

Galectins: a key intersection between glycobiology and immunology

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Abstract

Galectins are a family of evolutionarily conserved animal lectins, widely distributed from lower invertebrates to mammals. They share sequence and structure similarities in the carbohydrate recognition domain and specificity for polylectosamine-enriched glycoconjugates. In the last few years significant experimental data have been accumulated concerning their participation in different biological processes requiring carbohydrate recognition such as cell adhesion, cell growth regulation, inflammation, immunomodulation, apoptosis and metastasis. In the present review we will discuss some exciting questions and advances in galectin research, highlighting the significance of these proteins in immunological processes and their implications in biomedical research, disease diagnosis and clinical intervention. Designing novel therapeutic strategies based on carbohydrate recognition will provide answers for the treatment of autoimmune disorders, inflammatory processes, allergic reactions and tumor spreading.

Key words

- Galectins
- Immunomodulation
- Apoptosis
- Adhesion
- Embryogenesis
- Metastasis

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Presented at the XXVII Annual Meeting of the Brazilian Society of Biochemistry and Molecular Biology, Caxambu, MG, Brasil, May 23-26, 1998.

G.A. Rabinovich received the "Young Talent in Life-Sciences-98" award for this article from the Sociedade Brasileira de Bioquímica e Biologia Molecular and Amersham Pharmacia Biotech.

Research supported in part by CONICET, CONICOR, Fundación Antorchas and British Council.

Received December 2, 1998
Accepted February 1, 1999

Introduction

What are galectins?

Galectins are a family of evolutionarily conserved proteins widely distributed in nature from lower invertebrates to mammals (1-3). They are defined by their sequence and structure similarities in the carbohydrate recognition domain (CRD) and their specificity for polylectosamine-enriched glycoconjugates (1,2). Ten mammalian galectins have been well identified in a wide variety of tissues of several species (4).

How are galectins designed?











According to their architecture, Hiraba-

yashi and Kasai (5) classified this protein family into proto-type, chimera-type and tandem repeat type. Proto-type galectins include galectin-1 (6,7), -2 (8), -5 (9), -7 (10), human Charcot-Leyden crystal protein (11), frog galectins (12), electrolectin, which was the first galectin described (13), *C. elegans* 16-kDa galectin (14), chicken isolectins C-14 and C-16 (15,16), and sponge isolectins (17). They are non-covalent homodimers composed of two identical CRDs. On the other hand, chimera-type galectins exhibit a combined structure composed of a carboxy terminal CRD linked to a proline-, glycine-, and tyrosine-rich N-terminal domain. The only member of this family, galectin-3 (previously known as Mac-2, L-29, CBP-35 and IgEBP) (18), has been mainly found in mam-

mals, although it has also been detected in chickens (19). The third family of tandem-repeat galectins includes proteins with two distinct CRDs, such as galectins-4 (20), -6 (21), -8 (22) and -9 (23). The biochemical properties and tissue distribution of different galectins are summarized in Table 1. Several galectin genes have been cloned and charac-

Table 1 - Galectin family: properties and tissue distribution.

MM, Molecular mass; AA residues, amino acid residues.

Designation Structure	Species	Tissue/cell distribution	Architectural type	MM SDS/PAGE	AA residues
Gal-1 	human, rat, mouse, pig, hamster, monkey, bovine, ovine	muscle, heart, lung, placenta, brain, spleen, liver, lymph nodes, thymus, colon, prostate, macrophage	proto	14.5 kDa	134
Gal-2 	human, mouse	small intestine	proto	14.5 kDa	132
Gal-3 	human, rat, mouse, dog, hamster	macrophage, colon, leukemia cells, 3T3 fibroblast	chimera	29-35 kDa	>250
Gal-4 	human, rat, mouse, pig	alimentary tract, epithelial cells	tandem repeat	36 kDa	324
Gal-5 	rat	erythrocytes	proto	17-18 kDa	144
Gal-6 	mouse	gastro-intestine	tandem repeat	34 kDa	301
Gal-7 	human, rat	skin	proto	14.5 kDa	136
Gal-8 	human, rat	liver, lung, kidney	tandem repeat	34 kDa	316
Gal-9 	human, rat, mouse	kidney, thymus, Hodgkin's lymphoma	tandem repeat	35 kDa	322
Gal-10 	human	eosinophil, basophil	proto	17 kDa	142

terized and found to be associated with multiple regulatory elements.

