Unlocking the secrets of galectins: a challenge at the frontier of glyco-immunology

Gabriel A. Rabinovich, Natalia Rubinstein, and Leonardo Fainboim

Division of Immunogenetics, Hospital de Clínicas "José de San Martín" and Department of Microbiology, Parasitology and Immunology, School of Medicine, University of Buenos Aires, Argentina

Abstract: Over the last decade, we have witnessed an explosion of information regarding the function of glycoconjugates, carbohydrate-binding proteins, and the elucidation of the sugar code. This progress has yielded not only important insights into fundamental areas of glycobiology but has also influenced other fields such as immunology and molecular medicine. A family of galactoside-binding proteins, called galectins, has emerged recently as a novel kind of bioactive molecules with powerful, immunoregulatory functions. Different members of this family have been shown to modulate positively or negatively multiple steps of the inflammatory response, such as cell-matrix interactions, cell trafficking, cell survival, cell-growth regulation, chemotaxis, and proinflammatory cytokine secretion. To introduce a comprehensive overview of these new advances, here we will explore the molecular mechanisms and biochemical pathways involved in these functions. We will also examine the role of these proteins in the modulation of different pathological processes, such as chronic inflammation, autoimmunity, infection, allergic reactions, and tumor spreading. Understanding the intimate mechanisms involved in galectin functions will help to delineate selective and novel strategies for disease intervention and diagnosis. *J. Leukoc. Biol.* 71: 741–752; 2002.

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INTRODUCTION

Galectins, glycoligands, and the interpretation of the sugar code: state of the art

Galectins are members of a highly evolutionarily conserved family of animal lectins widely distributed in the animal kingdom [1]. They share structural similarities in the carbohydrate recognition domain (CRD) in addition to specificity for polylactosamine-enriched glycoconjugates [1, 2]. By virtue of their multivalency, galectins are able to cross-link cell-surface glycoconjugates and initiate cell biological responses [3]. The typical CRD is folded into a sandwich structure of five to six stranded β-sheets and recognizes the basic structure of *N*-acetyllactosamine (Gal β1→4GlcNAc) [2]. Galectins bind to *N*-acetyllactosamine with relatively low affinity [dissociation constant (K_d) in the range of 90–100 μM], but they bind to glycoproteins containing polylactosamine sequences with high affinity ($K_d \approx 1$ μM) [4].

Thirteen mammalian galectins have been identified to date in a wide variety of tissues from different species [5]. According to their structure, they have been classified by Hirabayashi and Kasai [3] into prototype galectins (galectins-1, -2, -5, -7, -10, -11, and -13), existing as monomers or noncovalent homodimers, consisting of two identical CRD, chimera-type (galectin-3), containing a nonlectin domain linked to a CRD, and tandem-repeat-type (galectins-4, -6, -8, -9, and -12), composed of two distinct CRDs in a single polypeptide chain (**Table 1**). Many of these β-galactoside-binding proteins have also received other names, according to their function, localization, or biochemical features, i.e., galectin-1 (L-14, BHL, or galaptin), galectin-3 [Mac-2, L-29, CBP-35, or εBP for immunoglobulin "(Ig)E-binding protein"], galectin-9 (ecalectin), galectin-10 (Charcot-Leyden crystal eosinophil protein), galectin-11 (GRIFIN for "galectin-related interfiber protein"), and galectin-13 (PP13) [2–7]. Analysis of GenBank databases has led to the identification of more galectin-like proteins in mammals, invertebrates, plants, and microorganisms, confirming that these carbohydrate-binding proteins are highly conserved throughout the evolution [5]. Approximately seven additional mammalian candidates for membership in this family have been identified recently, using search algorithms based on the structure of known galectins [5]. Almost all of them appear in human genomic DNA, and their mRNA is also expressed, suggesting that they are not pseudogenes [5]. Most galectins have been proposed to exert discrete biologic effects, according to subcellular compartmentalization, developmentally regulated expression, and cell-activation status [3]. Although galectins lack a signal peptide and a transmembrane domain, they are secreted by a nonclassical and novel apocrine mechanism, in which the synthesized protein becomes concentrated, after the perception of inflammatory stimuli, at the level of the plasma membrane, and are externalized further to form galectin-enriched extracellular vesicles [8, 9]. Although most galectins exert their functions extracellularly, intracellular functions

Correspondence: Dr. Gabriel A. Rabinovich, Laboratorio de Inmunogenética, Hospital de Clínicas "José de San Martín", Facultad de Medicina, Universidad de Buenos Aires, Córdoba 2351, 3er Piso (C 1120), Ciudad de Buenos Aires, Argentina. E-mail: gabyrabi@ciudad.com.ar

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TABLE 1. Schematic Representation, Biochemical and Functional Properties of Different Members of the Galectin Family

