Published in final edited form as:

Trends Endocrinol Metab. 2012 January ; 23(1): 23–31. doi:10.1016/j.tem.2011.09.003.

Galectins: guardians of eutherian pregnancy at the maternal-fetal interface

NG Than^{1,2,3}, R Romero¹, CJ Kim^{1,4}, MR McGown⁵, Z Papp³, and DE Wildman^{1,5,6}

¹Perinatology Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Detroit, MI, USA

²Wayne State University, School of Medicine, Detroit, MI, USA

³First Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary

⁴Department of Pathology, Wayne State University, School of Medicine, Detroit, MI, USA

⁵Center for Molecular Medicine and Genetics, Wayne State University, School of Medicine, Detroit, MI, USA

⁶Department of Obstetrics and Gynecology, Wayne State University, School of Medicine, Detroit, MI, USA

Abstract

Galectins are multifunctional regulators of fundamental cellular processes. They are also involved in innate and adaptive immune responses, and play a functional role in immune-endocrine crosstalk. Some galectins have attracted attention in the reproductive sciences because they are highly expressed at the maternal-fetal interface, their functional significance in eutherian pregnancies has been documented, and their dysregulated expression is observed in the ‘great obstetrical syndromes’. The evolution of these galectins has been linked to the emergence of eutherian mammals. Based on published evidence, galectins expressed at the maternal-fetal interface may serve as important proteins involved in maternal-fetal interactions, and the study of these galectins may facilitate the prediction, presentation, diagnosis, and treatment of pregnancy complications.

Galectins as regulators of multiple biological processes

Glycosylation is a common post-translational modification, and more than 50% of human proteins are glycosylated [1]. Glycans residing on glycoconjugates (e.g. glycoproteins, glycolipids) constitute a complex array, collectively termed the ‘glycome’, which can store biological information orders of magnitude greater than nucleic acids and proteins [2,3]. Lectins (Glossary) are sugar-binding proteins, and carbohydrate-lectin interactions are pivotal in the regulation of cellular interactions with other cells, the extracellular matrix (ECM), or pathogens [2–5]. Galectins are members of the lectin superfamily and regulators of a wide variety of fundamental biological processes including signal transduction, pre-mRNA splicing, cell growth, differentiation, apoptosis, and cell-cell/ECM interactions [3,6–10]. Vertebrate galectins are at the crossroads of innate and adaptive immunity because they

Corresponding Authors: Nandor Gabor Than, MD, PhD, Department of Obstetrics and Gynecology, Wayne State University, School of Medicine, 3990 John R St., #4 Brush, Detroit, MI, USA 48201, nthan@med.wayne.edu.; Roberto Romero, M.D., D.Med.Sci, Perinatology Research Branch, 3990 John R. St., #4 Brush, Detroit, MI, USA 48201, prbchiefstaff@med.wayne.edu; and Derek Wildman, PhD, Department of Obstetrics and Gynecology, Wayne State University, School of Medicine, 3990 John R St., #4 Brush, Detroit, MI, USA 48201, dwildman@med.wayne.edu.

are key regulators of acute and chronic inflammation, host-pathogen interactions, and immune tolerance [10–14], which all are important determinants of pregnancy outcome.

Some galectins have recently come into focus in the reproductive sciences and perinatal medicine because they are highly expressed at the maternal-fetal interface [11,15–38]. Their role in immune-endocrine crosstalk during pregnancy and in the establishment and maintenance of pregnancy (e.g. embryo implantation, placentation, maternal-fetal immune tolerance, danger signaling) has been suggested by *in vivo* and *in vitro* studies [11,15–17,19,20,22,24–26,28–31,35,37,38]. Furthermore, their dysregulated expression has been observed in the ‘great obstetrical syndromes’ (Glossary) [11,26,27,29–33,35,37,39–50]. Recent evidence suggests that the evolution of these galectins (Box 1) is tightly linked to the evolution of the placenta in eutherian mammals including primates [6,8,9,15,16,51]. This review aims to give an overview of galectins, especially in the context of their roles in eutherian pregnancies.

BOX 1

Evolutionary analyses have revealed a dynamic history of mammalian galectins, especially via the co-option of galectins expressed at the maternal-fetal interface [6,15,51]. Their shared exon-intron organization suggests that vertebrate galectins originated from an ancestral mono-CRD galectin by gene duplication, divergence and subfunctionalization (Glossary) [51]. Vertebrate galectin CRDs are encoded by three exons [6,7,51] and, based on the ending of exon 3, these can be classified into F3 and F4 groups [51]. Figure 3 shows a maximum-likelihood phylogeny generated from a 58-amino acid residue alignment derived from previously published sequences of the extended binding-site region of mammalian galectin CRDs [39,56]. Within the F4 group, galectins can be subdivided into two clades (Glossary) that contain galectins-1-3 or HSPC159 and galectins-4,-6,-8,-9,-12, respectively. Rat galectin-5 and sheep ‘galectin-14’ were nested within galectin-9 sequences, and mouse galectin-6 was closely related to galectin-4. Based on these phylogenetic trees [51], we can infer that there were several transitions to increased galectin expression at the maternal-fetal interface within eutherian mammals (Glossary), including the expression of galectins-1,-3,-8,-9, and members of the galectin-13-clade, in various cell types residing in the placenta, fetal membranes and decidua.

These transitions were mirrored by substantial changes in both coding sequences and regulatory regions. For example, galectin-1 underwent adaptive evolution at crucial residues on the lineage leading to the last common ancestor of placental mammals, including the gain of cysteine residues important in immune functions and maternal–fetal immune tolerance [16,84]. In addition, the gain of an estrogen response element among 10 novel promoter cis-regulatory elements suggests that estrogen-mediated regulation of galectin-1 in immune tolerance may have been gained during the emergence of placental mammals [11,16].

