‘Sweetening’ Pregnancy: Galectins at the Fetomaternal Interface

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Successful mammalian pregnancy relies upon acceptance of a semi-allogeneic fetus by the maternal immune system. Lessons learned from studies on protective immunity to microbial infections and tumours, prevention of autoimmunity, and allograft rejection have contributed to delineate the mechanisms leading to T-cell tolerance at the fetomaternal interface. Recent observations highlight the contribution of galectins, a family of endogenous glycan-binding proteins, to critical biological events occurring during mammalian gestation, including immune cell tolerance, inflammation, implantation, and angiogenesis. These multifunctional lectins can hierarchically control a cascade of immunoregulatory events including the expansion, recruitment, and function of regulatory T cells, the promotion of tolerogenic dendritic cells, and the execution of T-cell death programs. In addition, galectins can control cell adhesion and signaling events critical for implantation and are involved in fundamental processes linking tissue hypoxia to angiogenesis. In an attempt to integrate the regulatory roles of galectins to immunological and vascular programs operating during pregnancy. Here we outline the regulated expression and function of individual members of the galectin family within the fetoplacental unit and their biological implications for the development and preservation of successful pregnancies.

Mechanisms leading to fetomaternal tolerance

Homeostatic signals delivered in the form of immunosuppressive cytokines or inhibitory receptors are integrated into a cascade of regulatory circuits that sustain immune tolerance at the fetomaternal interface.[1,2] Several tolerogenic mechanisms contribute to prevent fetal damage resulting from overexuberant immune responses to fetal antigens.[1] These include a shift toward a T helper (Th) type-2 cytokine profile,[3,4] differentiation, and/or recruitment of $CD4^+$ $CD25^+$ $FoxP3^+$ T regulatory (T_{reg}) cells[5–8] and expansion of uterine immunomodulatory natural killer (uNK) cells.[9] Moreover, trophoblast cells express inhibitory signals such as programed death-ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase (IDO), synthesize immunosuppressive cytokines such as transforming growth factor-β (TGF-β) and IL-10[10] and promote death of uterine T cells via the Fas ligand (Fas L) and tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) pathways.[11,12] Furthermore, cells that integrate the fetoplacental unit can elude allorecognition by expression of ‘non-classical’ HLA molecules[1] and can

elaborate a variety of immunomodulatory neuropeptides including vasoactive intestinal peptide.[13–15] Additionally, a functional cross-talk between immune and endocrine systems, mediated by progesterone, progesterone-induced blocking factor, and human chorionic gonadotropin (hCG) has been reported to contribute to immune privilege during pregnancy.[16,17] Although there is still no integrated portrait of these regulatory circuits, disruption of single regulatory pathways may lead to substantial and unpredictable inflammatory responses that induce fetal loss.[18,19] Here, we outline the contribution of galectins to the promotion of fetomaternal tolerance and the development and preservation of a successful pregnancy.

Glycans and glycan-binding proteins in pregnancy

A multiplicity of regulatory mechanisms involving both mother and embryo cells govern embryo implantation, immune tolerance, and establishment of a functional vascularized placenta. These dynamic processes involve the secretion of a variety of cytokines, chemokines, hormones, and growth factors and a network of specialized cells, including trophoblast cells, epithelial cells, decidual stromal cells, endothelial cells and immune cells.[20] As glycans and glycan-binding proteins play key roles in immune cell communication and signaling, their involvement in pregnancy-related processes is not surprising. In fact, the enormous variety of glycans that cover the surfaces of mammalian cells have the potential of storing critical biological information that is decoded by a large number of glycan-binding proteins or lectins.[21] An interesting example illustrating this concept is the complex mucin MUC1. When a mammalian blastocyst penetrates the uterine cavity, the surface epithelium of the uterus is extensively covered by MUC1 which prevents the attachment of the highly adhesive blastocyst to an improper site. Indeed, in the human endometrium, MUC1 is upregulated during the implantation period.[22] This suggests that the human endometrial surface epithelium prevents blastocyst adhesion except for the precise place where the embryo attaches. It has been hypothesized that proinflammatory cytokines released in the uterine stroma induce local degradation of MUC1 which enables the blastocyst to attach to a specific area of the uterus.[22] Whether lectin–glycan interactions participate in MUC1-driven control of the implantation process remains unknown.

Galectins: key regulators of cellular homeostasis

Emerging evidence indicates that interactions between endogenous glycan-binding proteins and glycosylated receptors are integral for the control of immunological homeostasis.[21] Although distinct lectin families have been described that contribute to modulate innate and adaptive immune responses, including C-type lectins and siglecs,[23] we will focus here on an ancient family of glycan-binding proteins, termed galectins, which have emerged as central regulators of fetomaternal tolerance.