Galectins/ structure	Localization	Biochemical and functional properties
Galectin-1	Abundant in most organs: muscle, heart, liver, prostate, lymph nodes, spleen, thymus, placenta, testis, retina, macrophages, B cells, T cells, tumors	▪ Non-covalent homodimer ▪ Induces apoptosis of activated T cells and immature thymocytes ▪ Induces a polarized Th2 immune response ▪ Modulates cell-cell and cell-matrix interactions ▪ Inhibits acute inflammation: blocks arachidonic acid release, mast cell degranulation and neutrophil extravasation ▪ Suppresses chronic inflammation and autoimmunity
Galectin-2	Stomach epithelial cells	▪ Non-covalent homodimers ▪ Expressed at minor levels in tumor cells
Galectin-3	Mainly in tumor cells, macrophages, epithelial cells, fibroblasts, activated T cells	▪ Non-lectin domain linked to a CRD ▪ Anti-apoptotic and pro-inflammatory functions ▪ Modulates cell adhesion and migration ▪ Induces chemotaxis of monocytes ▪ Potentiates pro-inflammatory (IL-1) cytokine secretion ▪ Inhibits nitric oxide-induced apoptosis and anoikis ▪ Down-regulates IL-5 gene transcription
Galectin-4	Gastrointestinal tract	▪ Composed of two distinct CRDs in a single polypeptide chain ▪ Expressed at sites of tumor cell adhesion
Galectin-5	Erythrocytes	▪ Proto-type galectin: monomer ▪ No function assigned
Galectin-6	Gastrointestinal tract	▪ Composed of two distinct CRDs in a single polypeptide chain ▪ Closely linked to galectin-4
Galectin-7	Skin	▪ Proto-type galectin: monomer ▪ Used as a marker of stratified epithelium ▪ Increases susceptibility of keratinocytes to UVB-induced apoptosis
Galectin-8	Liver, kidney, cardiac muscle,prostate and brain	▪ Composed of two distinct CRDs in a single polypeptide chain ▪ Modulates integrin interactions with the extracellular matrix
Galectin-9	Thymus, T cells, kidney, Hodgkin´s lymphoma	▪ Composed of two distinct CRDs in a single polypeptide chain ▪ Induces eosinophil chemotaxis ▪ Induces apoptosis of murine thymocytes
Galectin-10	Eosinophils and basophils	▪ Proto-type galectin: monomer ▪ Mainly expressed by eosinophils, formally called "*Charcot-Leyden Crystal protein*"
Galectin-11	Lens	▪ Also called "GRIFIN " ▪ May represent a new lens crystalline ▪ Lacks affinity for β-galactoside sugars
Galectin-12	Adipocytes	▪ Composed of two distinct CRDs in a single polypeptide chain ▪ Induces apoptosis and cell cycle arrest
Galectin-13	Recently identified in human placenta	▪ Similar to "*proto-type* galectins"? ▪ Also called "PP-13"

have also been proposed for these proteins, such as regulation of the pre-mRNA splicing [10]. Elucidation of the nuclear or cytosolic ligands will be necessary for a more in-depth understanding of this first intracellular function.

Given their evolutionary conservation across living species, it is not surprising that galectins could be implicated in inflammatory processes and might regulate innate and adaptive-immune responses by cross-linking cell-surface or matrix glycoconjugates. In fact, research over the last few years suggested that many members of the galectin family play a crucial role in the homeostasis of the inflammatory response (Table 1; **Fig. 1**) by regulating cell survival, influencing cell growth and chemotaxis, or mediating cell-cell and cell-matrix interactions [11–17].

The wealth of new information promises a future scenario in which galectins or their antagonists will be used as powerful anti-inflammatory mediators, selective immunosuppressors, or antimetastatic agents.

MODULATION OF DIFFERENT STEPS OF THE INFLAMMATORY CASCADE

Galectins modulate cell growth and proliferation

Different members of the galectin family have been shown to exert critical but contradictory effects on cell growth and proliferation.