How were galectins identified?

Although the first galectins were identified by their binding to β -galactoside-sugars (5), the most recent galectins, such as galectins-5 and -8, were detected by immunoscreening cDNA libraries (9,22) or by crossreactivity with other galectins identified so far (24). In this context galectin-9 was found by screening tumor cDNA libraries with sera from tumor-bearing patients (23).

Why are galectins found in the extracellular milieu if they are designed as intracellular proteins?

From the viewpoint of protein structure, galectins have been designed to play key roles inside the cells with an acetylated N-terminus, lack of signal sequences and biosynthesis in free ribosomes. Although novel intracellular functions have been reported in the last few years, such as mRNA processing (25), most of the functions assigned to β -galactoside-binding proteins are confined to the cell surface or extracellular milieu (26), suggesting that galectins are externalized by a non-classical secretory mechanism (27).

The intracellular concentration of galectins under physiological conditions is as high as 0.01 mM in mammalian cells, but it can reach levels of approximately 0.1 mM in critical situations. Galectin expression was found to be modulated during embryogenesis (28), being a typical hallmark of specific developmental stages. Moreover, galectin expression and subcellular distribution were reported to be highly susceptible to modulation by diverse stimuli such as sodium butyrate (29), viral infections (30), tumor suppressor genes (31) or inflammatory agents (18). In the present paper we discuss in detail the regulated expression of RMGal, a rat

macrophage galectin-1-like protein, isolated and characterized in our laboratory, which showed immunomodulatory properties (24, 32). Gillenwater and colleagues (29) demonstrated that sodium butyrate, a known differentiating agent, was able to modulate galectin-1 content in human head and neck squamous carcinoma cells by a combination of transcriptional regulation and inhibition of histone deacetylation.

What are the mechanisms involved in galectin secretion?

This question still remains to be ascertained. However, two hypotheses have been considered regarding the externalization of galectin-1. The first involves the utilization of specific transmembrane carriers, like those used to export some bacterial toxins (33). The second considers the possibility that, after synthesis, galectin-1 becomes concentrated in plasma membrane evaginations prior to secretion and further externalized to form galectin-enriched extracellular vesicles (27). A kind of infrequent mechanism of externalization is also used by many cytokines and growth factors (34). Nevertheless, it is clear that galectins can be specifically targeted and secreted to exert their functions by interacting with intracellular or extracellular glycoconjugates.

Functions of galectins

What is the functional significance of this evolutionarily conserved family of animal lectins?

Conservation of galectins throughout animal evolution and their widespread distribution strongly suggest they could be implicated in critical biological functions requiring carbohydrate recognition. However, null mutant mice for galectin-1 and -3 genes presented no major phenotypic abnormalities (35,36), suggesting that they are not so cru-

cial for life. In this context, it may be proposed that other members of this family could potentially compensate for the absence of these proteins, as suggested for targeted disruption of other important genes (37).

Galectins have been involved in several *in vitro* physiopathological processes requiring carbohydrate recognition, such as cell adhesion (26), cell growth regulation (38), immunomodulation (39,40), apoptosis (32, 41), inflammation (42), embryogenesis (28), reproduction (43,44), tumor spreading (45) and pre-mRNA splicing (25). It should be emphasized that the vast majority of galectin functions have been assigned to galectins-1 and -3. Hence, functions corresponding to other members of this protein family remain to be elucidated in future work.

What are the molecular mechanisms involved in galectin functions?