Galectins specifically recognize complex glycan determinants on cell surface glycoproteins with relatively high affinity in the submicromolar range.[24] It has been demonstrated that it is the structure, number and density of glycan epitopes in multivalent glycoproteins, as well as the density of the glycoproteins expressed on the cell surface and the multivalent nature of some galectins, which together determine the avidity of lectin–glycan interactions.[25] Multivalent galectin-glycan complexes have been proposed to serve as scaffolds for organizing plasma membrane domains, which in turn modulate the signaling threshold of relevant surface glycoproteins including the T-cell receptor (TCR), B-cell receptor (BCR) and specific cytokine receptors.[26]

Although galectins do not have the signal sequence required for the classical secretory pathway, most of them are externalized through a nonclassical mechanism and function in the extracellular milieu by interacting with a myriad of glycosylated ligands on the cell surface and extracellular matrix.[24,27] However, these lectins also play roles inside the cells including modulation of signaling and splicing machineries.[28] According to their structure, galectins are classified into 'prototype' galectins (galectin-1, -2, -5, -7, -10, -11, -13, -14 and -15) which have one carbohydrate recognition domain (CRD) that can dimerize, 'tandem-repeat' galectins (galectin-4, -6, -8, -9 and -12) which contain two homologous CRDs in tandem in a single polypeptide chain and galectin-3, which is unique as it contains a CRD connected to a non-lectin N-terminal region that is responsible for oligomerization[29] (Fig. 1).

Although galectins were originally defined by their ability to recognize the disaccharide N-acetyllactosamine [Galβ(1–4)-GlcNAc; LacNAc], evidence obtained from glycan microarrays and frontal affinity chromatography analysis revealed substantial differences in the glycan-binding preferences of individual

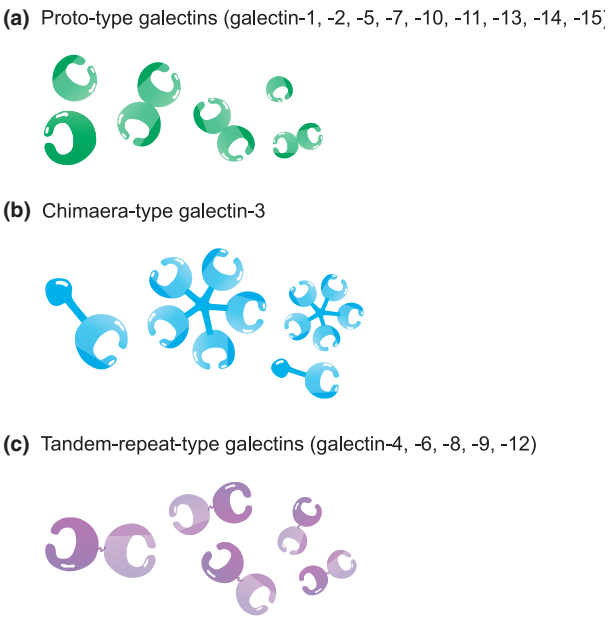

Fig. 1 Structure and classification of the galectin family. Galectin are subdivided into three groups: those containing one CRD; galectin-3, which consists of unusual tandem repeats of proline- and glycine-rich short stretches fused onto the CRD; and those containing two distinct CRDs in tandem, connected by a linker peptide. Many galectins are either bivalent or multivalent with regard to their carbohydrate-binding activities: one CRD galectins often exist as dimers; galectin-3 forms pentamers upon binding to multivalent carbohydrates and two CRD galectins have two carbohydrate-binding sites.

members of the galectin family, which might explain differences in biological activity.[27,30,31] Noteworthy, in addition to their regulated expression in immune-privileged sites and inflammatory microenvironments, different intrinsic and extrinsic factors may control the biological activity of galectins including (i) their subcellular compartmentalization, (ii) their oligomerization status, (iii) their stability in reducing or oxidative microenvironments and (iv) the active remodeling of N- and O-glycans on target cells.[27,32]

Interestingly, while some galectins are widely expressed, either constitutively or in an inducible fashion in immune cells and tissues, others have a more limited cellular distribution. For example, galectin-1 is considerably upregulated in activated T and B cells, inflammatory macrophages, tolerogenic DCs, decidual NK cells, and $CD4^+$ $CD25^+$ T_{Reg} cells, whereas galectin-10 expression appears to be restricted to eosinophils and $CD4^+$ $CD25^+$ T_{Reg} cells, galectin-5 is selectively expressed in rat reticulocytes, galectin-13 (so called 'placental protein 13' or PP13) is expressed in human placenta, and galectin-15 appears to be preferentially expressed in ovine placental tissue.[27] In the next sections, we will integrate pioneer work and recent findings that contributed to our understanding of the expression and function of galectins during inflammation and pregnancy.