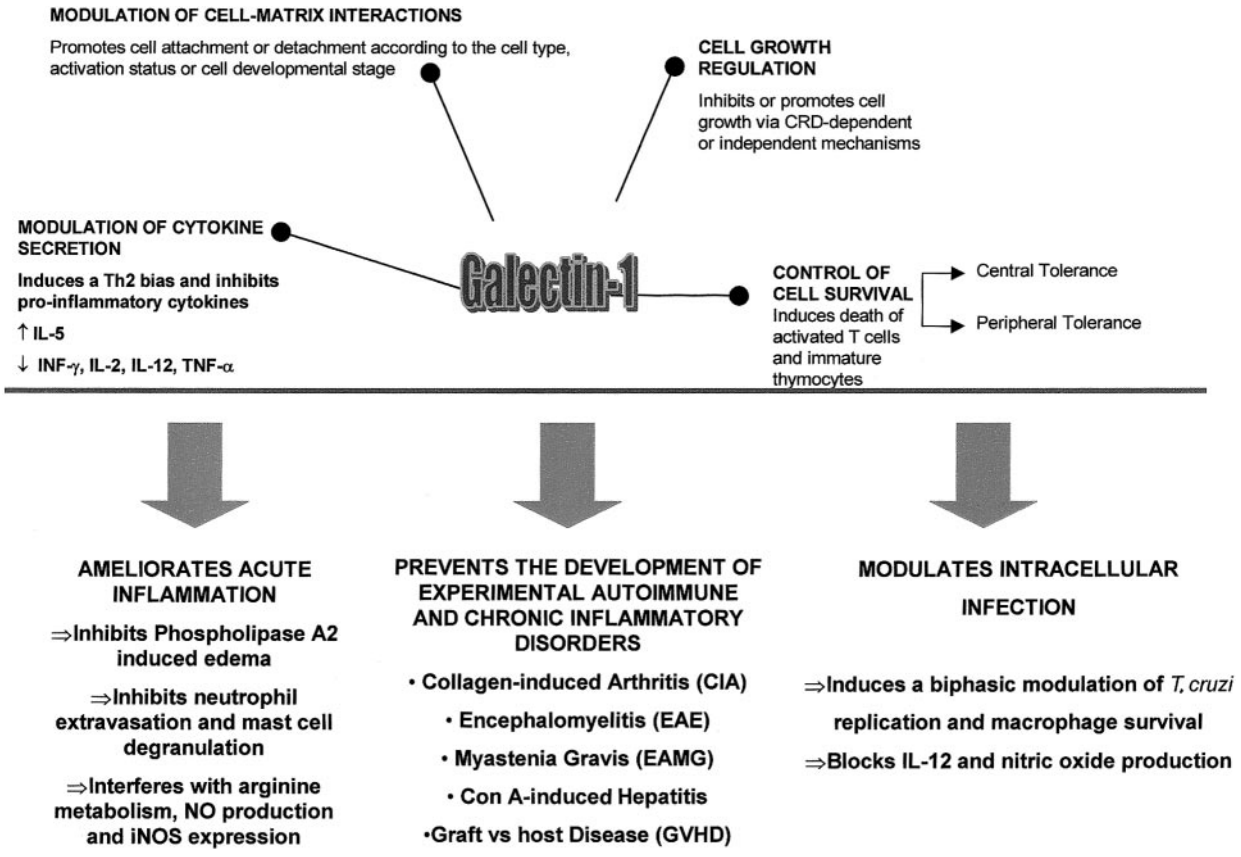

Fig. 1. Participation of galectin-1 in different physiological processes (upper panel) and pathological conditions (lower panel).

Galectin-1

Galectin-1, a monomeric or homodimeric protein composed by subunits of 14.5 kDa, is expressed widely within the central and peripheral immune compartments in thymic epithelial cells [18], antigen-stimulated T cells [19], macrophages [20, 21], and activated B cells [22]. This β-galactoside-binding protein has shown specific growth inhibitory properties at relatively low concentrations toward different cell types such as phytohemagglutinin (PHA)-activated human T cells [19, 22], concanavalin A (Con A)-stimulated rat T cells [23], human neuroblastoma cells [24], human leukemia T cells [25], and murine fibroblasts [26]. Although many of these effects were found to depend on the carbohydrate-binding activity of this lectin, some were mediated exclusively by protein-protein interactions. An overview of the biological effects induced by galectin-1 is shown in Figure 1.

A pioneer study conducted by Wells and Mallucci in 1991 [26] showed that galectin-1 acts as an autocrine-negative growth factor and inhibits the growth of mouse embryonic fibroblasts in a carbohydrate-independent way. We then demonstrated that this β-galactoside-binding protein induces a dose- and carbohydrate-dependent inhibition of proliferation of Con A-stimulated rat T cells but not lipopolysaccharide (LPS)-stimulated B cells [23]. Further investigation revealed that galectin-1 secreted by $CD8^+$ T cells reduces clonal expansion of antigen-experienced $CD8^+$ T cells and interleukin (IL)-2-induced proliferation of PHA-activated T lymphocytes in an autocrine-dependent manner by arresting the cells in the S and G_2/M phases of the cell cycle [19, 27]. Moreover, we have shown recently that galectin-1 is a negative regulator of the allogenic T-cell response (unpublished results). The controversial situation of galectins acting through carbohydrate-dependent or -independent mechanisms still remains to be investigated. The negative regulation of cell growth by galectin-1 has been extended recently to tumor cells. Kopitz and colleagues [24] showed that the cell density-dependent growth inhibition of human neuroblastoma cells is initiated by the increased ganglioside sialidase activity, leading to increased cell-surface expression of the ganglioside GM1, which acts as a ligand for galectin-1.

However, galectin-1 has also been shown to be a positive growth regulator toward other cell types, such as vascular endothelial cells [28]. It has been speculated that the growth inhibitory or stimulatory properties of this lectin are highly dependent on the cell type, cell-activation status, and concomitant environmental signals. In addition, these "double-edged" effects might be regulated alternatively by the relative levels of galectin-1 in the extracellular milieu and the equilibrium between its monomeric and dimeric forms (association constant≈7 μM), because dimeric galectin-1 is required to induce some, but not all, of the biological effects mediated by this carbohydrate-binding protein [4, 29].

Galectin-3

In contrast to galectin-1, most of the experimental evidence indicates that galectin-3, a 29-kDa member of the galectin

family, promotes cell growth and proliferation and acts as a mitogenic signal toward several cell types [30–33]. Yang et al. [31] demonstrated that human leukemia T cells transfected with galectin-3 cDNA showed higher rates of proliferation compared with control transfectants, which do not express this lectin. It has been suggested that cell-growth stimulatory activity of galectin-3 may be related to an intracellular function of this protein [31]. Moreover, Inohara et al. [30] provided clear-cut evidence that recombinant galectin-3 stimulates DNA synthesis, as well as cell proliferation, in quiescent cultures of normal human fibroblasts in a saccharide-inhibitable way. Increased cell proliferation triggered by galectin-3 was accompanied by an influx of extracellular Ca^{2+} in human Jurkat T-cells [34]. A recent study showed that inhibition of galectin-3 expression induced by an antisense oligodeoxynucleotide blocked proliferation of anti-CD3-stimulated T lymphocytes [35]. This 29-kDa chimeric protein also promoted proliferation of many cell types, other than immune cells, such as neurites from dorsal root ganglia explants and mesangial cells [2, 36].

Other galectins

In contrast to the substantial information about galectins-1 and -3, little is known about the influence of other members of the galectin family on cell growth. Galectin-12, a recently identified member of this family, has shown growth-inhibitory properties [37]. Ectopic expression of galectin-12 in cancer cells caused cell-cycle arrest at the G_1 phase and cell-growth suppression. Because targeted disruption of galectins-1 and -3 results in the absence of major phenotypic abnormalities [38], it might be speculated that other β-galactoside-binding lectins would compensate for the absence of these proteins. Further studies will address this issue and dissect the fine saccharide specificity and functional role of each member of the galectin family.

