynecol* 2001;792–7. [PubMed: 11641653]
43. Redman CW, Sargent IL. The pathogenesis of pre-eclampsia. *Gynecol Obstet Fertil* 2001;518–22. [PubMed: 11575148]
44. Chaiworapongsa T, Yoshimatsu J, Espinoza J, Kim YM, Berman S, Edwin S, Yoon BH, Romero R. Evidence of in vivo generation of thrombin in patients with small-for-gestational-age fetuses and pre-eclampsia. *J Matern Fetal Neonatal Med* 2002;362–7. [PubMed: 12389649]
45. Chaiworapongsa T, Gervasi MT, Refuerzo J, Espinoza J, Yoshimatsu J, Berman S, Romero R. Maternal lymphocyte subpopulations (CD45RA+ and CD45RO+) in preeclampsia. *Am J Obstet Gynecol* 2002;889–93. [PubMed: 12388971]

46. Bartha JL, Romero-Carmona R, Comino-Delgado R. Inflammatory cytokines in intrauterine growth retardation. *Acta Obstet Gynecol Scand* 2003;1099–102. [PubMed: 14616253]
47. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol* 1999;499–506. [PubMed: 9988826]
48. Kim YM, Romero R, Oh SY, Kim CJ, Kilburn BA, Armant DR, Nien JK, Gomez R, Mazor M, Saito S, et al. Toll-like receptor 4: a potential link between “danger signals,” the innate immune system, and preeclampsia? *Am J Obstet Gynecol* 2005;193:921–927. [PubMed: 16157088]
49. Bonney EA. Preeclampsia: a view through the danger model. *J Reprod Immunol* 2007;76:68–74. [PubMed: 17482268]
50. Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, et al. Galectins: A family of animal beta-galactosidebinding lectins. *Cell* 1994;76:597–598. [PubMed: 8124704]
51. Leonidas DD, Elbert BL, Zhou Z, Leffler H, Ackerman SJ, Acharya KR. Crystal structure of human Charcot–Leyden crystal protein, an eosinophil lysophospholipase, identifies it as a new member of the carbohydrate-binding family of galectins. *Structure* 1995;3:1379–1393. [PubMed: 8747464]
52. Visegrady B, Than NG, Kilar F, Sumegi B, Than GN, Bohn H. Homology modelling and molecular dynamics studies of human placental tissue protein 13 (galectin-13). *Protein Eng* 2001;14:875–880. [PubMed: 11742106]
53. Leffler H, Carlsson S, Hedlund M, Qian Y, Poirier F. Introduction to galectins. *Glycoconj J* 2004;19:433–440. [PubMed: 14758066]
54. Houzelstein D, Goncalves IR, Fadden AJ, Sidhu SS, Cooper DN, Drickamer K, Leffler H, Poirier F. Phylogenetic analysis of the vertebrate galectin family. *Mol Biol Evol* 2004;21:1177–1187. [PubMed: 14963092]
55. Buzas EI, Gyorgy B, Pasztoi M, Jelinek I, Falus A, Gabius HJ. Carbohydrate recognition systems in autoimmunity. *Autoimmunity* 2006;39:691–704. [PubMed: 17178566]
56. Lopez-Lucendo MF, Solis D, Andre S, Hirabayashi J, Kasai K, Kaltner H, Gabius HJ, Romero A. Growthregulatory human galectin-1: Crystallographic characterisation of the structural changes induced by single-site mutations and their impact on the thermodynamics of ligand binding. *J Mol Biol* 2004;343:957–970. [PubMed: 15476813]
57. Brewer FC. Binding and cross-linking properties of galectins. *Biochim Biophys Acta* 2002;255–62.
58. Rabinovich GA, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, Iacobelli S. Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* 2002;313–20. [PubMed: 12072371]
59. Elola MT, Chiesa ME, Alberti AF, Mordoh J, Fink NE. Galectin-1 receptors in different cell types. *J Biomed Sci* 2005;13–29. [PubMed: 15864736]
60. Kasai K, Hirabayashi J. Galectins: a family of animal lectins that decipher glycocodes. *J Biochem (Tokyo)* 1996;1–8. [PubMed: 8907168]
61. Villalobo A, Gabius H. Signaling pathways for transduction of the initial message of the glyocode into cellular responses. *Acta Anat (Basel)* 1998;110–29. [PubMed: 9780354]
62. Gabius HJ, Andre S, Kaltner H, Siebert HC. The sugar code: functional lectinomics. *Biochim Biophys Acta* 2002;165–77. [PubMed: 12223267]
63. Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: a small protein with major functions. *Glycobiology* 2006;137–57.
64. Hirabayashi J, Kasai K. Human placenta beta-galactoside-binding lectin. Purification and some properties. *Biochem Biophys Res Commun* 1984;938–44. [PubMed: 6477573]
65. Hirabayashi J, Kasai K. Complete amino acid sequence of a beta-galactoside-binding lectin from human placenta. *J Biochem (Tokyo)* 1988;1–4.
66. Couraud PO, Casentini-Borocz D, Bringman TS, Griffith J, McGrogan M, Nedwin GE. Molecular cloning, characterization, and expression of a human 14-kDa lectin. *J Biol Chem* 1989;1310–6. [PubMed: 2910856]
67. Bevan BH, Kilpatrick DC, Liston WA, Hirabayashi J, Kasai K. Immunohistochemical localization of a beta-D-galactoside-binding lectin at the human maternofetal interface. *Histochem J* 1994;582–6. [PubMed: 7960935]