Galectins in pregnancy: decision-makers in fetomaternal tolerance, inflammation, and implantation

During the past few years, there has been increasing appreciation for the impact of galectin–glycan interactions in the control of immune cell homeostasis.[21] Although galectins have been mostly studied in the context of pathologic responses including autoimmune pathology, microbial infection, or tumour growth, their biological roles in physiologic settings, including pregnancy, are just emerging. Here, we will dissect the general roles of individual members of the galectin family with particular emphasis on their function in the context of reproduction and pregnancy.

Galectin-1

Galectin-1, a one CRD galectin, functions as a monomer or a dimer and is highly sensitive to the redox fluctuations in inflammatory microenvironments.[32] This endogenous lectin elicits a broad spectrum of immunoregulatory activities *in vivo* including the attenuation of autoimmune pathology,[33–36] control of inflammation-induced neurodegeneration,[37] resolution of cardiac inflammation during myocardial infarction,[38] and promotion of tumour immune escape.[39–43] Mice lacking galectin-1 show greater Th1 and Th17 responses, higher frequency of immunogenic dendritic cells, aberrant microglia activation and display more severe autoimmune pathology compared with their wild-type counterpart.[35,37,44,45] Mechanistically, galectin-1 selectively kills Th1 and Th17 cell subsets through glycosylation-dependent mechanisms,[35] modulates T-cell adhesion and trafficking,[46–48] promotes the expansion of $FoxP3^+$ or $FoxP3^-$ regulatory T (T_{reg}) cells,[34,40,43] induces IL-10 secretion[49–51] and promotes the differentiation of IL-27^+ IL-10^+ tolerogenic dendritic cells.[44]

Research over the past few years has identified essential roles of galectin-1 during reproduction and pregnancy.[52,53] Galectin-1 is abundant in the

placenta, in reproductive tracts and developing embryo of various species.[54–61] Moreover, this 14.5-kDa β-galactoside-binding lectin is substantially increased in FoxP3$^+$ T_{reg} cells,[62] tolerogenic dendritic cells,[44] activated T cells,[63] inflammatory macrophages,[64] and uterine NK cells.[9,65] Within reproductive tracts and the fetoplacental unit, galectin-1 is mainly expressed in the late-secretory phase endometrium,[59] invasive extravillous trophoblast cells, maternal decidual cells and to some extent also in the villous mesenchyme of human first trimester and term placenta.[66,67] Moreover, human embryos undergoing pre-implantation development express galectin-1 on the trophectoderm and inner cell mass.[61] Galectin-1 is initially synthesized in the trophectoderm of expanded blastocysts immediately prior to implantation, suggesting that it may be involved in the attachment of the embryo to the uterine epithelium.[68]

Although original studies showed that Gal-1-deficient mice developed normally and did not show any apparent phenotypic abnormality,[69] recent studies showed that mice lacking this endogenous lectin had higher rates of fetal loss compared with their WT counterpart in allogeneic, but not in syngeneic pregnancies.[70] Administration of recombinant galectin-1 prevented fetal loss and restored tolerance *in vivo* through expansion and recruitment of uterine tolerogenic dendritic cells which in turn promote the differentiation of IL-10-producing T_{reg} cells.[70] Accordingly, these effects were prevented in mice depleted of T_{reg} cells or in mice devoid of IL-10.[70] Moreover, galectin-1 confers immune privilege to the human trophoblast through modulation of a number of immunosuppressive pathways including induction of FoxP3$^+$ T_{reg} cells and suppression of Th1 cytokines[67] (Fig. 2). Targeted inhibition of galectin-1 expression through antibody-mediated blockade, disruption of the carbohydrate recognition domain using specific disaccharides or retroviral-mediated siRNA strategies prevented these immunoregulatory effects.[67] Interestingly, secretion of galectin-1 by uNK cells has been shown to contribute to the generation of an immune-privileged environment by inducing apoptosis of decidual T cells.[71] More recently, galectin-1 produced by uterine mast cells (uMC) has been identified as a decisive factor which positively influences spiral artery formation and placentation and prevents fetal abnormalities.[72] Also, recent studies underscored a role for galectin-1 in promoting immune privilege by regulating expression of HLA-G on human trophoblast cells.[61] Exogenous galectin-1 upregulated synthesis of the membrane-bound HLA-G isoforms (HLA-G1 and G2) in a cytotrophoblast cell line, whereas endogenous galectin-1 induced expression of the soluble isoforms (HLA-G5 and -G6),[61] adding an additional galectin-1-mediated mechanism of immune tolerance.