Galectin-sugar interactions modulate cell apoptosis: the sweet kiss of death

Evidence now suggests that the balance between T-cell proliferation and apoptosis is the key determinant of healthy immune responses and peripheral tolerance. Apoptosis occurs during lymphopoiesis to remove potentially autoreactive cells and those lymphocytes that have failed to produce a functional antigen receptor. Moreover, activated mature T cells also undergo apoptosis by a propriocidal mechanism to prevent immunopathology associated with an overactive immune response [39].

Galectin-1

Investigation of the molecular mechanisms responsible for the inhibitory effects of galectin-1 revealed that this carbohydrate-binding protein induces apoptosis of T cells during their development in the thymus and after immune stimulation in the periphery [21, 29, 40] (Fig. 1). The first evidence suggesting that galectin-1 could regulate T-cell apoptosis was the detection of tenfold up-regulation of its gene expression in a human leukemia cell line during glucocorticoid-induced cell death [41]. Undoubtedly, one of the most leading contributions was completed by Baum and colleagues [18, 40], who showed that human-thymic epithelial cells express galectin-1, which binds to core 2-*O*-glycans on immature cortical thymocytes and modulates their survival at two different steps of thymic maturation. The degree of galectin-1 binding to thymocytes correlated with the maturation stage of the cells, because immature thymocytes bound more galectin-1 than did mature thymcytes [18]. In addition, the authors showed that galectin-1 induced apoptosis of two subsets of $CD4^{lo}$ $CD8^{lo}$ thymocytes, representing non-selected and negatively selected, immature thymocytes [40]. These results suggested that galectin-1 could be a potential candidate molecule to provide a second apoptotic signal required for negative selection. Experimental models of galectin-1 knockout mice or the use of mice carrying an antisense galectin-1 transgene expressed in thymic stroma might be helpful for further investigation of the role of this protein during T-cell development.

Galectin-1 also induced apoptosis of activated but not resting peripheral T cells [29, 42]. However, this protein did not trigger death via known T-cell surface receptors such as CD3, Fas, or tumor necrosis factor (TNF) receptors [29]. The first candidate proposed to be a galectin-1 ligand on T cells was CD45, particularly the polylactosamine-enriched CD45R0-splicing product, which is expressed mainly on the surface of activated T cells, memory lymphocytes, and immature thymocytes [29, 40]. CD45 is expressed in multiple isoforms as a result of alternative splicing of variable CD45 exons, which vary extensively in their glycosylation patterns [43]. Because galectin-1 can bind to CD45, and this binding appears to depend on the expression of CD45 isoforms, it was tempting to speculate that galectin-1-induced apoptosis might be regulated by the affinity of the interaction of distinct CD45 isoforms with this carbohydrate-binding protein. The relevance of CD45 in mediating apoptosis was supported further by the fact that CD45-positive Jurkat T cells were more susceptible to the apoptotic effect than were CD45-negative cells [29, 44].

Although CD45 appeared to be the "perfect" candidate ligand, during the last few years, many other T-cell surface receptors have been shown to bind galectin-1, such as CD43, CD2, CD4, CD3, and CD7 [44–48]. To address whether these receptors act cooperatively or independently to deliver the apoptotic signal, Pace and colleagues [47] demonstrated that galectin-1 binding to T cells results in a dramatic redistribution of these glycoproteins into segregated membrane microdomains on the cell surface. Whereas CD45 and CD3 colocalize on apoptotic blebs, CD7 and CD43 are distributed in small patches away from the membrane blebs [47]. The authors demonstrated further that CD7 is a "critical" coreceptor necessary to deliver the proapoptotic signal during galectin-1-induced T-cell death [48]. It has been speculated that the loss of CD7 in several pathologies, such as lymphoid tumors and autoimmune disease, might contribute to enhance survival and expansion of malignant and autoreactive lymphocytes [49]. Because susceptibility to galectin-1 may be regulated by the presentation of lactosamine on specific oligosaccharide structures created by glycosyltransferase enzymes, Galvan and colleagues [50] investigated the requirement of transferases for the apoptotic process and demonstrated that expression of the core 2-beta-1,6-*N*-acetylglucosaminyltransferase (C2GnT) is necessary to create a branched structure on *O*-glycans of T-cell

surface receptors and is critical for galectin-1 to kill activated T cells. Overexpression of C2GnT increased the susceptibility of double-positive thymocytes to galectin-1 death signals [50]. We have demonstrated that under certain inflammatory conditions, activated macrophages, stimulated B cells, and alloreactive T cells secrete high levels of galectin-1 to induce apoptosis of activated T cells and shut off T-cell effector functions after the completion of an immune response [19–22].

To gain insight into the intracellular signals transduced by galectin-1 upon binding to activated T cells, we have demonstrated that apoptosis induced by galectin-1 requires activation of the AP-1 transcription factor and modulation of Bcl-2 but not Bcl-xL protein expression [51]. Recent studies have provided evidence that galectin-1 cooperates with T-cell receptor (TCR) engagement to enhance extracellular signal-regulated kinase-2 (ERK-2) activation in a T-cell hybridoma and freshly isolated mouse thymocytes [42]. In an elegant study, Chung et al. [52] have suggested recently that the molecular mechanisms implicated in the inhibitory effects of galectin-1 are even more complex and interesting. They proposed that galectin-1 acts as a partial ligand that antagonizes TCR responses known to require costimulation and complete tyrosine phosphorylation, such as IL-2 production, but permits those TCR responses that require only partial signals, such as CD69 up-regulation and apoptosis [52].

Because galectin-1 is highly expressed in sites of immune privilege such as placenta [53, 54], testis [55], and retina [56], one may speculate that this protein might operate to ensure rapid elimination of inflammatory T cells to protect these vulnerable sites from tissue damage. Accordingly, galectin-1 has been detected mainly in trophoblastic tissue and endometrium from first-trimester human placenta [54]. We have shown that galectins-1 and -3, copurified from ovine placental tissue, exert antagonic inhibitory and stimulatory effects, which are neutralized reciprocally in a natural mixture of these proteins, suggesting that the interplay between these two carbohydrate-binding proteins might be critical in the establishment of immune homeostasis [32, 53, 57]. The physiopathological consequences of galectin-1-induced apoptosis will be discussed extensively in the next sections.