Although galectins are supposed to exert their biological roles by crosslinking glycoconjugate ligands, the precise mechanisms of action of these proteins and their signal transduction pathways remain largely unknown.

Galectins in cell adhesion

What experimental evidence suggests that galectins could be involved in cell adhesion?

Poly-lactosamine residues in extracellular matrix (ECM) glycoproteins such as laminin (46) and fibronectin (47) have been proposed to be candidate ligands for galectin-1. In view of this specific recognition, galectins have been postulated as powerful modulators of cell-cell and cell-ECM interactions.

In this context, are galectins pro-adhesive or anti-adhesive proteins?

Controversial results have been reported

as to whether galectin-1 exerts a positive or a negative effect on cell adhesion to ECM glycoconjugates, raising the possibility that this dimeric protein could promote cell attachment or detachment according to cell type or cell developmental stage (26). At first sight, galectin-1 promoted cell adhesion in various cell types, such as melanoma cell lines (48), F9 teratocarcinoma cells (46), olfactory neurons (49), rhabdomyosarcoma cells (47) and CHO fibroblasts (46), by bridging oligosaccharides between specific cell surface glycoconjugates and ECM components. On the other hand, the presence of galectin-1 inhibited myoblast interaction with laminin by sterically blocking the laminin receptor $\alpha_7\beta_1$ integrin from recognizing laminin, thus allowing myoblasts to fuse into myotubes (50).

Similarly, the effects of galectin-3 on cell adhesion were antagonistic, depending on the physiopathological environment. While galectin-3 promoted neutrophil adhesion to laminin (51) in the context of inflammation, it showed a dramatic inhibitory effect on melanoma cell adhesion to ECM in metastases (52). In this context, expression of galectins-1 and -3 in tumor cells was found to be correlated with high metastatic potential and low survival (53).

Galectins in cell growth regulation

What is the role of galectins in cell growth regulation and differentiation?

Galectins have shown critical antagonistic effects on cell growth. Sanford and Harris-Hooker (54) reported a mitogenic effect of galectin-1 on vascular cells during alveolarization of postnatal rat lung. On the other hand, Wells and Mallucci (38) demonstrated that galectin-1 acted as a powerful inhibitory growth factor on murine embryonic fibroblasts by controlling the exit from the G0 and G2 stages of the cell cycle.

How can it be explained that antagonistic stimulatory and inhibitory properties could be present within the same molecule?

To answer this question, an interesting study reports a biphasic modulation of cell growth by recombinant galectin-1 (55). While this β -galactoside-binding protein was found to be mitogenic at low physiological concentrations, the growth-inhibitory properties were apparent in a higher concentration range, when tested on human cells *in vitro*. Moreover, galectin-1 may trigger either proliferation or cell growth arrest depending on the presence of concomitant environmental signals, cell cycle stages, or the expression of its carbohydrate receptors on the cell surface (55). Other signaling molecules, such as TGF β (56) and FasL (57), have been shown to trigger different effects depending on the type and functional state of the cells.

Galectins in the immune system

The ultimate goal of immunologists is to manipulate the immune response, so that deficient responses can be potentiated, while harmful responses are suppressed.

Are galectins important in the context of innate immunity?

The innate immune system is important because it provides the early phases of host defense to protect the organism. Galectins have been implicated in different functions of innate immunity such as the respiratory burst (58), chemotaxis of eosinophils (59) and neutrophil migration during inflammatory episodes (51).

What was the first clue suggesting that galectins could play a role in the regulation of the adaptive immune response?

That galectins could be implicated in the

regulation of the immune response was first inferred from galectin-1 localization in immune privileged sites of the body, such as the placenta (6,43,44) and the eye (60). In immune privileged sites multiple factors interact to prevent immunopathogenic processes from disrupting the critical functions of these particularly vulnerable organs. It has been recently demonstrated that FasL expression in immune privileged sites may have a key role in preserving tolerance by selectively killing inflammatory T cells by apoptosis (61). Accordingly, galectin-1 could be proposed as an alternative regulatory signal to regulate immune privilege. Expression of this protein in the first term gestation placenta would prevent inflammatory T cells from harming the fetus (43). Furthermore, galectin-1 expression in the eye would protect this sensory organ from the devastating effects of an inflammatory response (61,62). In agreement, a protein related to the galectin family called GRIFIN (galectin-related interfiber protein) was recently identified in the lens and in simple cellular structures of the eye (60).