Interestingly, a progesterone–galectin synergism has been reported in both mouse and human settings.[67,70] This cross-talk was confirmed by phylogenetic footprinting studies highlighting steroid-responsive elements in the galectin-1 gene (*LGALS1*) that were gained after the emergence of mammalian placentation.[73] Supporting these findings, recent studies revealed that galectin-1 markedly reduces the incidence of resorptions in mice missing the immunophilin FK506-binding protein (FKB)52, a co-chaperone that optimizes progesterone receptor (PR) signaling in the uterus.[74] Notably, galectin-1 was significantly downregulated in both *Fkbp52*$^{-/-}$ and *Pgr*$^{-/-}$ uteri compared with WT uteri, suggesting that uterine galectin-1 is an important downstream target of progesterone-FKBP52-PR signaling in the uterus.[74] In addition, galectin-1 has been shown to act as a regulator of progesterone and hCG synthesis by the chorionic carcinoma cell line BeWo.[75]

In addition to its role in fetomaternal tolerance, galectin-1 has been implicated in non-immunological processes including trophoblast cell invasion and sincytium formation.[76,77] In the choriocarcinoma-derived BeWo cells, galectin-1 inhibits proliferation through binding to the Thomsen–Friedenreich disaccharide on MUC1 and modulation of the receptor tyrosine kinases rearranged during transfection (RET) and Janus kinase (JAK)2 as well as modulation of the vascular endothelial growth factor-3 (VEGFR3).[77] Moreover, galectin-1–N-glycan interactions have been proposed to play key roles in tumour neovascularization.[78–81] Tissue expression of this lectin is upregulated during hypoxia through mechanisms involving hypoxia-inducible factor (HIF)-1α or nuclear factor kappa B (NF-κB).[80,82] Although these effects were demonstrated in tumour settings, it is surmised that galectin-induced angiogenesis may also be important in reproductive biology and implantation where hypoxia is a critical regulatory factor. In addition, galectin-1 plays a key role in modulation of innate immunity, host–pathogen interactions, and platelet physiology.[83–85] These effects may contribute to protective immunity and normal physiology during pregnancy. Thus, in

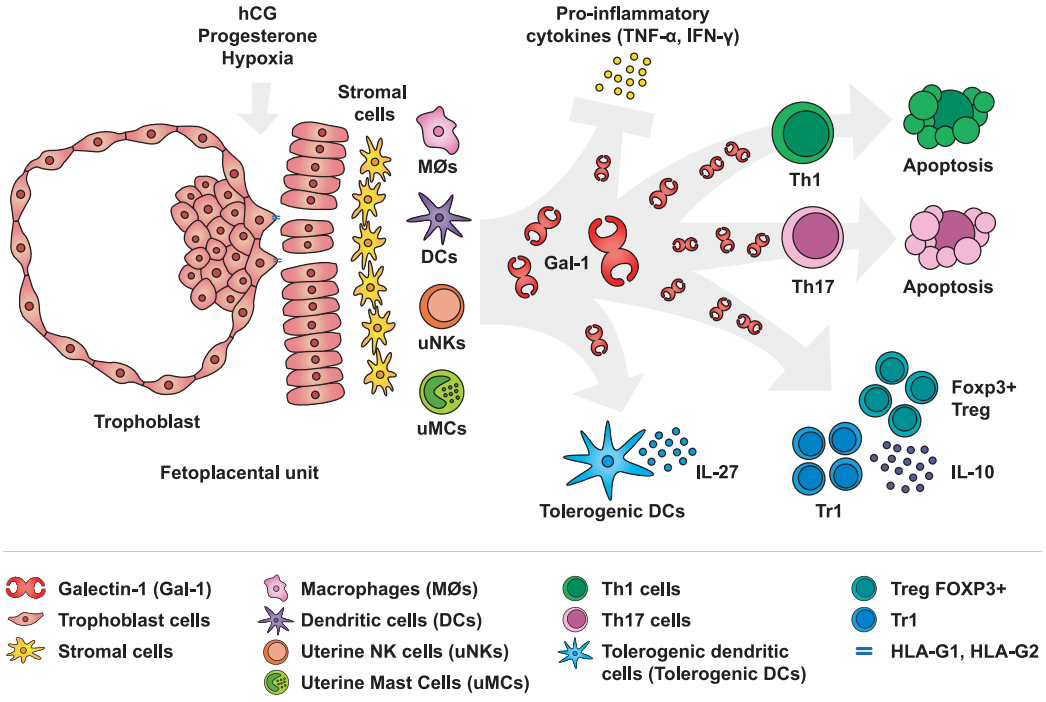

Fig. 2 The multifaceted roles of galectin-1 in pregnancy. Galectin-1 (Gal-1), an endogenous glycan-binding protein, is synthesized by trophoblast cells, stromal cells, uterine NK cells, uterine mast cells, $CD4^+$ $CD25^+$ regulatory T (T_{reg}) cells, macrophages (Mϕ), and dendritic cells (DC) within the fetoplacental unit. Its expression is regulated by several factors including progesterone, human chorionic gonadotrophin, and hypoxia. Through extracellular mechanisms, this lectin signals apoptosis of Th1 and Th17 cells, suppresses pro-inflammatory cytokine production, drives the differentiation of tolerogenic dendritic cells (tolerogenic DC), promotes the differentiation of $FoxP3^+$ IL-10-producing T_{reg} cells and $FoxP3^-$ IL-10-producing T_{reg} (Tr1) cells and induces HLA-G1 and G2 isoforms. In addition, this lectin contributes to trophoblast cell invasion, sincytium formation, placentation, and angiogenesis.

addition to the major role of galectin-1 as an essential component of the tolerogenic circuit activated during pregnancy, this endogenous lectin might also regulate other critical processes including placentation, angiogenesis, hemostasis, and control of pathogen invasion.