Within the subfamily containing galectin-13, multiple gains and losses of genes via birth-and-death evolution (Glossary) occurred specifically in primates: galectin-14 is found in anthropoids, galectins-13,-16,-17 are present in catarrhines (Old World monkeys and apes, including humans), whereas galectins-19,-20 have been found only in New World monkeys [15]. Galectins-13,-14,-16,-19,-20 expression is predominantly placental in anthropoids [15], which have an invasive hemochorial type of placentation (Glossary) [82], whereas galectin-15 expression is predominantly endometrial in ruminants [17], which have non-invasive epitheliochorial placentation (Figure 3) [82] – suggesting that there are significant regulatory differences between these genes in species with substantial differences in placentation.

Moreover, pervasive adaptive evolution occurred shortly after many new genes in the galectin-13 clade arose, suggestive of neofunctionalization (Glossary), and especially at residues involved in sugar binding (Figure 3) [15,18,39,53,54]. This intense positive selection was followed by conservation of residues in descendant lineages, implying that placenta-expressed galectins in anthropoids have acquired and sustained important new functions [15].

Common structural characteristics of galectins

Galectins are characterized by carbohydrate-recognition domains (CRDs) possessing consensus amino acid sequences with binding-affinity for β -galactoside-containing glycoconjugates [7–10]. Galectins were originally termed ‘S-type lectins’, referring to their free cysteine residues [7–10]. The first galectin (electrolectin) was isolated from the eel *Electrophorus electricus* [10]. Subsequent studies found galectins to be the most widely expressed animal lectins, and family members have been identified in species ranging from sponges to human [6,9,10]. Subsequently, galectins or galectin-like proteins were also found in plants, fungi and viruses [6,10]. Because of this diversity it is challenging to infer homology between mammalian and non-mammalian galectins; therefore, nomenclature has diverged with non-mammalian galectins retaining specific names, whereas mammalian galectins have been named using sequential numbering [7]. Proteins with structural similarity to galectins but lacking affinity for β -galactosides are only ‘related to’ but not members of this lectin family [6,10].

In mammals, 19 galectins have been identified, of which 13 are expressed in human tissues [6,12,15]. Mammalian galectins can be divided into three structural groups [7–10]. ‘Tandem-repeat-type’ galectins (galectins-4,-6,-8,-9,-12) contain two homologous CRDs (which may differ in their carbohydrate-binding affinities) connected by a short linker sequence, thereby enabling multivalent binding activities [7–9,52]. ‘Chimera-type’ galectins possess a C-terminal CRD and an N-terminal non-lectin domain consisting of proline- and glycine-rich short tandem repeats, which are important for multimerization and crosslinking, as well as for functional regulation via proteolysis [7–9,52]. Galectin-3 is the only chimera-type galectin in vertebrates, but these are much more common in invertebrates [10]. Most mammalian galectins are ‘prototype’ (galectins-1,-2,-5,-7,-10,-13,-14,-15,-16,-17,-19,-20), which contain a single CRD of ~130 residues that may homodimerize [7–10,52].

Although the primary structure of galectins has diverged, their CRD topologies are very similar to each other and to some other classes of lectins (e.g. legume lectins, pentraxins) [10]. The monomer galectin CRD has a distinctive ‘jelly-roll structure’ (Glossary), which may dimerize in prototype galectins [7,9,10,53–55]. Sequence alignments revealed eight conserved residues in galectin CRDs, which are directly involved in carbohydrate-binding via hydrogen bonds and electrostatic- and van der Waals interactions [53,55]. Although their interactions with mono- or disaccharides are generally weak, multivalent galectins have a high affinity for extended poly-N-acetyllactosamine- or ABO blood-group-containing glycans, the latter being responsible for their hemagglutinin activity [39,52,56,57]. Galectin-10 seems to be an exemption because it binds to β -mannosides rather than to β -galactosides [53].

Common functional characteristics of galectins

Galectins display a unique combination of intra- and extracellular activities [10,58,59]. They are predominantly localized to the cytoplasm where they modulate various pathways, including the regulation of cell growth, differentiation, apoptosis, and migration [10,58,59]. These effects are mediated by protein-protein interactions because galectins’ glycan-ligand

pairs are not present inside the cell [10,58,59]. Some galectins (e.g. galectins-1,-3) are also transported to the nucleus where they function in pre-mRNA [10,58,60].

As a link between their intra- and extracellular functions, galectins can be exported from cells by non-classical secretion, a characteristic of a very restricted set of proteins (e.g. interleukin-1 β ; high-mobility group box 1 protein) [61]. Galectins do not have a secretory signal sequence; however, they can be incorporated into intracellular vesicles under the plasma membrane and can be secreted, avoiding the endoplasmic reticulum and Golgi vesicles [61]. On cell surfaces, mammalian galectins often localize to lipid rafts (Glossary) and crosslink receptor ligands [10,37,52,62].

Extracellular functions of galectins depend on their sugar-binding abilities [3,9,10,12,14,52,60]. Galectins do not have specific receptors, but bind to an array of cell-surface or ECM molecules which carry their carbohydrate ligands [10,52]. By crosslinking these distinct ligands on cell surfaces, galectins can form ordered arrays of galectin-glycan lattices, altering cell surface residency of their ligands, and having effects on cell growth and survival, metabolic responses, cytokine secretion, and cell-cell/ECM interactions [10,12,13,52,60,63]. Of importance, various galectins have been shown to influence cell adhesion, induce or prevent apoptosis, and, due to their cytokine-like properties, they are major activators or inhibitors of immune responses (Figure 1) [10,12,14,52,60].

An intriguing feature of galectins is that their vesicular secretion is increased in response to cellular stress (e.g. inflammation, infection) and damage (e.g. necrosis); thus, they have been implicated to function as stress sensors or ‘alarmins’ (Glossary), which signal tissue damage and elicit effector responses from innate and adaptive immune cells, contributing to the activation and/or resolution of immune responses [13,31,37,64].