68. Walzel H, Neels P, Bremer H, Kohler H, Raab N, Barten M, Brock J. Immunohistochemical and glycohistochemical localization of the beta-galactoside-binding S-type lectin in human placenta. *Acta Histochem* 1995;97:33–42. [PubMed: 7539568]
69. Maquoi E, van den Brule FA, Castronovo V, Foidart JM. Changes in the distribution pattern of galectin-1 and galectin-3 in human placenta correlates with the differentiation pathways of trophoblasts. *Placenta* 1997;18:433–439. [PubMed: 9250706]
70. Vicovac L, Jankovic M, Cuperlovic M. Galectin-1 and -3 in cells of the first trimester placental bed. *Hum Reprod* 1998;13:730–735. [PubMed: 9572443]
71. Jeschke U, Mayr D, Schiessl B, Mylonas I, Schulze S, Kuhn C, Friese K, Walzel H. Expression of galectin-1, -3 (gal-1, gal-3) and the Thomsen–Friedenreich (TF) antigen in normal, IUGR, preeclamptic and HELLP placentas. *Placenta* 2007;28:1165–1173. [PubMed: 17664004]
72. Dettin L, Rubinstein N, Aoki A, Rabinovich GA, Maldonado CA. Regulated expression and ultrastructural localization of galectin-1, a proapoptotic beta-galactoside-binding lectin, during spermatogenesis in rat testis. *Biol Reprod* 2003;51–9. [PubMed: 12493695]
73. Sakaguchi M, Shingo T, Shimazaki T, Okano HJ, Shiwa M, Ishibashi S, Oguro H, Ninomiya M, Kadoya T, Horie H, et al. A carbohydrate-binding protein, Galectin-1, promotes proliferation of adult neural stem cells. *Proc Natl Acad Sci U S A* 2006;7112–7. [PubMed: 16636291]
74. Rubinstein N, Alvarez M, Zwirner NW, Toscano MA, Ilarregui JM, Bravo A, Mordoh J, Fainboim L, Podhajcer OL, Rabinovich GA. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; A potential mechanism of tumor-immune privilege. *Cancer Cell* 2004;241–51. [PubMed: 15050916]
75. He J, Baum LG. Presentation of galectin-1 by extracellular matrix triggers T cell death. *J Biol Chem* 2004;4705–12. [PubMed: 14617626]
76. Rabinovich GA, Sotomayor CE, Riera CM, Bianco I, Correa SG. Evidence of a role for galectin-1 in acute inflammation. *Eur J Immunol* 2000;1331–9. [PubMed: 10820379]
77. Offner H, Celnik B, Bringman TS, Casentini-Borocz D, Nedwin GE, Vandenbark AA. Recombinant human beta-galactoside binding lectin suppresses clinical and histological signs of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 1990;177–84. [PubMed: 1694534]
78. Rabinovich GA, Daly G, Dreja H, Tailor H, Riera CM, Hirabayashi J, Chernajovsky Y. Recombinant galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. *J Exp Med* 1999;385–98. [PubMed: 10430627]
79. Santucci L, Fiorucci S, Cammilleri F, Servillo G, Federici B, Morelli A. Galectin-1 exerts immunomodulatory and protective effects on concanavalin A-induced hepatitis in mice. *Hepatology* 2000;399–406. [PubMed: 10655263]
80. Toscano MA, Commodaro AG, Ilarregui JM, Bianco GA, Liberman A, Serra HM, Hirabayashi J, Rizzo LV, Rabinovich GA. Galectin-1 suppresses autoimmune retinal disease by promoting concomitant Th2- and T regulatory-mediated anti-inflammatory responses. *J Immunol* 2006;6323–32. [PubMed: 16670344]
81. Baum LG, Blackall DP, Irias-Magallano S, Nanigian D, Uh SY, Browne JM, Hoffmann D, Emmanouilides CE, Territo MC, Baldwin GC. Amelioration of graft versus host disease by galectin-1. *Clin Immunol* 2003;295–307. [PubMed: 14697744]
82. Baum LG, Seilhamer JJ, Pang M, Levine WB, Beynon D, Berliner JA. Synthesis of an endogenous lectin, galectin-1, by human endothelial cells is up-regulated by endothelial cell activation. *Glycoconj J* 1995;63–8. [PubMed: 7795414]
83. Kim CW, Cho EH, Lee YJ, Kim YH, Hah YS, Kim DR. Disease-specific proteins from rheumatoid arthritis patients. *J Korean Med Sci* 2006;478–84. [PubMed: 16778393]
84. Rabinovich GA, Iglesias MM, Modesti NM, Castagna LF, Wolfenstein-Todel C, Riera CM, Sotomayor CE. Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells: biochemical and functional characterization. *J Immunol* 1998;4831–40. [PubMed: 9590230]
85. Zuniga E, Gruppi A, Hirabayashi J, Kasai KI, Rabinovich GA. Regulated expression and effect of galectin-1 on *Trypanosoma cruzi*-infected macrophages: modulation of microbicidal activity and survival. *Infect Immun* 2001;6804–12. [PubMed: 11598053]