Galectin-2

Similar to galectin-1, galectin-2 is a 'prototype' galectin that exists as symmetric dimers containing two extended antiparallel β-sheets.[86] Recombinant galectin-2 induces apoptosis of mucosal T cells and displays anti-inflammatory activity in a model of colitis,[87] suggesting its major role in mucosal immunity. Human macrophages express significant amounts of galectin-2 which interacts with lymphotoxin α (LTA) and play a key role in the development of innate immunity and inflammation during myocardial infarction. Moreover, galectin-2 binds to epithelial cells at the E-cadherin/β-catenin complex and significantly enhances epithelial cell restoration and cellular migration *in vitro*,[88] suggesting its possible involvement in placentation-related events. However, the precise function of this lectin during pregnancy remains unexplored.

Galectin-3

Galectin-3 is the only 'chimera-type' member of the galectin family. It shows an extended N-terminal region composed of tandem repeats of short amino acid segments (a total of approximately 120 amino acids) connected to a C-terminal CRD. Unlike galectin-1 and -2, this CRD does not exist as homodimers in the crystal. By nuclear magnetic resonance spectroscopy, galectin-3 was found to exist as monomers in solution, and the N-terminal fragment has an unfolded, extended structure. However, galectin-3 can oligomerize in the presence of multivalent carbohydrate ligands and is capable of cross-linking glycans on the cell surface, thereby initiating

transmembrane signaling events and affecting various cellular functions.[28] This self-association property is dependent on the N-terminal region of the protein. As this region is sensitive to proteases, such as collagenase and matrix metalloproteinases (MMPs), the *in vivo* biological activities of galectin-3 are likely to be modulated by these enzymes.[89,90] Compared with other galectins, intracellular functions of galectin-3 are more extensively documented. Intracellularly, galectin-3 functions as an antiapoptotic protein and induces pre-mRNA splicing.[28] Extracellularly, galectin-3 binds in a carbohydrate-dependent manner to extracellular matrix proteins, including integrins, thereby influencing cell–cell adhesion, migration, cytokine production, and signaling.[28] In addition, galectin-3 can form lattices with selected cell surface glycans and modulate signaling, mobility, and endocytosis of different glycosylated receptors including the TCR, epidermal growth factor receptor and transforming growth factor.[26] Galectin-3 affects the differentiation and growth of various immune cells; it induces apoptosis of T cells and neutrophils and activates several lymphoid and myeloid cells, including mast cells, neutrophils, and T cells, resulting in production of reactive oxygen species, degranulation, and cytokine production.[91] *In vivo* studies using galectin-3-deficient (*Lgals3*$^{-/-}$) mice supported the notion that galectin-3 amplifies inflammatory responses, suggesting that this endogenous lectin might play a role during early implantation, where proinflammatory cytokines, such as TNF and IFN-γ play decisive roles. In addition, a number of studies supported a role of galectin-3 in the modulation of Th1-Th2 cytokine balance.[29] Galectin-3, probably the best studied member of the family, has also been proposed to play key roles in several physiologic and pathologic processes including angiogenesis,[92] tumour development and progression,[93] and wound healing.[94] Collectively, these observations support a major role for galectin-3, acting either intracellularly or extracelllularly, at different stages of pregnancy development.