Expression of mammalian galectins

Gene- and protein-expression studies show that galectins have distinct and overlapping expression profiles in adult mammalian tissues [7,10,15,65,66]. Galectin-1 is widely expressed at both the mRNA and protein levels in human tissues, being most abundant in the endometrium/decidua and highly expressed in the placenta [10,16,65]. Galectin-3 has a similarly broad tissue distribution with peak expression in activated immune cells [10,65]. Tandem-repeat and prototype galectin-8 isoforms, encoded by at least seven different mRNAs, are differentially expressed in various tissues [10,36,65]. The expression of galectin-9, encoded by three genes in humans, is predominant in immune cells, intestine and endometrium/decidua (Figure 2) [65]. Each of these galectins is known to be highly expressed at the maternal-fetal interface, and possible roles in mammalian pregnancies have been proposed [10,11,15,16,19,20,22,24–28,30,31,33–36,67].

Other vertebrate galectins (galectins-2,-4,-5,-6,-7,-12) have restricted expression patterns [10,65,68]. Galectin-10 (Charcot-Leyden crystal protein) expression is characteristic for suppressive T regulatory (Treg) cells and for eosinophil and basophil lineages in bone marrow and in the periphery [53,69]. Galectin-10 is the most abundant protein in eosinophils, and this lectin forms characteristic crystals in tissues and secretions in sites of eosinophil-associated inflammation [53,69]. At the maternal-fetal interface, galectins-13,-14,-16 are only predominantly expressed by the placenta, whereas the expression of galectin-17 is generally low in the human tissues investigated [15,21,65,70] (Figure 2). Sheep *LOC443162* encodes ‘galectin-14’, a protein that is more closely related to galectin-9 rather than to eosinophils where it plays a role in allergic inflammation [71]. Galectin-15 is highly expressed in the endometrium of ruminants [17]. Expression of HSPC159 (i.e. galectin-related protein, GRP) and griffin is restricted to hematopoietic stem cell precursors and lens, respectively [10].

Galectins in support of eutherian mammal pregnancies

A striking feature of most galectins is that their expression is not only induced upon cell stress or activation but is also spatiotemporally regulated during cell differentiation and embryo development [10]. For example, galectins-1,-3,-9 are widely expressed during human and mouse embryo-genesis (galectin-1: connective tissues, epithelia; galectin-3: chondrocytes, notochord, liver, myocardium; galectin-9: liver, thymus), suggesting that they may play important roles in embryo development in mammals [10]. However, there may be some redundancy in their functions because mouse null mutants for either galectin-1 or galectin-3 are viable [11,60].

Tumor cells and placental trophoblasts have several overlapping characteristics [72]. Consistent with their oncodevelopmental significance, galectins-1,-3,-9 are also expressed in different tumors and their expression correlates with poor clinical outcome [10,23,66,73]. Overexpression of galectin-1 led researchers to suggest that it participates in tumor progression and metastasis, including (i) promotion of tumor angiogenesis, (ii) weakening of antitumor responses by facilitating the apoptosis of Th1 cells, and (iii) the expansion in the number of Treg cells and the increase in their immunosuppressive activity, leading to tumor immune escape [10,73].

These functions of galectin-1 are also important in the establishment and maintenance of eutherian pregnancies [11,28]. Indeed, galectin-1 is abundantly expressed at the maternal-fetal interface (Glossary; Figure 2) in humans, mice, and other mammals (e.g. galectin-3 in mice and sheep, galectin-9 in mice, and galectin-13-clade galectins in primates) [10,15,24,25], and their expression is often dysregulated in human pregnancy complications (Figure 2) [26,27,29–35,37].

The role of galectins expressed in maternal cells at the maternal-fetal interface

Systematic exploration of the expression of galectins in reproductive tissues has opened a window into their role in pregnancy. As evidence for their role in immune-endocrine crosstalk in the maintenance of mammalian pregnancies [11], the expression of galectins in the uterine endometrium and decidua is strictly regulated by sex steroids [11,17,19,24,25]. In human endometrium, galectin-3 expression is increased in the secretory phase of the menstrual cycle in glandular epithelial cells, whereas galectin-1 expression is increased in the last secretory phase in stromal cells, where its expression further increases in decidua [25]. A similar temporal expression pattern during the estrus cycle was observed for galectin-1 in mice [19], and its dependence on estrogen and progesterone has been established *in vivo* [11,19]. Similarly, galectin-9 expression increases in the mid- and late-secretory phases in endometrial epithelial cells in human decidua [24]. Because the peak endometrial expression of these galectins coincides with the window of implantation, a possible sex-steroid-induced role in blastocyst attachment and in the regulation of endometrial immune cell homeostasis during implantation has been proposed [19,24,25]. Galectin-15 expression is also regulated by progesterone in endometrial epithelial cells in sheep and goat, where it promotes blastocyst development, attachment to the uterine epithelium, and implantation [17].

Decidual expression of galectin-1 was significantly decreased in a stress-induced fetal-loss model in mice, but this could be reversed by progesterone treatment, underlining the progesterone-dependent regulation of decidual galectin-1 expression [11]. Stressed mice has low decidual galectin-1 expression and, similar to galectin-1-deficient mice, they had a significantly higher rate of fetal loss in allogeneic pregnancies (Figure 2) [11]. Treatment of

mice with galectin-1 in parallel with stress induction restored immune tolerance and rescued pregnancies in these animals through the induction of tolerogenic dendritic cells and CD4+ CD25+ IL-10+ regulatory T cells [11]. Of note, galectin-1 treatment prevented the decrease of progesterone and progesterone-induced blocking factor (PIBF) serum concentrations observed in stressed animals, leading the authors to suggest a synergistic effect of galectin-1 and progesterone in sustain pregnancies, and a key position for galectin-1 in the cascade of messengers involved in pregnancy maintenance [11].