Are galectins also localized within the immune system?

Galectin-1 has been identified not only in peripheral lymphoid organs such as lymph nodes (63) and spleen (24), but also in the thymus (64). Baum et al. (64) found that human thymic epithelial cells synthesized galectin-1, which bound to oligosaccharide ligands on the surface of thymocytes. Recently, Blaser et al. (65) found that galectin-1 gene expression was strongly up-regulated in effector T cells. In addition, this protein was able to inhibit antigen-induced proliferation of naive and memory CD8⁺ T cells, acting as an autocrine negative growth factor (65). When this inhibitory mechanism was studied in detail, it was clear that galectin-1 switched off T lymphocyte effector functions by arresting cell cycle progression at

the level of the S and G₂/M stages (66).

What is the association between galectins and atopic diseases?

Recent studies have shown that galectin-3, formerly defined as an Ig E-binding protein, was able to inhibit IL-5 gene transcription and protein release from human eosinophils and allergen-specific T cell lines (67). The possibility to shut down the IL-5 pathway could be determinant in the regulation of the T helper 2-dependent allergic reactions. Further investigations will be needed to clarify the mechanisms involved in this process.

What is the association between galectins and autoimmunity?

Galectin-1 has shown immunosuppressive properties in two experimental models of autoimmunity. Administration of recombinant galectin-1 prevented clinical and histopathological manifestations of experimental autoimmune encephalomyelitis (EAE) in susceptible Lewis rats (39) and autoimmune myasthenia gravis (EAMG) in rabbits (40).

What kind of molecular mechanisms are involved in these immunomodulatory properties?

Although the precise mechanisms involved in these properties *in vivo* still remain to be elucidated, hypotheses have been raised concerning the ability of galectin-1 to affect processes in T cell suppressor commitment and in sensitization or deletion of antigen-specific T cells (39,40). Interestingly, in the last few years evidence has been obtained concerning the implications of galectin-1 in apoptosis of mature T cells (31,41,43) and particular subsets of immature cortical thymocytes (59), thus providing a breakthrough in glycoimmunology.

What was the first clue indicating that galectins could be implicated in programmed cell death?

The first clue was raised when Goldstone and Lavin (68) reported in 1991 that the galectin-1 gene was overexpressed during glucocorticoid-induced cell death.

What is the role of galectin-1 in central tolerance?

Concerning the immune system, cell death programs promote tolerance by deletion of autoreactive lymphocytes in the central lymphoid organs and termination of immune responses in the peripheral immune system. At the central level, lymphocytes are susceptible to positive (69) and negative (70) selection. Immature T cells that recognize self-peptides with high affinity in the context of autologous major histocompatibility complex (MHC) molecules undergo negative selection by programmed cell death (70). T lymphocytes that fail to receive antigen stimulation (neglected cells) also undergo a default apoptotic program (69). The remaining T cells, which bind to peptide MHC complexes with low affinity, are positively selected to survive. In the context of new molecules involved in the machinery of central death, galectin-1 expressed by human thymic epithelial cells (64) has been recently shown to induce apoptosis of cortical thymocytes (71). The model of galectin-1 gene knock-out mice (35) will be useful to elucidate the precise role of galectin-1 in the elimination of non-selected and negatively selected cells during thymocyte development.

What is the role of galectin-1 in peripheral tolerance?