86. Garin MI, Chu CC, Golshayan D, Cernuda-Morollon E, Wait R, Lechler RI. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. *Blood* 2007;2058–65. [PubMed: 17110462]
87. Gil CD, Cooper D, Rosignoli G, Perretti M, Oliani SM. Inflammation-induced modulation of cellular galectin-1 and -3 expression in a model of rat peritonitis. *Inflamm Res* 2006;99–107. [PubMed: 16673152]
88. Rabinovich GA, Gruppi A. Galectins as immunoregulators during infectious processes: from microbial invasion to the resolution of the disease. *Parasite Immunol* 2005;103–14. [PubMed: 15910418]
89. Sibai BM, Ewell M, Levine RJ, Klebanoff MA, Esterlitz J, Catalano PM, Goldenberg RL, Joffe G. Risk factors associated with preeclampsia in healthy nulliparous women. The Calcium for Preeclampsia Prevention (CPEP) Study Group. *Am J Obstet Gynecol* 1997;1003–10. [PubMed: 9396883]
90. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 2000;S1–S22.
91. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol* 1996;163–8. [PubMed: 8559516]
92. Fu WJ, Hu J, Spencer T, Carroll R, Wu G. Statistical models in assessing fold change of gene expression in real-time RT-PCR experiments. *Comput Biol Chem* 2006;21–6. [PubMed: 16321570]
93. Bonferroni C. Il calcolo delle assicurazioni su gruppi di teste. In *Studi in Onore del Professore Salvatore Ortu Carboni*. Rome 1935;13–60.
94. Redline RW, Boyd T, Campbell V, Hyde S, Kaplan C, Khong TY, Prashner HR, Waters BL. Maternal vascular underperfusion: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol* 2004;237–49. [PubMed: 15022063]
95. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation--a workshop report. *Placenta* 2005;S114–S117. [PubMed: 15837060]
96. Ozeki Y, Matsui T, Yamamoto Y, Funahashi M, Hamako J, Titani K. Tissue fibronectin is an endogenous ligand for galectin-1. *Glycobiology* 1995;255–61. [PubMed: 7780201]
97. Phillips B, Knisley K, Weitlauf KD, Dorsett J, Lee V, Weitlauf H. Differential expression of two beta-galactoside-binding lectins in the reproductive tracts of pregnant mice. *Biol Reprod* 1996;548–58. [PubMed: 8862771]
98. Iglesias MM, Rabinovich GA, Ivanovic V, Sotomayor C, Wolfenstein-Todel C. Galectin-1 from ovine placenta--amino-acid sequence, physicochemical properties and implications in T-cell death. *Eur J Biochem* 1998;400–7. [PubMed: 9546655]
99. Prior IA, Muncke C, Parton RG, Hancock JF. Direct visualization of Ras proteins in spatially distinct cell surface microdomains. *J Cell Biol* 2003;165–70. [PubMed: 12527752]
100. Nickel W. Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells. *Traffic* 2005;607–14. [PubMed: 15998317]
101. Jeschke U, Karsten U, Wiest I, Schulze S, Kuhn C, Frieze K, Walzel H. Binding of galectin-1 (gal-1) to the Thomsen-Friedenreich (TF) antigen on trophoblast cells and inhibition of proliferation of trophoblast tumor cells in vitro by gal-1 or an anti-TF antibody. *Histochem Cell Biol* 2006;437–44. [PubMed: 16607538]
102. Moiseeva EP, Williams B, Goodall AH, Samani NJ. Galectin-1 interacts with beta-1 subunit of integrin. *Biochem Biophys Res Commun* 2003;1010–6. [PubMed: 14550305]
103. Peters DG, Kassam AB, Feingold E, Heidrich-O'Hare E, Yonas H, Ferrell RE, Brufsky A. Molecular anatomy of an intracranial aneurysm: coordinated expression of genes involved in wound healing and tissue remodeling. *Stroke* 2001;1036–42. [PubMed: 11283408]
104. Lotan R, Belloni PN, Tressler RJ, Lotan D, Xu XC, Nicolson GL. Expression of galectins on microvessel endothelial cells and their involvement in tumour cell adhesion. *Glycoconj J* 1994;462–8. [PubMed: 7696849]
105. Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. *Nature* 1995;736–9. [PubMed: 7501023]
106. La M, Cao TV, Cerchiaro G, Chilton K, Hirabayashi J, Kasai K, Oliani SM, Chernajovsky Y, Perretti M. A novel biological activity for galectin-1: inhibition of leukocyte-endothelial cell interactions in experimental inflammation. *Am J Pathol* 2003;1505–15. [PubMed: 14507657]