Galectins expressed by maternal immune cells infiltrating the uterus may also play a key role in mammalian pregnancies [22,28,250,69,74]. Galectin-1 is selectively upregulated in a subset of natural killer cells in the uterus (uNK cells) compared to circulating NK cells [22,28]. Uterine NK cells have unique immunomodulatory potential and comprise ~70% of maternal lymphocytes at the implantation site, where these innate immune cells promote angiogenesis and trophoblast invasion, fundamental processes in placentation [22,75]. Moreover, human uNK cell-expression galectin-1 induces apoptosis of activated T cells [28], contributing to the immune-privileged environment at the maternal-fetal interface [28,75]. Of importance, compared to other T cell types, galectins-1 and -10 are overexpressed in CD4+ CD25+ Treg cells, in which they have a key role in suppressive functions [69,74]. Indeed, the pool of maternal Treg cells normally expands during pregnancy, and they 'suppress an aggressive maternal allogeneic response directed against the fetus' [76]. Not surprisingly, the proportion of galectin-1 expressing peripheral blood NK cells and Treg cells is decreased in pre-eclampsia [50], a syndrome in which exaggerated maternal systemic immune activation leads to serious maternal and/or fetal complications [77].

The role of galectins expressed in fetal cells at the maternal-fetal interface

Several galectins are also expressed in fetal membranes [30,34,35]. Galectin-1 is abundantly expressed in all cell types (i.e. amnion epithelial cells, chorionic trophoblasts, and chorioamniotic mesenchymal cells) where it may regulate tissue development and cell-cell/ECM interactions, have antimicrobial effects, and participate in the counter-regulation of inflammatory responses in fetal membranes [30]. Because the amniotic layer of these membranes has strong immunosuppressive properties, it is used for skin transplantation, biological bandaging of skin, and treatment of ocular surface disorders, where it reduces inflammation, prevents graft rejection, and promotes wound healing, possibly through the effect of secreted immunosuppressive factors [30]. Consistent with these observations, gene and protein expression data showed that galectin-1 is upregulated in inflamed fetal membranes, especially in fetal macrophages, where it was supposed to promote the recognition and phagocytic removal of invading maternal neutrophils, as well as the resolution of inflammation (Figure 2) [30]. Moreover, proteomics studies showed that galectin-1 is also upregulated in fetal membranes in preterm labor [35]. There is evidence that galectins may also participate in various pathways leading to term labor in fetal membranes; for example, microarray studies have demonstrated that galectin-7 is upregulated in human amnion in oxytocin-induced labor, and galectin-9 is downregulated in the chorion at the site of its weakening and rupture (Figure 2) [34].

Human galectins-1,-3,-8 are expressed in extravillous trophoblast cells (EVTs) of the first trimester placenta throughout their invasive pathway of differentiation [20,36,72]. These galectins are expressed in EVT cell columns that are active in ECM deposition, and are ligands of major structural glycans (e.g. laminin, fibronectin) that are constituents of the placental bed [10,20,36,78]. Thus, galectins-1,-3,-8 have been suggested to participate in the organization of the ECM and to act as physiological modulators of cell adhesion in EVT columns [20,36]. Of importance, galectins-1 and -3 are absent from the non-proliferating,

highly invasive, interstitially migrating cytotrophoblasts, and this has been proposed to reflect their role in the regulation of EVT cell cycle [20]. Notably, galectins-1 and -3 are upregulated in EVTs in pre-eclampsia and in HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, conditions where there is a failure of EVT invasion in the placental bed [27] (Figure 2).

Galectins-1,-3,-8-13,-14,-16 are expressed by villous trophoblasts (VTs) of the placenta in humans [15,16,18,26,27,29,31–33,36–39,67]. VTs undergo the fusion pathway of differentiation, in which postmitotic cytotrophoblasts differentiate and fuse to form the multinucleated syncytium [72,79]. This outermost cell-layer of the placenta is responsible for the exchange of gases, nutrition and waste products between mother and fetus, and for the production of hormones and immunosuppressive molecules important in the regulation of maternal-fetal interactions [72,79]. Galectin-1 is expressed by all cell types in the placental villi, where it has been suggested to participate in ECM organization, host-pathogen immune responses and the establishment of maternal-fetal immune tolerance [31]. Of importance, a proteomics study identified galectin-1 among the immunosuppressive molecules secreted by VTs that inhibit T lymphocyte proliferation and adaptive immune responses [67]. Furthermore, another proteomics study identified galectin-1 as being downregulated in VTs in early pregnancy loss, reflecting anomalies in mechanisms supporting the maintenance of pregnancy [26]. Galectins-1 and -8 are upregulated in VTs in pre-eclampsia and HELLP syndrome [27,31,33], a condition with increased stress of the trophoblast at the time of exaggerated maternal systemic inflammation, and in this condition these galectins may function as trophoblastic ‘alarmins’ (Glossary; Figure 2) [13,31].

Galectin-13 is a placenta-specific galectin that is mainly expressed in the differentiated syncytiotrophoblast but not in the underlying proliferating cytotrophoblasts [15,18,29]. Galectin-13 is also considered to be a placental alarmin because it is increasingly secreted/shed from the syncytiotrophoblast through lipid rafts at the time of the clinical onset of pre-eclampsia and HELLP syndrome [29,37]. Lipid rafts are important microdomains of cellular signaling, and indeed, galectin-13 was shown to elicit Ca^{2+} -signaling in cultured trophoblast [40]. Moreover, galectin-13 was demonstrated to induce the apoptosis of activated T cells, suggesting that it may reduce the risk of attach on fetal tissues by maternal immune cells in primates – which have long gestation times and highly invasive placentation [15]. In accordance with these findings, a recent study has revealed that syncytiotrophoblast-secreted galectin-13 plays a unique role in early placentation by forming decidual crystal-like aggregates, and attracting, activating and killing maternal immune cells, diverting them from spiral arterioles and invading trophoblasts [38]. It has been suggested that decreased galectin-13 expression leads to deficient trophoblast invasion and failure of spiral arteriole conversion, and eventually to the development of pre-eclampsia [38].