Galectin-3 mRNA and protein were higher in pregnant compared to non-pregnant mouse endometrium, and their maximum levels were reached on days 2 and 4, being localized at the luminal and glandular epithelia. Interestingly, the numbers of embryos implanted decreased substantially when galectin-3 was knocked down selectively in mouse endometrium.[95] Northern blot analysis of total RNA prepared from separated fetal and maternal components of utero-placental complexes demonstrated different patterns of galectin-3 expression. Relative levels of galectin-3 mRNA peaked at midgestation in the implantation site and during the second half of gestation remain elevated in the placenta but declined in the uterus.[96] Moreover, in bovine placentomas, galectin-3 was confined to uterine epithelial cells.[97] In human endometrium, galectin-3 expression increased significantly during the secretory phase of the menstrual cycle.[59] This lectin was observed in endometrial cells of the primary decidual zone immediately after implantation and at later stages of pregnancy in the decidua basalis and metrial gland and all trophoblastic elements of the placenta.[54] In fact, both galectin-1 and galectin-3 expression correlated with the differentiation pathways of trophoblasts. Galectin-3 was detected in all trophoblastic lineages including villous cytotrophoblasts and extravillous trophoblasts (trophoblastic cell columns, infiltrating trophoblasts, endovascular trophoblasts, and placental bed giant cells).[56] However, despite considerable evidence on the expression pattern of galectin-3 at the fetoplacental unit of several species, its physiologic roles during pregnancy remain obscure. Crider-Pirkle and colleagues identified cubilin, as an endogenous binding partner for galectin-3. Cubilin occurred in yolk sac epithelium throughout pregnancy but was endocytosed by uterine NK cells, apparently via interaction with galectin-3.[98] Whether cubilin–galectin-3 interactions play any role in uNK cell physiology remains to be determined. In addition, in ovine placental tissue, galectin-1 and -3 have demonstrated antagonic immunomodulatory effects *in vitro*. While galectin-3 favored T-cell proliferation and activation, galectin-1 displayed T-cell inhibitory activity.[57,99]

Galectin-4

Galectin-4, a tandem-repeat two CRD galectin, is predominantly expressed in mucosal tissues including the intestines.[100] The role of galectin-4 in lipid raft formation has been studied in different settings. Sulphatides with long-chain hydroxylated fatty acids, which are enriched in lipid rafts, were identified as high-affinity ligands for galectin-4. By interacting with sulphatides, galectin-4 fosters the clustering of lipid rafts and contributes to apical delivery of proteins.[101] Galectin-4 regulates the development of inflammatory bowel disease by stimulating CD4^{+} T cells to produce IL-6.[102] Recently,

Nishida et al. identified an inducible colitis-associated glycome that can be identified through the binding of galectin-4 in local, but not systemic, memory $CD4^+$ T cells exposed to intestinal inflammatory conditions. The inflammation-associated glycome represents an immature core 1-expressing O-glycan structure selectively expressed in inflamed intestinal tissue.[103] In addition, galectin-4 contributes to wound healing and regulates apoptosis of mucosal T cells.[88] These observations suggest a major role of galectin-4 in immune cell activation, inflammation, and expansion of memory T cells in mucosal tissue. Although scarce information is available regarding the function of galectin-4 in feto-placental tissue, its expression has been found to be restricted to uterine epithelial cells and blood vessel walls in the bovine endometrium.[97] Furthermore, during rat placentation, galectin-4 was downregulated in differentiated trophoblasts. Immunohistochemical analysis showed that galectin-4 was preferentially located in the maternal–fetal junctional zone, suggesting a possible role for this lectin in the promotion of trophoblast cell differentiation.[104]

Galectin-7

Galectin-7, a one CRD 'prototype' galectin, is found mainly in stratified squamous epithelium and its expression correlates with keratinocyte differentiation. The crystal structure of human galectin-7 revealed formation of complexes with galactose, lactose, and N-acetyllactosamine.[105] Galectin-7 is an early transcriptional target of the tumour suppressor protein p53, its expression is upregulated rapidly after ultraviolet B irradiation of epidermal keratinocytes and promotes apoptosis of these cells.[106] This lectin is also implicated in wound healing and epithelial cell migration.[94] However, the regulated expression and function of this lectin during pregnancy has not yet been explored.

Galectin-8

Three mRNAs encode six different galectin-8 isoforms; three with two CRDs in tandem and three with only one CRD.[107] Recombinant galectin-8 inhibits epithelial cell adhesion through binding to $\alpha 1\beta 3$ integrin. Binding of galectin-8 results in strong activation of integrin-mediated signaling which regulates cell adhesion and survival.[108] By cross-linking specific glycoconjugates on αM integrin, galectin-8 stimulates innate immunity through induction of superoxide production by neutrophils and control of neutrophil adhesion to extracellular matrix.[109] Moreover, both galectin-8 and galectin-4, which are expressed in mucosal tissue, recognize and kill human blood group antigen-expressing invading bacteria replacing recognition by specific antibodies.[110] Recent findings demonstrated that galectin-8 is required for T-cell proliferation[111], platelet activation,[112] and angiogenesis.[113] Collectively, these findings suggest a possible role for galectin-8 in cellular migration, host–pathogen interactions, immunity, and neovascularization during pregnancy. Although galectin-8 is selectively expressed by villous and extravillous trophoblast in human placenta,[114] the functional relevance of this lectin during pregnancy has not yet been ascertained.