Galectin-3

Although galectin-1 has been shown to induce T-cell apoptosis, galectin-3 has conversely been shown to prevent cell death induced by Fas ligation and staurosporine [31]. In contrast to the proapoptotic effect of galectin-1, which was demonstrated by adding recombinant galectin-1 to activated T cells, the antiapoptotic activity of galectin-3 seems to be an intracellular function of this protein. Of particular interest, galectin-3 shows a significant sequence similarity to Bcl-2, mainly concentrated in the functional BH1 (NWGR) domain. Accordingly, recent studies demonstrated successfully that galectin-3 protects human breast carcinoma cells from cisplatin- and nitric oxide (NO)-induced apoptosis and also prevents cell death induced by the loss of cell anchorage (anoikis) [33, 58–60]. Galectin-3 has been shown to rescue cells from apoptosis by protecting against alterations of the mitochondrial membrane and formation of reactive oxygen species [57]. A definitive evidence of the antiapoptotic activity of galectin-3 has been provided by studies in galectin-3 knockout mice [16]. Peritoneal granulocytes from these mice underwent accelerated apoptosis after treatment with apoptotic stimuli, compared with wild-type mice [16]. These encouraging results suggest that galectins may have profound effects in leukocyte function in vivo.

Other galectins

It has been postulated that other galectins may also have effects on cell survival, and their apoptotic activity seems to be tissue-specific. Overexpresion of galectin-7 on keratinocytes increased their susceptibility to apoptosis induced by ultraviolet B (UVB) irradiation [61]. Moreover, Hadari et al. [62] showed that galectin-8 binds to $\alpha_3\beta_1$ integrin and regulates adhesion and survival of carcinoma cells in the context of the extracellular matrix (ECM). Similar to galectin-1, galectin-9 has also been shown to induce apoptosis of murine thymocytes [63], and more recently, galectin-12, expressed by adipocytes, has been proposed to induce cell-cycle arrest and apoptosis [37].

Galectins as modulators of cell-cell and cell-matrix interactions: just bridging oligosaccharides?

The ECM is the major milieu in which immune cells function during inflammatory processes. Adhesion and migration of immune cells through ECM are multistep processes orchestrated by receptors that recognize a number of glycoproteins [64].

Galectin-1

Galectin-1 has been implicated in cellular interactions with the subendothelial ECM through recognition of poly-*N*-acetyllactosamine residues on ECM glycoproteins such as laminin and fibronectin [65]. Whether galectin-1 exerts a positive or a negative effect on cell adhesion to ECM glycoproteins remains controversial, raising the possibility that this protein could promote cell attachment or detachment according to the cell type, cell's activation status, or cell developmental stage (reviewed in ref. [8]). Moreover, the binary actions of galectin-1 could also be associated to the high or low concentrations of galectin-1 in the extracellular milieu or the glycosylation state of counter-receptors (Fig. 1, upper panel). Although this lectin has been shown to inhibit myoblast interactions with laminin by blocking $\alpha_7\beta_1$ integrin, it has also shown proadhesive effects toward other cell types such as melanocytes, teratocarcinoma cells, olfactory neurons, rhabdomiosarcoma cells, and fibroblasts (reviewed in refs. [2, 8]). In the context of an inflammatory response, we have demonstrated that galectin-1, at concentrations less than 1 μM, inhibits IL-2-induced T-cell adhesion to ECM glycoproteins and affects reorganization of the activated cell's actin cytoskeleton [14]. To integrate the antiadhesive, anti-inflammatory, and proapoptotic activity of galectin-1, a hypothetical model could be proposed in which galectin-1 is secreted in low concentrations from immunocompetent or bystander cells after the completion or exacerbation of an inflammatory response. The presence of galectin-1 in the extracellular milieu would contribute to inhibit T-cell adhesion to ECM and proinflammatory cytokine secretion as a compensatory mechanism [14]. If this first regulatory checkpoint is not sufficient to achieve homeostasis, enhanced secretion of galec-

tin-1, together with prolonged stimulation and persistence in the extracellular milieu, would finally affect the survival of activated T cells [29].

Galectin-3

Controversial results have also been found regarding the pro- or antiadhesive role of galectin-3 [2]. Although this protein promoted neutrophil adhesion to laminin in the context of an inflammatory response [66], it showed a drastic, inhibitory effect on melanoma cell adhesion to ECM [67]. Moreover, in the context of antigen presentation and T-cell activation, Swarte et al. [68] have demonstrated recently that galectin-3 mediates dendritic cell adhesion to lymphocytes following entry via high endothelial venules and after activation via L-selectin.

Other galectins

Galectin-8 has been shown to modulate integrin interactions with the ECM [62]. Hadari et al. [62] showed that galectin-8 secreted from human carcinoma cells forms complexes with various members of the integrin family to inhibit cell adhesion. The role of other members of the galectin family on cell adhesion and trafficking still remains to be ascertained.

Modulation of the cytokine balance by galectins-1 and -3: novel signals or just a tree in the woods?