Indeed, galectin-13 mRNA expression is decreased in the syncytiotrophoblast in cases of pre-eclampsia and HELLP syndrome, especially in their early-onset forms [29,32,37], and this decrease has been suggested to reflect abnormal VT differentiation and/or genetic variations in the gene encoding galectin-13 [29,32,37,80]. Of importance, patients who develop pre-eclampsia in the second half of their pregnancies have both decreased placental galectin-13 mRNA expression and decreased maternal serum galectin-13 protein and mRNA concentrations as early as the first trimester [29,32,37,39–49]. Not surprisingly, galectin-13 is currently one of the most promising first trimester maternal serum biomarkers for the prediction of preterm pre-eclampsia (Figure 2) [39–49].

Concluding remarks

This review has described that galectins expressed at the maternal-fetal interface have a dynamic evolutionary history. It has been shown that uterine endometrium-expressed galectins are regulated by sex steroids and play an important role in immune-endocrine crosstalk and maternal-fetal immune responses. Galectin-expression adaptive and innate immune cells in the uterine decidua promote angiogenesis and trophoblast invasion, and also contribute to an immune-privileged environment at the maternal-fetal interface. It has been suggested that galectins in the fetal membranes may regulate tissue development, display antimicrobial effects and participate in the counter-regulation of inflammatory responses. The role of galectins in EVT's undergoing invasive differentiation has been proposed to include the organization of the ECM and the regulation of the cell cycle. Galectins expressed by VTs undergoing the fusion differentiation pathway have been suggested to function as alarmins, suppress maternal anti-fetal immune response, and participate in early placentation events. Because dysregulated expression of particular galectins has been observed in pregnancy complications, their utilization as biomarkers is under investigation.

Acknowledgments

We thank Prof Dr. Peter Zavodszky and Dr. Andras Szilagyi (Hungarian Academy of Sciences) for constructing Figures 3c and 3d and for their permission to re-use these images; Dr. Adi Tarca and Gaurav Bhatti (Wayne State University) for their assistance in computations; Pat Schoff (Wayne State University) and Valerie Richardson for art work; and Maureen McGerty and Sara Tipton (Wayne State University) for their critical readings of the manuscript. This research was supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS; the National Science Foundation (BCS-0827546); and the European Union FP6 Program (Pregenesys; 037244).

GLOSSARY

Alarmin	an endogenous danger signal secreted via non-classical pathways by activated cells, or released by necrotic cells, which signals tissue damage, contributing to the activation and/or resolution of immune responses [64]
Anthropoid primates	a clade of primates consisting of New World monkeys, Old World monkeys, and apes
Birth-and-death evolution	the differential gain through duplication and loss of closely-related genes in descendant lineages
Clade	a group consisting of a common ancestor and all its descendant taxa
Eutheria	a clade that consists of all extant placental mammals and closely related extinct taxa
Great obstetrical syndromes	pregnancy complications (e.g. preterm labor, preeclampsia) responsible for the majority of perinatal mortality and morbidity. These syndromes have multiple etiologies, are often late clinical manifestations of diverse pathophysiologic processes, and result from adaptive responses of the maternal-fetal unit [81]
Jelly-roll structure	galectin carbohydrate-recognition domains that form a b-sandwich consisting of five- and six-stranded antiparallel b-sheets (Figure 1)

Lectin	a sugar-binding protein (Latin: legere, to select) that is not an antibody or enzyme, which specifically recognizes and binds to glycans without catalyzing their modification [2,4]
Lipid rafts	dynamic receptor-, cholesterol- and sphingolipid-rich microdomains (10–200 nm) in mammalian cell membranes that compartmentalize cellular processes and provide a platform for protein–protein/lipid interactions, influencing membrane trafficking, cell signaling and immune responses [62]
Maternal–fetal immune tolerance	multiple mechanisms in eutherian mammals that promote the establishment of immunological privilege within the pregnant uterus, and antigen-specific, local and systemic maternal tolerance to the fetus. These mechanisms are also reflected by the type of ‘placentation’ – interactions between apposed trophoblasts and maternal immune cells at the ‘maternal–fetal interface’ [75,76,79]
Maternal–fetal interface	the site of contact between maternal and fetal cells, which can vary among taxa. In humans, the syncytiotrophoblast of the embryo is opposed to maternal cells in the decidua for a few days post-implantation and then to the intervillous space. By the end of the first trimester, the syncytiotrophoblast is bathed in maternal blood, whereas invasive extravillous cytotrophoblasts in the placental bed and trophoblasts in the chorion come into contact with maternal cells in the decidua (Figure 2) [75–77,79]
Neofunctionalization	the acquisition of a new function in a duplicated gene
Placentation	the major features that characterize placental morphology including (i) the placental interface (e.g. epitheliochorial, endotheliochorial, hemochorial), (ii) fetomaternal interdigitation (e.g. villous, trabecular, labyrinthine), and (iii) placental shape (e.g. diffuse, cotyledonary, zonary, discoidal). Humans have highly invasive, hemochorial, villous, discoidal placentae [82]
Subfunctionalization	the acquisition by duplicated genes of a complementary subset of functions performed by the ancestral gene [83]

References

1. Apweiler R, et al. On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. *Biochim Biophys Acta*. 1999; 1473:4–8. [PubMed: 10580125]
2. Varki, A., et al. *Essentials in Glycobiology*. Cold Spring Harbor Laboratory Press; 2008.
3. Gabius HJ, et al. The sugar code: functional lectinomics. *Biochim Biophys Acta*. 2002; 1572:165–177. [PubMed: 12223267]
4. Barondes SH. Bifunctional properties of lectins: lectins redefined. *Trends Biochem Sci*. 1988; 13:480–482. [PubMed: 2855286]
5. Jones CJ, Aplin JD. Glycosylation at the fetomaternal interface: does the glycode play a critical role in implantation? *Glycoconj J*. 2009; 26:359–366. [PubMed: 18677581]
6. Cooper DN. Galectinomics: finding themes in complexity. *Biochim Biophys Acta*. 2002; 1572:209–231. [PubMed: 12223271]