Galectin-9

Three isoforms of galectin-9 differing in the lengths of their linker sequences have been identified.[115] Similar to other two CRD galectins, galectin-9 has also been found in lipid rafts. Galectin-9 can either initiate or terminate adaptive immunity through modulation of T-cell survival or activation of antigen-presenting cells. In fact, recombinant galectin-9 induces selective apoptosis of Th1 cells and terminates autoimmune inflammation but, paradoxically, promotes dendritic cell maturation and macrophage activation through binding to the cell surface molecule TIM-3.[116,117] However, TIM-3-independent mechanisms of immune cell activation, Th1/Th17 cytokine production, and apoptosis have also been described.[118] Moreover, galectin-9 regulates glucose homeostasis by facilitating retention of GLUT-2 on the surface of pancreatic β cells.[119] These observations suggest potential roles for galectin-9 in immunological and metabolic pathways during pregnancy.

Notably, galectin-9 is found in human endometrium, specifically in endometrial epithelial cells, but not in stromal cells or immune cells. It is expressed at very low concentrations during the proliferative phase and the early secretary phase and showed a sharp increase in the mid and late-secretory phase, the window of implantation, as well as in the deciduas.[120] Moreover, a recent study profiled expression of galectin-9 (*LGALS9*) isoforms at the fetomaternal interface in mouse and human pregnancy. Decidual galectin-9 was considerably altered in a mouse model of spontaneous abortion. The *LGALS9* D5

isoform selectively dampened IFN-γ production by decidual NK cells. In human patients, six *LGALS9* splice variants were detected, and a decrease in *LGALS9* D5/10 was associated with recurrent fetal loss.[121] Given the inhibitory role of TIM-3 during microbial and tumour immunity, the role of galectin-9–TIM-3 interactions during gestation remains to be investigated.

Galectin-10

Galectin-10 has a single CRD, sharing only six of eight residues directly involved in lactose binding. Surprisingly, it has affinity for mannose but not for β-galactosides.[122] In spite of its modest sequence homology, the overall structural fold of galectin-10 is very similar to other galectins, especially galectin-7.[122] This lectin is constitutively expressed in eosinophils, basophils, and $CD4^+$ $CD25^+$ T_{reg} cells. Inhibition of endogenous galectin-10 by RNA interference abrogated the suppressive activity of T_{reg} cells.[123] These effects appear to be mediated via an intracellular mechanism that is not completely understood. Although unexplored in pregnancy settings, the selective expression of galectin-10 in T_{reg} cells suggests its potential role in fetomaternal tolerance in human pregnancies.

Galectin-12

While the N-terminal domain of galectin-12, a 'tandem-repeat' galectin, contains all sequence elements predicted to form the two β-sheets found in other galectins as well as conserved carbohydrate-interacting residues, its C-terminal domain shows considerable divergence from the consensus sequence.[124] Galectin-12 is over-represented in adipocytes compared to other tissues and has been proposed to play key roles in cell cycle regulation, adipocyte differentiation, and insulin sensitivity.[125,126] The role of this 'tandem-repeat' lectin during pregnancy remains to be investigated.

Galectin-13

Placental protein 13 (PP13) was cloned from human term placenta. As sequence analyses, alignments, and computational modeling showed its conserved structural and functional homology to members of the galectin family, the protein was designated as galectin-13. Similar to human eosinophil galectin-10 but not other galectins, its weak lysophospholipase activity was confirmed.[127] It has been proposed that PP13 serves as a placental alarmin and undergoes lipid raft-associated subcellular redistribution in the syncytiotrophoblast.[128] Sugar-binding assays revealed that N-acetyl-lactosamine, mannose, and N-acetyl-glucosamine residues widely expressed in human placenta had the strongest binding affinity to both the purified and the recombinant PP13/galectin-13. PP13 was found to be localized to syncytiotrophoblasts in the chorionic villi and to occasional multinucleated luminal trophoblasts within converted decidual spiral arterioles.[127] Extracellular PP13 aggregates were found around decidual veins associated with immune cell-containing decidual zones of necrosis,[129] suggesting their role in trophoblast invasion, conversion of maternal spiral arterioles, and modulation of immunity. Exposure of cultured trophoblasts to PP13 resulted in depolarization of calcium ions, followed by liberation of linoleic and arachidonic acids from the trophoblast membrane, and subsequent elevation of prostacyclin and tromboxanes,[130] supporting its potential role in the control of trophoblast physiology.

Galectin-14

Ovine galectin-14 is selectively expressed in eosinophils and is released into lung and gastrointestinal tissues following allergen or parasite challenge. Galectin-14 is active in carbohydrate-binding assays and is involved in promoting cellular adhesion and changing mucus properties during parasite infection and allergies.[131]

The possibility that eosinophil-derived galectin-14 could modulate mucus properties during implantation or could influence host–pathogen interactions during pregnancy remains to be explored.