Activation-induced cell death of mature peripheral T cells is indeed one of the mechanisms aimed at preventing the expansion of dominant autoaggressive clones (72). In this

context, galectin-1 has also been reported to induce apoptosis of mature T cells (32,41,43). The apoptotic effect of soluble galectin-1 depended upon the activation state of T cells. Perillo et al. (41) clearly showed that galectin-1 induced apoptosis of mature T cells bearing the CD45RO splicing product, which was found to be differentially glycosylated (73). This provides an alternative mechanism for explaining the immunosuppressive properties exhibited by this highly conserved protein family.

What is the link between apoptosis and autoimmunity?

The importance of decreased apoptosis in the etiology of autoimmune disorders has been highlighted by the occurrence of lupus-like disorders in MRL-*lpr/lpr* or C3H-*gld/gld* mouse strains, carrying spontaneous mutations in *Fas* or *FasL* genes (74), and in particular by the dominant *Fas* gene mutation associated with Canale-Smith syndrome, a human autoimmune lymphoproliferative disorder (75).

Galectins-1 and -3: why a novel paradigm?

Antagonistic functions have been assigned to galectin-1 and galectin-3, providing clues for a novel paradigm. While galectin-1 has been shown to induce T cell apoptosis (32,41,43), galectin-3 has been conversely shown to prevent cell death (76). Similarly to members of the *Bcl-2* family, galectins-1 and -3 belong to an additional family of

proteins with high sequence homologies but opposite effects on cell survival (Figure 1). As clearly shown in the Figure, *Bcl-2* and *Bcl-X_L* are negative regulators of apoptosis, whereas *Bax*, *Bad*, and *Bak* contribute to a positive signal in the regulation of cell death (77). Transfection of galectin-3 cDNA into leukemia T cells conferred resistance to apoptosis induced by Fas ligation and staurosporine (76). Of particular interest, galectin-3 showed a significant sequence similarity to *Bcl-2*, mainly concentrated in the functional BH1 (NWGR) domain. Although this paradigm between galectins-1 and -3 seems to be attractive, it should not be conceived as a general principle. Future studies should challenge this paradigm in the context of other physiological systems. Consistently, expression of galectins-1 and -3 was found to be differentially regulated during mammalian gestation (43,44). Iglesias et al. (44) reported the coexistence of mitogenic galectin-3 and apoptotic galectin-1 (43) in ovine placenta. Interestingly, galectin-3 expression was found to be decreased in term ovine placenta, in comparison with the middle of the gestation period. In contrast, no significant decrease was observed in galectin-1 expression. In this context, one might speculate that differential expression of both β -galactoside-binding lectins in ovine placenta could be associated with selective requirements at different developmental stages of gestation.

Furthermore, the participation of other members of the galectin family in the control of programmed cell death should also be considered. In this sense, galectin-9, a novel

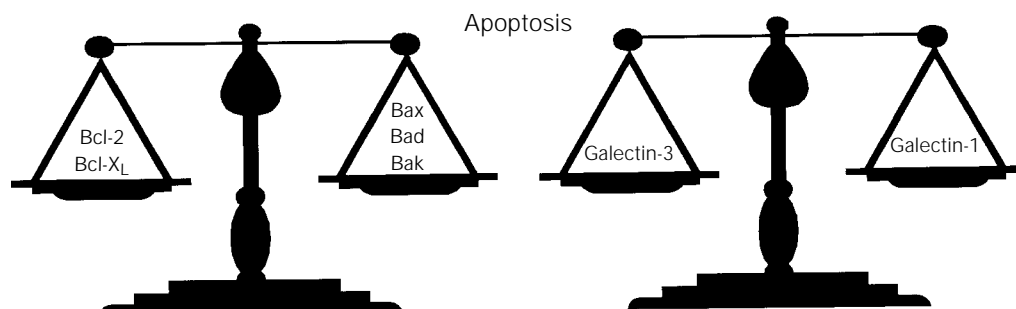


Figure 1 - Galectins-1 and -3: a novel paradigm in the regulation of programmed cell death. Galectins-1 and -3 represent an additional family of proteins similar to the *Bcl-2* family, where different members exhibit sequence and structure similarities, but opposite effects on cell survival.

tandem repeat lectin, has recently shown strong apoptotic activity toward murine thymocytes (78).