7. Barondes SH, et al. Galectins. Structure and function of a large family of animal lectins. *J Biol Chem.* 1994; 269:20807–20810. [PubMed: 8063692]
8. Hirabayashi J, Kasai K. The family of metazoan metal independent beta-galactoside-binding lectins: structure, function and molecular evolution. *Glycobiology.* 1993; 3:297–304. [PubMed: 8400545]
9. Kasai K, Hirabayashi J. Galectins: a family of animal lectins that decipher glycodes. *J Biochem (Tokyo).* 1996; 119:1–8. [PubMed: 8907168]
10. Cummings, RD.; Liu, FT. Galectins. In: Varki, A., et al., editors. *Essentials of Glycobiology.* 2. Cold Spring Harbor Laboratory Press; 2009. p. 475–488.
11. Blois SM, et al. A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med.* 2007; 13:1450–1457. [PubMed: 18026113]
12. Rabinovich GA, Toscano MA. Turning ‘sweet’ on immunity: galectin–glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol.* 2009; 9:338–352. [PubMed: 19365409]
13. Sato S, et al. Galectins in innate immunity: dual functions of host soluble beta-galactoside-binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen associated molecular patterns (PAMPs). *Immunol Rev.* 2009; 230:172–187. [PubMed: 19594636]
14. Liu FT, Rabinovich GA. Galectins: regulators of acute and chronic inflammation. *Ann N Y Acad Sci.* 2010; 1183:158–182. [PubMed: 20146714]
15. Than NG, et al. A primate subfamily of galectins expressed at the maternal–fetal interface that promote immune cell death. *Proc Natl Acad Sci USA.* 2009; 106:9731–9736. [PubMed: 19497882]
16. Than NG, et al. Emergence of hormonal and redox regulation of galectin-1 in placental mammals: implication in maternal–fetal immune tolerance. *Proc Natl Acad Sci USA.* 2008; 105:15819–15824. [PubMed: 18824694]
17. Lewis SK, et al. Galectin 15 (LGALS15): a gene uniquely expressed in the uteri of sheep and goats that functions in trophoblast attachment. *Biol Reprod.* 2007; 77:1027–1036. [PubMed: 17855730]
18. Than NG, et al. Functional analyses of placental protein 13/galectin-13. *Eur J Biochem.* 2004; 271:1065–1078. [PubMed: 15009185]
19. Choe YS, et al. Expression of galectin-1 mRNA in the mouse uterus is under the control of ovarian steroids during blastocyst implantation. *Mol Reprod Dev.* 1997; 48:261–266. [PubMed: 9291476]
20. Vicovac L, et al. Galectin-1 and -3 in cells of the first trimester placental bed. *Hum Reprod.* 1998; 13:730–735. [PubMed: 9572443]
21. Than NG, et al. Isolation and sequence analysis of a cDNA encoding human placental tissue protein 13 (PP13), a new lysophospholipase, homologue of human eosinophil Charcot–Leyden crystal protein. *Placenta.* 1999; 20:703–710. [PubMed: 10527825]
22. Koopman LA, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med.* 2003; 198:1201–1212. [PubMed: 14568979]
23. Bozic M, et al. Galectin-1 and galectin-3 in the trophoblast of the gestational trophoblastic disease. *Placenta.* 2004; 25:797–802. [PubMed: 15451194]
24. Popovici RM, et al. Galectin-9: a new endometrial epithelial marker for the mid- and late-secretory and decidual phases in humans. *J Clin Endocrinol Metab.* 2005; 90:6170–6176. [PubMed: 16105962]
25. von Wolff M, et al. Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation. *Mol Hum Reprod.* 2005; 11:189–194. [PubMed: 15681515]
26. Liu AX, et al. Proteomic analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. *Biol Reprod.* 2006; 75:414–420. [PubMed: 16738225]
27. Jeschke U, et al. 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]
28. Kopcow HD, et al. T cell apoptosis at the maternal–fetal interface in early human pregnancy, involvement of galectin-1. *Proc Natl Acad Sci USA.* 2008; 105:18472–18477. [PubMed: 19011096]