Galectin-15

Galectin-15 (also known as OVGAL11) is expressed specifically in the endometrial luminal and superficial glandular epithelia of the uterus in concert with blastocyst elongation during the pre-implantation period in the sheep, but it is also expressed in the uterus of goats and pigs.[132] Carbohydate-binding assays revealed its ability to bind lactose and mannose sugars. Progesterone upregulates galectin-15 mRNA in the endometrial epithelium of pre-implantation conceptus.[133] In pregnant sheep, expression

appeared in the epithelia between days 10 and 12 and increased between days 12 and 16. Interestingly, galectin-15 forms crystals in the trophectoderm and regulates implantation and placentation by functioning as a heterophilic cell adhesion molecule between the conceptus trophectoderm and endometrium luminal epithelium.[134]

Galectins in pathologic pregnancies

In human pregnancy, the implantation period is characterized by an initial inflammatory response that later shifts toward a tolerogenic state.[135] An inappropriate dysregulation of proinflammatory/anti-inflammatory mediators may represent a major cause of fetal loss. Interestingly, a significant reduction in circulating galectin-1 and a higher prevalence of antigalectin-1 autoantibodies were found in sera from patients with recurrent spontaneous abortions (RSA) compared with fertile women, suggesting a possible mechanism to explain pregnancy loss in RSA patients.[67] Decreased levels of galectin-1 were also found in RSA patients who suffered subsequent miscarriages compared with normal pregnant women.[61] Supporting these findings, proteomic analysis of placental villous showed the lower expression of galectin-1 in placental tissue from patients with early pregnancy loss compared with placental tissue from normal pregnant women.[136] However, the levels of serum galectin-1 and antigalectin-1 autoantibodies did not differ significantly between healthy pregnant women and patients with pre-eclampsia[137]; yet, galectin-1 expression was markedly upregulated in placental tissue from patients with pre-eclampsia.[138] These results underscore possible differences in the expression of local versus systemic galectin-1 in different pathophysiologic settings. Moreover, increased galectin-1 mRNA expression was found in chorioamnionitis when analyzed in patients with pre-term pre-labor rupture of the membranes (PPROM). Here, its expression was mainly localized in amniotic epithelium, chorioamniotic mesodermal cells, and apoptotic bodies.[139] Importantly, both galectin-1 and galectin-3 were upregulated on placental tissue from patients with intrauterine growth retardation (IUGR), whereas only galectin-1 was upregulated in decidual tissue from pre-eclamptic placentas.[140] Moreover, immunoreactivity for both galectin-1 and galectin-3 was found to be increased in gestational trophoblastic disease (GTD), suggesting possible roles of these lectins in the invasiveness of the transformed trophoblastic cells.[58] On the other hand, PP13 (galectin-13) has been proposed to be a practical, individual predictor of women at risk of developing pre-eclampsia.[141] Levels of PP13 in first-trimester pregnancies increased with gestational age in controls and pre-eclampsia cases, but at different rates. PP13 levels were decreased in women with pre-eclampsia compared to controls.[142] Thus, changes in the expression and function of different galectins may contribute to the transition from normal to pathologic pregnancies. Alternatively, selective variations in galectin expression levels could serve as possible biomarkers of pregnancy complications including RSA, pre-eclampsia, IUGR, and GTD.

Conclusions and implications

The present review aims at integrating scattered information on the role of galectins, a family of endogenous glycan-binding proteins, in the development of normal and pathologic pregnancies. Galectins play diverse roles during the initiation, amplification and resolution of inflammatory response and can also regulate non-immunological processes including wound healing, cellular adhesion, migration, and angiogenesis. Although many of these functions have not yet been explored at the fetomaternal interface, galectins have been shown to play key roles in implantation and fetomaternal tolerance. Particularly, galectin-1 produced by trophoblast cells, uMC and uNK cells hierarchically regulates immunosuppression during pregnancy including differentiation of tolerogenic dendritic cells, expansion of T_{reg} cells, and apoptosis of decidual T cells, but also contributes to placentation through non-immunological mechanisms.

Future studies should examine the *in vivo* role of individual members of the galectin family (in addition to galectin-1) during pregnancy. Given the diverse range of galectin-deficient mice that are now available, it will be feasible to determine the impact of galectins and their glycosylated receptors in fetomaternal tolerance, implantation, and placentation. These data will set the basis to improve clinical outcomes in pathologic pregnancies and/or to provide novel biomarkers of successful gestation or early fetal loss. However, before galectin-based therapeutic agents can be extrapolated to clinical settings, a more thorough understanding of the mechanisms involved in galectin functions is essential. In this

regard, it will be critical to evaluate the results of side-by-side studies of the immunoregulatory activities of different members of the galectin family and to study the cross-talk between galectins and other established regulatory pathways including PD-L1, STAT-3, and IDO. Finally, given the complexity of galectin–glycan boundaries and the multiple parameters influencing these molecular interactions, further work is required, involving multidisciplinary approaches, to achieve a global comprehensive view of the role of endogenous galectins and their specific carbohydrate ligands during pregnancy.

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