What is the functional significance of galectin-1 in activated macrophages?

Macrophages (M ϕ s) play important roles in a wide spectrum of events from innate immunity to adaptive immune response (24). Since galectin-1 had shown such immunomodulatory properties, we wondered whether it could be possible to identify its presence in key immunoregulatory cells such as M ϕ s and further demonstrate its functional meaning for target cells.

By means of immunochemical and immunocytochemical studies, we have identified the presence of a 15-kDa soluble protein with key features of mammalian galectin-1 in rat peritoneal M ϕ s by using a polyclonal Ab raised against the extensively studied C-16 chicken isolectin (24). Rat macrophage galectin (RMGal) was purified from chemically activated macrophages by a single step affinity chromatography on a lactosyl-Sepharose matrix, resulting in the isolation of a sharp protein band of 15 kDa, an isoelectric point of 4.8 and high hemagglutinating activity specifically inhibited by saccharides bearing a β -D-galactoside configuration (32). This protein behaved as a dimer under nondenaturing conditions, and internal amino acid sequencing revealed extensive homologies (80-90%) with other members of the galectin-1 subfamily. Taken together, all the properties described resembled those exhibited by mammalian galectin-1 (32).

Resident, inflammatory or chemically activated M ϕ s must be viewed as distinct cell subpopulations, since they show different phenotypes according to their activation status (24). In light of this observation, the next question we tackled was related to the regulation of total and surface RMGal expression in different M ϕ subpopulations. By Western blot analysis and further densitometric

quantitation, we found a 5-fold increase in total galectin expression in chemically activated M ϕ s and a 2-fold increase in inflammatory M ϕ s in comparison to peritoneal resident cells (24). Moreover, modulation of RMGal expression was accompanied by an increase in cell surface immunostaining as shown by FACS analysis of non-permeabilized cells (24). Modulation of galectin expression and subcellular distribution strongly suggested that it could be implicated in crucial immunological processes mediated by M ϕ s. By using current techniques to evaluate apoptosis, such as TUNEL assay, transmission electron microscopy and DNA fragmentation assay, we concluded that RMGal was clearly involved in apoptosis of mature T-cells and that this effect was dependent on its carbohydrate recognition domain. These results were more significant than those obtained using a heterologous system (79). Consistently, it has been reported that monocytes and dendritic cells from peripheral blood of healthy individuals can induce apoptosis of mitogen-activated T-cells (80).

Concluding remarks and future perspectives

Elucidation of the molecular mechanisms involved in galectin functions will provide new insights in biomedical research, disease diagnosis, prognosis and clinical therapy.

At the level of disease diagnosis, Lutomski et al. (81) reported the presence of autoantibodies against galectin-1 in sera of patients with neurological disorders, particularly multiple sclerosis. Moreover, aberrant expression of galectins on the surface of tumor cells seems to be indicative of a worse prognosis (53).

At the level of clinical therapy, future research should focus on the identification of new strategies for treatment of autoimmune diseases, inflammatory processes, allergic reactions and tumor spreading using galectins or galectin antagonists. First, the

possibility to regulate the apoptotic threshold of autoreactive T cells using galectin-1 would be beneficial in autoimmunity. Second, down-regulation of the IL-5 gene by galectin-3 would be important in the treatment of allergic processes. Finally, neutralizing antibodies or antisense oligonucleotides aimed at inhibiting galectins-1 and -3 expression would potentially decrease the metastatic potential of tumor cells

Acknowledgments

We would like to give special thanks to all the people who contributed to our work during the last few years, especially to Drs. Mercedes Iglesias, Nidia Modesti, Carlota Wolfenstein-Todel, Yuti Chernajovsky, Ofer Lider, Hanna Dreja, Gordon Daily, José Luis Bocco and Leonardo Castagna.

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