Galectin-1

In addition to modulating cell adhesion and survival, galectin-1 has been shown to prevent synthesis or release of proinflammatory cytokines [12, 14, 15] (Fig. 1). We have demonstrated in vivo, using gene and protein therapy strategies, that galectin-1 suppresses the inflammatory response in a murine experimental model of rheumatoid arthritis [12]. Within the arthritogenic process, lymph node cells from galectin-1-treated mice exhibited a T-helper cell type 2 (Th2)-polarized immune response with increased IL-5 production and a dramatic reduction of interferon-γ (IFN-γ) secretion, compared with the control group [12]. In vitro experiments revealed that galectin-1 treatment inhibits TCR-mediated IL-2 production [12, 42] and T-cell-derived IFN-γ and TNF-α but not IL-10 secretion [14, 22] in a manner independent of its proapoptotic properties. Galectin-1-transfected fibroblasts as well as recombinant galectin-1 induced a carbohydrate-dependent inhibition of IL-2 production by a collagen type-II-specific, T-cell hybridoma clone [12]. Recently, in the context of an intracellular infection, we have shown that galectin-1 blocks IL-12 almost completely but not IL-10 secretion from in vivo or in vitro *Trypanosoma cruzi*-infected macrophages [69]. Because IL-12 is necessary to block parasite cell cycle, selective inhibition of this cytokine was reflected by the enhanced survival and replication of intracellular amastigotes in cultured macrophages [69]. It is interesting that the apoptotic threshold of these macrophages was unaffected by low concentrations of this carbohydrate-binding protein. It should be highlighted that in almost all the situations studied, the apoptotic effects of galectin-1 and its inhibitory effects on cytokine production appeared to be independent and mediated by different signaling pathways [12, 22, 42, 69].

Galectin-3

Whether galectin-3 shows overall opposite functions to galectin-1 remains to be ascertained still. However, in the context of cytokine production, this paradigm is supported by several experimental observations. Galectin-3 has been shown to potentiate LPS-induced IL-1 production by human monocytes [70]. Moreover, Cortegano et al. [71] have shown recently that galectin-3 inhibits IL-5 gene expression specifically by human eosinophils and allergen-specific T-cell lines [71]. It is interesting that the inhibitory effect was found to be mediated by the increased activity of the REIII site, a regulatory transcription element [72].

Galectins and chemotaxis: novel chemokine-like proteins with sugar-binding activity?

Galectins-3 and -9

The multifaceted role of galectins in inflammation has been demonstrated clearly by the identification of galectin-9 (ecalectin) as a powerful T-cell-derived regulator of eosinophil recruitment in tissues during inflammatory reactions [73]. This biological effect was specific because it did not induce chemotaxis of neutrophils, monocytes, or lymphocytes. The authors demonstrated further that galectin-9 is overexpressed in mononuclear cells from allergic patients [73]. In addition, Sano et al. [74] have recently provided evidence that galectin-3 acts as a novel chemoattractant for monocytes and macrophages. This protein was found to be chemotactic at high concentrations (1.0 μM) but chemokinetic at low concentrations (10 nM). Similar to chemokines, the chemotactic activity of galectin-3 was abolished by pertussis toxin, suggesting that it acts through a G protein-coupled pathway [74].

GALECTINS IN CONCERT: TUNERS OF THE INFLAMMATORY RESPONSE?

Galectin-1: a novel immunosuppressive strategy for the treatment of autoimmune and chronic inflammatory disorders

The immunosuppressive and anti-inflammatory activities of galectin-1 are supported by several in vivo studies (Fig. 1, lower panel). Offner et al. [11] provided clinical and histopathological evidence that galectin-1 prevents the development of experimental autoimmune encephalomyelitis in rats. Recently, we have shown, using gene and protein therapy strategies, that galectin-1 ameliorates the inflammatory and autoimmune response in collagen-induced arthritis, an experimental model of rheumatoid arthritis [12]. A single injection of syngeneic fibroblasts engineered to secrete galectin-1 at the day of the disease onset was able to abrogate clinical and histopathological manifestations of arthritis almost completely. Galectin-1 treatment increased T-cell susceptibility to activation-induced cell death and resulted in a strong shift from a Th1 toward a Th2 cytokine profile [12]. Moreover, the anti-inflammatory effects of galectin-1 have been tested in Con A-induced hepatitis in mice, a T-cell dependent model of liver injury [15]. Galectin-1 pretreatment prevented liver injury and T-cell liver

infiltration. The effect was associated with selective apoptosis of activated T cells and inhibition of Con A-induced TNF-α secretion. Furthermore, recent observations suggest that galectin-1 could ameliorate the development and severity of graft versus host disease (GVHD), a multiorgan system damage mediated by donor-derived, alloreactive T cells [75]. Accordingly, evidence has been provided recently for an inhibitory role of galectin-1 in the course of the human allogenic T-cell response (unpublished results). In addition to the immunosuppressive effects of galectin-1 on T-cell-mediated disorders, a β-galactoside-binding lectin, purified from electric eel, has been shown to prevent the development of myasthenia gravis in rabbits, an experimental model of an antibody-mediated autoimmune disorder [76]. Moreover, Tsuchiyama et al. [77] have demonstrated recently the efficacy of galectins-1, -3, and -9 in the amelioration of nephrotoxic nephritis, induced by injection of rabbit antiglomerular basement-membrane serum in Wistar Kyoto rats.