107. Danese S, Dejana E, Fiocchi C. Immune regulation by microvascular endothelial cells: directing innate and adaptive immunity, coagulation, and inflammation. *J Immunol* 2007;6017–22. [PubMed: 17475823]
108. He J, Baum LG. Endothelial cell expression of galectin-1 induced by prostate cancer cells inhibits T-cell transendothelial migration. *Lab Invest* 2006;578–90. [PubMed: 16607379]
109. Frauli M, Ludwig H. Demonstration of the ability of Hofbauer cells to phagocytose exogenous antibodies. *Eur J Obstet Gynecol Reprod Biol* 1987;135–44. [PubMed: 3311844]
110. Zaccheo D, Pistoia V, Castellucci M, Martinoli C. Isolation and characterization of Hofbauer cells from human placental villi. *Arch Gynecol Obstet* 1989;189–200. [PubMed: 2482706]
111. Matsubara S, Takayama T, Yamada T, Usui R, Izumi A, Watanabe T, Ohkuchi A, Shibahara H, Sato I, Suzuki M. Hofbauer cell activation and its increased glucose-6-phosphate dehydrogenase activity in second trimester-spontaneous abortion: an ultrastructural dual staining enzyme-cytochemical study. *Am J Reprod Immunol* 2003;202–9. [PubMed: 12852494]
112. Taniguchi T, Matsuzaki N, Kameda T, Shimoya K, Jo T, Saji F, Tanizawa O. The enhanced production of placental interleukin-1 during labor and intrauterine infection. *Am J Obstet Gynecol* 1991;131–7. [PubMed: 1853890]
113. Saito S, Kasahara T, Sakakura S, Umekage H, Harada N, Ichijo M. Detection and localization of interleukin-8 mRNA and protein in human placenta and decidual tissues. *J Reprod Immunol* 1994;161–72. [PubMed: 7738906]
114. Blumenstein M, Keelan JA, Bowen-Shauver JM, Mitchell MD. Suppressors of cytokine signaling proteins in human preterm placental tissues 169. *J Mol Endocrinol* 2005;165–75. [PubMed: 16087730]
115. Moussa M, Mognetti B, Dubanchet S, Menu E, Roques P, Dormont D, Barre-Sinoussi F, Chaouat G. Expression of beta chemokines in explants and trophoblasts from early and term human placentae. *Am J Reprod Immunol* 2001;309–17. [PubMed: 11712758]
116. Correa SG, Sotomayor CE, Aoki MP, Maldonado CA, Rabinovich GA. Opposite effects of galectin-1 on alternative metabolic pathways of L-arginine in resident, inflammatory, and activated macrophages. *Glycobiology* 2003;119–28. [PubMed: 12626408]
117. Barrionuevo P, Beigier-Bompadre M, Ilarregui JM, Toscano MA, Bianco GA, Isturiz MA, Rabinovich GA. A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. *J Immunol* 2007;436–45. [PubMed: 17182582]
118. Than, GN.; Bohn, H.; Szabo, DG. *Advances in pregnancy-related protein research*. CRC Press; Boca Raton, FL: 1993. p. 1-333.
119. Chaouat G, Ledee-Bataille N, Zourbas S, Ostojic S, Dubanchet S, Martal J, Frydman R. Cytokines, implantation and early abortion: re-examining the Th1/Th2 paradigm leads to question the single pathway, single therapy concept. *Am J Reprod Immunol* 2003;177–86. [PubMed: 14629021]
120. Keelan JA, Mitchell MD. Placental cytokines and preeclampsia. *Front Biosci* 2007;2706–27. [PubMed: 17127274]
121. Hunt JS, Vassmer D, Ferguson TA, Miller L. Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. *J Immunol* 1997;4122–8. [PubMed: 9126971]
122. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 1998;1191–3. [PubMed: 9712583]
123. Phillips TA, Ni J, Pan G, Ruben SM, Wei YF, Pace JL, Hunt JS. TRAIL (Apo-2L) and TRAIL receptors in human placentas: implications for immune privilege. *J Immunol* 1999;6053–9. [PubMed: 10229846]
124. Kayisli UA, Selam B, Guzeloglu-Kayisli O, Demir R, Arici A. Human chorionic gonadotropin contributes to maternal immunotolerance and endometrial apoptosis by regulating Fas-Fas ligand system. *J Immunol* 2003;2305–13. [PubMed: 12928375]
125. Niederkorn JY. See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat Immunol* 2006;354–9. [PubMed: 16550198]