29. Than NG, et al. Placental protein 13 (galectin-13) has decreased placental expression but increased shedding and maternal serum concentrations in patients presenting with preterm pre-eclampsia and HELLP syndrome. *Virchows Arch.* 2008; 453:387–400. [PubMed: 18791734]
30. Than NG, et al. Chorioamnionitis and increased galectin-1 expression in PPROM – an anti-inflammatory response in the fetal membranes? *Am. J Reprod Immunol.* 2008; 60:298–311.
31. Than NG, et al. Severe preeclampsia is characterized by increased placental expression of galectin-1. *J Matern Fetal Neonatal Med.* 2008; 21:429–442. [PubMed: 18570123]
32. Sekizawa A, et al. PP13 mRNA expression in trophoblasts from preeclamptic placentas. *Reprod Sci.* 2009; 16:408–413. [PubMed: 19087972]
33. Winn VD, et al. Severe preeclampsia-related changes in gene expression at the maternal–fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2. *Endocrinology.* 2009; 150:452–462. [PubMed: 18818296]
34. Nhan-Chang CL, et al. Characterization of the transcriptome of chorioamniotic membranes at the site of rupture in spontaneous labor at term. *Am J Obstet Gynecol.* 2010; 202:462–541. [PubMed: 20452490]
35. Shankar R, et al. Molecular markers of preterm labor in the choriodecidua. *Reprod Sci.* 2010; 17:297–310. [PubMed: 20009011]
36. Kolundzic N, et al. Galectin-8 is expressed by villous and extravillous trophoblast of the human placenta. *Placenta.* 2011; 10.1016/j.placenta.2011.07.087
37. Balogh A, et al. Placental protein 13 (PP13/galectin-13) undergoes lipid raft-associated subcellular redistribution in the syncytiotrophoblast in preterm preeclampsia and HELLP syndrome. *Am J Obstet Gynecol.* 2011; 205:156.e1–156.e14. [PubMed: 21596368]
38. Kliman HJ, et al. Placental protein 13 and decidual zones of necrosis: an immunologic diversion that may be linked to preeclampsia. *Reprod Sci.* 2011; 10.1177/1933719111424445
39. Than NG, et al. PP13, maternal ABO blood groups and the risk assessment of pregnancy complications. *PLoS ONE.* 2011; 6:e21564. [PubMed: 21799738]
40. Burger O, et al. Placental protein 13 (PP-13): effects on cultured trophoblasts, and its detection in human body fluids in normal and pathological pregnancies. *Placenta.* 2004; 25:608–622. [PubMed: 15193867]
41. Nicolaides KH, et al. A novel approach to first-trimester screening for early pre eclampsia combining serum PP-13 and Doppler ultrasound. *Ultrasound Obstet Gynecol.* 2006; 27:13–17. [PubMed: 16374755]
42. Chafetz I, et al. First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol.* 2007; 197:35–37. [PubMed: 17618748]
43. Spencer K, et al. First-trimester maternal serum PP-13, PAPP-A and second-trimester uterine artery Doppler pulsatility index as markers of pre-eclampsia. *Ultrasound Obstet Gynecol.* 2007; 29:128–134. [PubMed: 17149788]
44. Huppertz B, et al. Longitudinal determination of serum placental protein 13 during development of preeclampsia. *Fetal Diagn Ther.* 2008; 24:230–236. [PubMed: 18753763]
45. Gonen R, et al. Placental protein 13 as an early marker for pre-eclampsia: a prospective longitudinal study. *BJOG.* 2008; 115:1465–1472. [PubMed: 19035985]
46. Romero R, et al. First-trimester maternal serum PP13 in the risk assessment for preeclampsia. *Am J Obstet Gynecol.* 2008; 199:122. [PubMed: 18539259]
47. Akolekar R, et al. Maternal serum placental protein 13 at 11–13 weeks of gestation in preeclampsia. *Prenat Diagn.* 2009; 29:1103–1108. [PubMed: 19777530]
48. Khalil A, et al. First-trimester markers for the prediction of preeclampsia in women with a-priori high risk. *Ultrasound Obstet Gynecol.* 2010; 35:671–679. [PubMed: 20069559]
49. Wortelboer EJ, et al. First-trimester placental protein 13 and placental growth factor: markers for identification of women destined to develop early-onset pre-eclampsia. *BJOG.* 2010; 117:1384–1389. [PubMed: 20840693]
50. Molvarec A, et al. Peripheral blood galectin-1-expressing T and natural killer cells in normal pregnancy and preeclampsia. *Clin Immunol.* 2011; 139:48–56. [PubMed: 21292557]

51. Houzelstein D, et al. Phylogenetic analysis of the vertebrate galectin family. *Mol Biol Evol.* 2004; 21:1177–1187. [PubMed: 14963092]
52. Brewer FC. Binding and cross-linking properties of galectins. *Biochim Biophys Acta.* 2002; 1572:255–262. [PubMed: 12223273]
53. Swaminathan GJ, et al. Selective recognition of mannose by the human eosinophil Charcot–Leyden crystal protein (galectin-10): a crystallographic study at 1.8 Å resolution. *Biochemistry.* 1999; 38:13837–13843. [PubMed: 10529229]
54. Visegrady B, et al. Homology modelling and molecular dynamics studies of human placental tissue protein 13 (galectin-13). *Protein Eng.* 2001; 14:875–880. [PubMed: 11742106]
55. Lopez-Lucendo MF, et al. Growth-regulatory 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]
56. Horlacher T, et al. Determination of carbohydrate-binding preferences of human galectins with carbohydrate microarrays. *Chembiochem.* 2010; 11:1563–1573. [PubMed: 20572248]
57. Stowell SR, et al. Innate immune lectins kill bacteria expressing blood group antigen. *Nat Med.* 2010; 16:295–301. [PubMed: 20154696]
58. Liu FT, et al. Intracellular functions of galectins. *Biochim Biophys Acta.* 2002; 1572:263–273. [PubMed: 12223274]
59. Camby I, et al. Galectin-1: a small protein with major functions. *Glycobiology.* 2006; 16:137–157.
60. Hernandez JD, Baum LG. Ah, sweet mystery of death! Galectins and control of cell fate. *Glycobiology.* 2002; 12:127R–136R.
61. Nickel W. Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells. *Traffic.* 2005; 6:607–614. [PubMed: 15998317]
62. Danielsen EM, Hansen GH. Lipid raft organization and function in brush borders of epithelial cells. *Mol Membr Biol.* 2006; 23:71–79. [PubMed: 16611582]
63. Dennis JW, et al. Metabolism, cell surface organization, and disease. *Cell.* 2009; 139:1229–1241. [PubMed: 20064370]
64. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol.* 2007; 81:1–5. [PubMed: 17032697]
65. Su AI, et al. A gene atlas of the mouse and human protein encoding transcriptomes. *Proc Natl Acad Sci USA.* 2004; 101:6062–6067. [PubMed: 15075390]
66. Saal I, et al. Human galectin-2: expression profiling by RT-PCR/immunohistochemistry and its introduction as a histochemical tool for ligand localization. *Histol Histopathol.* 2005; 20:1191–1208. [PubMed: 16136502]
67. Dong M, et al. The effect of trophoblasts on T lymphocytes: possible regulatory effector molecules – a proteomic analysis. *Cell Physiol Biochem.* 2008; 21:463–472. [PubMed: 18453754]
68. Gitt MA, et al. Galectin-4 and galectin-6 are two closely related lectins expressed in mouse gastrointestinal tract. *J Biol Chem.* 1998; 273:2954–2960. [PubMed: 9446608]
69. Kubach J, et al. Human CD4+CD25+ regulatory T cells: proteome analysis identifies galectin-10 as a novel marker essential for their anergy and suppressive function. *Blood.* 2007; 110:1550–1558. [PubMed: 17502455]
70. Yang QS, et al. Cloning and expression of a novel human galectin cDNA, predominantly expressed in placenta(1). *Biochim Biophys Acta.* 2002; 1574:407–411. [PubMed: 11997112]
71. Young AR, et al. Functional characterization of an eosinophil specific galectin, ovine galectin-14. *Glycoconj J.* 2009; 26:423–432. [PubMed: 18810635]
72. Bischof P, Irminger-Finger I. The human cytotrophoblastic cell, a mononuclear chameleon. *Int J Biochem Cell Biol.* 2005; 37:1–16. [PubMed: 15381142]
73. Rabinovich GA, Ilarregui JM. Conveying glycan information into T-cell homeostatic programs: a challenging role for galectin-1 in inflammatory and tumor microenvironments. *Immunol Rev.* 2009; 230:144–159. [PubMed: 19594634]
74. Garin MI, et al. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. *Blood.* 2007; 109:2058–2065. [PubMed: 17110462]