To integrate all of these findings, an interesting study provided indirect evidence of the role of galectins in the regulation of T-cell homeostasis in vivo. Demetriou et al. [78] showed that null mutations in β1,6 *N*-acetylglucosaminyltransferase V (Mgat5), a key enzyme in the *N*-glycosylation pathway, results in enhanced, delayed-type hypersensitivity and increased susceptibility to autoimmune disorders [78]. Mgat5 is responsible for initiating GlcNAc β 1,6 branching on *N*-glycans, thereby increasing *N*-acetyllactosamine residues (the ligands for galectins). The authors propose that galectin-3 normally forms multivalent connections with glycoproteins of the TCR and thereby restrains the lateral mobility of the TCR complex. In the case of Mgat5 knockout mice, dysregulation of galectin-glycoprotein associations will increase TCR-mediated activation and susceptibility to autoimmune disease because of the absence of specific saccharides provided by the enzyme [78]. Another attractive explanation could be that the absence of *N*-acetyllactosamine residues on cell-surface glycoproteins might render autoreactive T cells resistant to the apoptotic and homeostatic effects of galectin-1. It should be pointed out that the functions so far demonstrated in vitro may not be operative in vivo. Nevertheless, some in vivo observations have been forthcoming, including those from mice injected with galectin-1-transfected cells [12] and experiments in galectin knockout mice [16], supporting the relevance of at least some of the in vitro demonstrated functions.

In an attempt to extrapolate our experimental data to the understanding of human autoimmune disease, we have demonstrated recently that defective mononuclear apoptosis in synovial inflammatory infiltrates from patients with juvenile rheumatoid arthritis could be explained in part by decreased galectin-1 and increased galectin-3 expression [79]. Moreover, we have recently found the occurrence of anti-galectin-1 autoantibodies in sera from patients with autoimmune disorders such as uveitis (unpublished results) and chronic Chagas' cardiomiopathy [80], which correlate with the severity of the disease. Whether these autoantibodies have blocking activity or just represent epiphenomena to polyclonal activation still remains to be elucidated.

Galectins in acute inflammatory processes and innate immunity

Galectin-1

In addition to the role of galectin-1 in chronic inflammatory disorders, we have also demonstrated that this β-galactoside-binding lectin plays a key role in acute inflammation [13]. This protein was able to inhibit phospholipase A_2 but not histamine-induced edema in a selective, specific, and dose-dependent manner when coinjected together with the enzyme (Fig. 1). Moreover, galectin-1 was found to inhibit arachidonic acid release and prostaglandin E_2 production from LPS-stimulated macrophages [13].

The anti-inflammatory properties of galectin-1 have also been established at the cellular level, because administration of this protein inhibited neutrophil extravasation and mast-cell degranulation [13]. Recently, we have shown that this protein interferes with macrophage arginine metabolism and consequent release of NO, a critical mediator of inflammatory, apoptotic, and autoimmune processes (unpublished results).

Galectin-1 expression, secretion, and subcellular distribition have been demonstrated to be highly susceptible to modulation by different inflammatory stimuli such as phorbol esters (phorbol 12-myristate 13-acetate), chemotactic peptides (formyl-Met-Leu-Phe), LPS [20–22], and signals involved in B- or T-cell activation [19, 22]. Moreover, intracellular infection has been shown to be a potent regulator of galectin-1 secretion [69].

Mechanisms of galectin secretion

It is interesting that all known galectins lack a signal sequence and are synthesized on free ribosomes. The mechanism by which galectins are secreted or exported from cells is unknown, but it may be related to the atypical secretory pathway used by several growth factors and cytokines, including fibroblast growth factor and IL-1 [8, 9]. Prior to secretion, galectins become concentrated under plasma membrane and in plasma membrane evaginations, which appear to pinch-off to form galectin-enriched extracellular vesicles [8].

Galectin-3

In contrast to the negative, regulatory effects of galectin-1 in acute inflammation, galectin-3 has shown proinflammatory activity in vitro and in vivo. Galectin-3 has been shown to stimulate superoxide production from peripheral blood leukocytes [81] and activate the reduced nicotinamide adenine dinucleotide phosphate oxidase in neutrophils that experienced extravasation [82]. Studies of galectin-3-deficient mice have provided clear-cut evidence for the proinflammatory effects of this lectin [16, 17]. Colnot and colleagues [17] examined the effect of gene targeting toward an inflammatory challenge in a model of acute peritonitis. Four days after thioglycollate injection, galectin-3 knockout mice exhibited a significantly reduced number of peritoneal leukocytes, compared with wild-type animals. Hsu et al. [16] also provided strong evidence for reduced peritoneal inflammation in galectin-3 knockout mice. When compared with cells from galectin-3 wild-type mice, thioglycollate-elicited inflammatory macrophages from galectin-3 mutant mice exhibited significantly lower levels of nuclear factor-κB activation [16].

Galectin-3 expression was found to be modulated by several inflammatory stimuli such as thioglicolate, LPS, and the calcium ionophore A23187 in peritoneal murine macrophages [83, 84]. Moreover, proinflammatory cytokines and TCR cross-linking were able to up-regulate expression of this protein on T cells [35]. It is interesting that galectin-3 expression levels decreased significantly in mice exposed to immobilization stress, suggesting that this protein might also be implicated in the physiological response to psychological stress [85].

Galectins in allergic inflammation

Galectin-3

Galectin-3 was first shown as an εBP able to activate and degranulate rat basophilic leukemia cells and mast cells [86]. As mentioned above, Cortegano et al. [71, 72] have shown that galectin-3 selectively inhibits IL-5 gene transcription and protein release from human eosinophils and allergen-specific T-cell lines by interfering at the level of the REIII site, a negative regulatory transcription element. The possibility to shut down the IL-5 pathway opens new avenues in the regulation of Th2-dependent allergic reactions.

Other galectins

The chemotactic activity of galectin-9 for eosinophils, its overexpression in peripheral blood mononuclear cells from allergic patients [73], and the selective localization of galectin-10 (Charcot-Leyden crystal protein) in eosinophils and basophils [87] suggest that these β-galactoside proteins may also play a crucial role in allergic inflammation. Induction of galectin-10 by butyric acid in eosinophils has been demonstrated recently [88].