126. Lei ZM, Yang M, Li X, Takikawa O, Rao CV. Upregulation of placental indoleamine 2,3-dioxygenase by human chorionic gonadotropin. *Biol Reprod* 2007;639–44. [PubMed: 17182891]
127. Rabinovich GA, Ariel A, Hershkovich R, Hirabayashi J, Kasai KI, Lider O. Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. *Immunology* 1999;100–6. [PubMed: 10447720]
128. van der Leij J, van den Berg A, Harms G, Eschbach H, Vos H, Zwiers P, van Weeghel R, Groen H, Poppema S, Visser L. Strongly enhanced IL-10 production using stable galectin-1 homodimers. *Mol Immunol* 2007;506–13. [PubMed: 16581128]
129. Rorive S, Belot N, Decaestecker C, Lefranc F, Gordower L, Micik S, Maurage CA, Kaltner H, Ruchoux MM, Danguy A, et al. Galectin-1 is highly expressed in human gliomas with relevance for modulation of invasion of tumor astrocytes into the brain parenchyma. *Glia* 2001;241–55. [PubMed: 11241742]
130. Camby I, Belot N, Rorive S, Lefranc F, Maurage CA, Lahm H, Kaltner H, Hadari Y, Ruchoux MM, Brotchi J, et al. Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. *Brain Pathol* 2001;11:12–26. [PubMed: 11145198]
131. Le QT, Shi G, Cao H, Nelson DW, Wang Y, Chen EY, Zhao S, Kong C, Richardson D, O'Byrne KJ, et al. Galectin-1: A link between tumor hypoxia and tumor immune privilege. *J Clin Oncol* 2005;23:8932–8941. [PubMed: 16219933]
132. Saussez S, Lorfevre F, Lequeux T, Laurent G, Chantrain G, Vertongen F, Toubeau G, Decaestecker C, Kiss R. The determination of the levels of circulating galectin-1 and -3 in HNSCC patients could be used to monitor tumor progression and/or responses to therapy. *Oral Oncol* 2008;44:86–93. [PubMed: 17350328]
133. Daroqui CM, Ilarregui JM, Rubinstein N, Salatino M, Toscano MA, Vazquez P, Bakin A, Puricelli L, Bal de Kier JE, Rabinovich GA. Regulation of galectin-1 expression by transforming growth factor beta1 in metastatic mammary adenocarcinoma cells: implications for tumor-immune escape. *Cancer Immunol Immunother* 2007;491–9. [PubMed: 16900348]
134. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003;1201–12. [PubMed: 14568979]
135. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004;266–71. [PubMed: 14758358]
136. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. *Nat Immunol* 2006;241–6. [PubMed: 16482172]
137. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, et al. A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* 2007;1450–7. [PubMed: 18026113]
138. Than NG, Wildman DE, Erez O, Edwin SS, Espinoza J, Kim CJ, Han YM, et al. Trophoblast, galectin-1 and pre-eclampsia. *Am J Obstet Gynecol* 2006;195.
139. Wilczynski JR. Immunological analogy between allograft rejection, recurrent abortion and pre-eclampsia - the same basic mechanism? *Hum Immunol* 2006;492–511. [PubMed: 16829304]
140. van der Leij J, van den Berg A, Blokzijl T, Harms G, van Goor H, Zwiers P, van Weeghel R, Poppema S, Visser L. Dimeric galectin-1 induces IL-10 production in T-lymphocytes: an important tool in the regulation of the immune response. *J Pathol* 2004;511–8. [PubMed: 15538736]
141. Fuertes MB, Molinero LL, Toscano MA, Ilarregui JM, Rubinstein N, Fainboim L, Zwirner NW, Rabinovich GA. Regulated expression of galectin-1 during T-cell activation involves Lck and Fyn kinases and signaling through MEK1/ERK, p38 MAP kinase and p70S6 kinase. *Mol Cell Biochem* 2004;177–85. [PubMed: 15663199]
142. Keelan JA, Marvin KW, Sato TA, Coleman M, McCowan LM, Mitchell MD. Cytokine abundance in placental tissues: evidence of inflammatory activation in gestational membranes with term and preterm parturition. *Am J Obstet Gynecol* 1999;1530–6. [PubMed: 10601939]
143. Starzl TE, Zinkernagel RM. Antigen localization and migration in immunity and tolerance. *N Engl J Med* 1998;1905–13. [PubMed: 9862947]