75. Moffett A, Loke C. Immunology of placentation in eutherian mammals. *Nat Rev Immunol.* 2006; 6:584–594. [PubMed: 16868549]
76. Aluvihare VR, et al. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol.* 2004; 5:266–271. [PubMed: 14758358]
77. Redman CW, Sargent IL. Immunology of pre-eclampsia. *Am J Reprod Immunol.* 2010; 63:534–543. [PubMed: 20331588]
78. Elola MT, et al. Galectin-1 receptors in different cell types. *J Biomed Sci.* 2005; 12:13–29. [PubMed: 15864736]
79. Aplin JD. Developmental cell biology of human villous trophoblast: current research problems. *Int J Dev Biol.* 2010; 54:323–329. [PubMed: 19876840]
80. Gebhardt S, et al. A novel exonic variant (221delT) in the LGALS13 gene encoding placental protein 13 (PP13) is associated with preterm labour in a low risk population. *J Reprod Immunol.* 2009; 82:166–173. [PubMed: 19818512]
81. Romero R. Prenatal medicine: the child is the father of the man. *Prenat Neonatal Med.* 1996; 1:8–11.
82. Wildman DE, et al. Evolution of the mammalian placenta revealed by phylogenetic analysis. *Proc Natl Acad Sci USA.* 2006; 103:3203–3208. [PubMed: 16492730]
83. Lynch M, Force A. The probability of duplicate gene preservation by subfunctionalization. *Genetics.* 2000; 154:459–473. [PubMed: 10629003]
84. Inagaki Y, et al. Oxidized galectin-1 promotes axonal regeneration in peripheral nerves but does not possess lectin properties. *Eur J Biochem.* 2000; 267:2955–2964. [PubMed: 10806394]

Figure 1.

Galectins promote a diverse range of responses when they contact leukocytes. Galectin-effects on innate and adaptive immune cells are depicted around the 3D model of galectin-1 dimer (1GZW) [31,55]. These effects are context-dependent and relate to the type of galectins and their intra- or extracellular localization, as well as to the type of cells and their activation and/or differentiation status [10,12,14]. Galectins as well as most types of immune cells (e.g. T cells, neutrophils, macrophages) depicted in the figure are abundant at the maternal-fetal interface. Figure adapted from [31], © Taylor & Francis.

Figure 2.

Galectin expression at the maternal–fetal interface. The figure represents three interfaces where maternal and fetal cells are in direct contact from the end of the first trimester of human pregnancy. The syncytiotrophoblast of the villous placenta (depicted with gold, right side) is bathed in maternal blood, whereas invasive extravillous cytotrophoblasts in the placental bed (depicted in red, right side) and chorionic trophoblasts in the fetal membranes (depicted in red, left side) are in contact with maternal cells in the decidua (depicted in dark blue, both sides). The dysregulated expression of highly expressed galectins at the maternal–fetal interface (depicted by arrows at sites of dysregulation) is often observed in pregnancy complications. Figure adapted from [16], © National Academy of Sciences of the U.S.A.

Figure 3. Galectin evolution. (a) Maximum-likelihood phylogeny of mammalian galectin CRD amino acid sequences inferred using RaxML with a Dayhoff matrix. Prototype galectins are numbered with red (o14: sheep ‘galectin-14’), chimera-type galectin with magenta, tandem-repeat-type galectins with black (F3 domains) and blue (F4 domains), galectin-related proteins (hs, HSPC159; gr, griffin) with orange. (b) Phylogenetic relationship among closely-related genes within the galectin-13-clade cluster [15]. Genes with predominant placental expression are highlighted in red. (c) Evidence for adaptive evolution of galectins in the anthropoid cluster is represented on the molecular backbone of galectin-16. Site-specific v values are indicated by the width of the molecular backbone and by a color spectrum [15]. v values $<$, $=$, $>$ 1 indicate purifying selection, neutral evolution, and positive selection, respectively; v values ranged between 0.2 and 2.2. Residues with greater v are wider and nearer the red range of the color spectrum. (d) The same color-coding shows that four conserved residues (53, 65, 72, 75) in the CRDs of cluster galectins are under strong purifying (i.e. negative) selection, others on the opposite side of the CRDs (55, 57, 63, 77) show more variability [15]. Figures adapted from [15], © National Academy of Sciences of the U.S.A.