144. Matzinger P. The danger model: A renewed sense of self. *Science* 2002;296:301–305. [PubMed: 11951032]
145. Sato S. Galectins as molecules of danger signal, which could evoke an immune response to infection. *Trends Glycosci Glyc* 2002;14:285–301.
146. Moffett A, Loke C. Immunology of placentation in eutherian mammals. *Nat Rev Immunol* 2006;6:584–594. [PubMed: 16868549]

Figure 1. Pleiotropic effects of galectin-1 on immune cells

Galectin-1 is up-regulated in inflammation and infection and contributes to the regulation of immune cells in physiological and pathological conditions. Galectin-1 is involved in both the adaptive and innate immune responses and predominantly exerts anti-inflammatory effects on different immune cell types. [X-ray crystallographic data of galectin-1 (1GZW)[56] was accessed at the MMDB Database (NCBI, NLM, NIH, Bethesda, MD, USA) and the ribbon diagram was generated with Cn3D and Adobe Photoshop 7.0. The jelly-roll structure of galectin-1 includes two antiparallel β -sheets (F1–F5 in yellow; S1–S6a/b in red); the carbohydrate-recognition domains encompass the S4–S6a/S6b sheets on the concave face of the subunits].

Figure 2. Localization of galectin-1 mRNA and protein in normal term placentas

(A) Galectin-1 mRNA hybridization signal was distinct in villous stromal cells and in the trophoblastic layers, especially in cytotrophoblasts. Inlet: there was no hybridization signal with antisense control (mRNA *in situ* hybridization, 20x magnification). (B) The syncytiotrophoblast apical membrane, villous capillary endothelial cells and stromal cells were strongly immunopositive, while the villous stroma was weakly stained. Galectin-1 staining is shown in red, nuclei are in green. Inlet: there was only nuclear staining when applying isotype control primary antibody (Immunofluorescence confocal microscopy, 40x magnification, fluorescence and DIC combination images).

Figure 3. Placental galectin-1 mRNA expression is increased in severe preeclampsia

Placental galectin-1 mRNA expression was significantly increased in severe preeclampsia (1.44 fold, $p=0.003$) and in severe preeclampsia complicated by SGA (1.47 fold, $p=0.004$) when compared to gestational age matched controls. Galectin-1 expression was significantly higher in severe preeclampsia with or without SGA than in SGA without preeclampsia (1.68 fold, $p=0.001$; 1.64 fold, $p=0.001$; respectively). There was no difference in galectin-1 mRNA expression between SGA and controls ($p=1.00$) and the two severe preeclampsia groups ($p=1.00$).

Figure 4. Placental galectin-1 immunofluorescence signal is increased in severe preeclampsia
(A) In term controls (38 weeks), the syncytiotrophoblast apical membrane, capillary endothelial cells and stromal cells had the strongest galectin-1 immunopositivity. **(B)** In term SGA (39 weeks), galectin-1 immunopositivity was similar in its extent and pattern to that seen in controls. **(C)** In severe preeclampsia complicated with SGA (39 weeks), galectin-1 immunofluorescence signal was intense, predominantly in the trophoblastic layer, stromal cells and the villous stroma. **(D)** Similarly, in severe preeclampsia without SGA (37 weeks), trophoblastic, stromal cell and stromal galectin-1 immunofluorescence staining was the strongest. Galectin-1 staining is shown in red, nuclei are in green (Immunofluorescence confocal microscopy, 40x magnifications, fluorescence and DIC combination images).

Figure 5. Placental galectin-1 is increased in severe preeclampsia

15 ng of recombinant human galectin-1 and 30 μ g of villous tissue lysates were electrophoresed on 15% (w/v) SDS-polyacrylamide gel. Proteins were electroblotted and probed with anti-human galectin-1 IgG. All samples used for the representative image were taken from placentas delivered at 39 weeks of gestation. Lane 1: recombinant galectin-1; lane 2: control; lane 3: SGA; lane 4: severe preeclampsia; lane 5: severe preeclampsia complicated with SGA. Galectin-1 migrated as a single 15 kDa band in all lanes. Galectin-1 immunopositive signal was the strongest in severe preeclampsia complicated with SGA.

Table 1

Demographic and clinical characteristics of the study population

	Controls (n=10)	SGA (n=10)	Severe PE (n=10)	Severe PE with SGA (n=10)	p-value
Preterm cases (< 37 weeks)	5	5	5	5	-
Term cases (≥ 37 weeks)	5	5	5	5	NS ²
Maternal age (years)	21 [20–33]	27 [19–29]	30 [23–35]	24 [21–34]	NS ¹
Nulliparity	4	1	3	7	NS ³
Maternal weight (kg)	86 [65–112]	71 [60–94]	97 [89–103]	106 [82–119]	NS ³
Body mass index (kg/m ²)	33.2 [22.4–41.2]	27.3 [24.1–33.7]	35.7 [33.7–36.9]	35.5 [30.1–43.3]	NS ¹
African-American origin	5	6	7	9	NS ¹
Gestational age at delivery (weeks)	37.1 [31.4–39.9]	36.6 [31.8–38.5]	36.1 [29.3–38]	36.4 [30.6–37.9]	NS ²
Birth-weight (g)	2970 [1694–3358]	2165 [1378–2464]	2805 [1193–3343]	1765 [1130–2213]	NS ²
Highest measured systolic blood pressure (mm Hg)	117 [111–130]	112 [108–137]	177 [171–182]	178 [159–198]	<0.001 ^{2*}
Highest measured diastolic blood pressure (mm Hg)	72 [61–79]	72 [60–87]	104 [99–111]	107 [96–109]	<0.001 ^{2*}
Chronic hypertension	-	2	1	5	<0.05 ^{2*}
Cesarean section	2	4	6	7	NS ¹

SGA, small-for-gestational age; PE, preeclampsia; NS, not significant. Values are presented as median [interquartile range] or number.

Comparisons were performed among all groups with:

* Statistically significant.

¹ Fisher's exact test

² Kruskal-Wallis test

³ one-way ANOVA test.