Galectins in the battle against foreign invaders

Galectin-1

The balance between galectins-1 and -3 might also be relevant in the context of microbial infection. Regulation of apoptosis has been shown to be a critical determinant factor during host-pathogen interactions. Infectious agents manipulate host-cell apoptosis to increase their lifespan within infected cells or to spread infection. Conversely, the host-immune response induces apoptosis of infected target cells to damage intracellular microbial pathogens [89].

Recently, we have provided experimental evidence of the immunomodulatory effects of galectin-1 in the context of an infectious process by studying macrophages isolated from *T. cruzi*-infected mice or by infecting cells in vitro with living trypomastigotes [69]. A biphasic modulation of parasite replication and cell viability was observed when macrophages isolated from *T. cruzi*-infected mice were exposed to increasing concentrations of galectin-1. Although low concentrations of this protein increased parasite replication and did not affect macrophage survival, higher inflammatory doses of galectin-1 were able to commit cells to apoptosis and hence inhibit parasite replication. Furthermore, low concentrations of galectin-1 were sufficient to down-regulate critical mediators for parasite killing, such as IL-12 and NO, and did not affect IL-10 production. Further experiments will be required using galectin-1-deficient mice to evaluate the role of endogenous galectin-1 in the evolution of in vivo parasite infection. Endogenous galectin-1 was found to be up-regulated and secreted markedly by the J774 macrophage cell line exposed to the presence of living trypomastigotes [69]. Furthermore, increased expression of this protein was detected in heart tissue from patients with severe chronic Chagas' disease, an endemic, cardiac illness caused by the protozoan *T. cruzi* [80]. Anti-galectin-1 IgG autoantibodies also occurred in sera from these patients and correlated with the severity of cardiac damage [80]. The presence of these antibodies was specific, because they were absent in sera from patients with other nonrelated cardiomyopathies [80].

Galectin-3

Although there is still no direct evidence of a role for galectin-3 in infectious processes, its expression has been shown to be modulated by infection with human T-cell lymphotropic virus 1 retrovirus [90] or by the TAT protein of HIV-1 [91]. In cotransfection experiments, the 5′-regulatory sequence of the galectin-3 gene was up-regulated significantly by expression vectors encoding the TAT protein [91]. In the context of microbial invasion, it has been proposed that binding *T. cruzi* trypomastigotes to laminin is enhanced in the presence of galectin-3 [92].

Galectins and tumor spreading: modulation at different steps of the metastatic cascade

Several lines of evidence suggest that galectins play a nontrivial role in the modulation of different steps of the metastatic cascade (reviewed in ref. [93]).

Galectin-1

Increased expression of galectin-1 has been found to correlate with the malignant potential of many tumor types, such as human-thyroid carcinoma [94], glioma [95], and prostate adenocarcinoma [96, 97]. Regarding modulation of galectin-1 expression in tumor cells, sodium butyrate, a potent, differentiating agent, has been shown to increase transcription of this prototype lectin in head and neck squamous carcinoma [98]. Furthermore, we have demonstrated recently that the antimetastatic effect of a single low-dose cyclophosphamide involves modulation of galectin-1 and Bcl-2 expression by lymphoma and spleen cells [99]. Galectin-1 expressed on tumor cells may act as a modulator of different steps of the metastatic cascade, and it may increase adhesion of cancer cells to ECM or also induce homotypic or heterotypic adhesion between cells through interaction with complementary glycoconjugates. In addition, galectin-1 may act as an immunological shield by inducing apoptosis of cytotoxic T cells (the counterattack hypothesis), or it may modulate cytokine production or TCR-mediated signal transduction at the level of tumor-leukocyte interface. Although the precise role of galectin-1 during the metastatic process still remains to be established, a line of evidence indicates that galectin-1 modulates attachment or detachment of cancer cells during tumor spreading [100].

Galectin-3

A growing body of evidence supports the involvement of galectin-3 in tumor growth and metastasis (reviewed in ref. [93]).

Expression of this endogenous β-galactoside-binding protein has been shown to correlate with the malignant potential of several tumorigenic cells, possibly by affecting cell motility and invasion of ECM [101–103]. As has been mentioned above, galectin-3 has recently been shown to protect against apoptosis induced by the loss of cell anchorage (anoikis) [59], suggesting that this protein may provide a critical determinant for cell survival of disseminating cancer cells in the circulation during metastasis. Accordingly, inhibition of galectin-3 expression in human colon cancer cells resulted in reduced liver colonization [101]. Moreover, down-regulation of this protein in human breast carcinoma cells provoked a significant suppression of tumor growth in nude mice [103]. However, the generality of these findings to all tumors is not fully clear. For example, decreased expression of this lectin has been associated with the malignant potential of cancer cells in breast, endometrial, and ovary carcinomas [104]. Thus, the ability of galectin-3 to promote or inhibit invasion and metastasis may depend on the tumor microenvironment and cell-cycle stage. To integrate proadhesive and antiapoptotic functions, Mataresse et al. [105] demonstrated that galectin-3 overexpression protects cancer cells from apoptosis by improving cell-adhesion properties. It is interesting that galectin-3 gene expression was found to be up-regulated upon cell transfection with a *c-Ha-ras* oncogene [106]. Conversely, it was down-modulated upon transfection with wild-type but not mutated p53 [106].

Serum levels of circulating galectin-3 and its 90-kDa ligand have been shown to be of relative value to predict biological aspects of tumor behavior associated with a metastatic and invasive phenotype in several types of cancer [107, 108].

Other galectins

The interpretation of the results obtained for galectins-1 and -3 will only be unequivocal if no additional galectins with overlapping or opposing functions would be expressed by the tumor [109]. To avoid an oversimplified picture, the contribution of other galectins to the metastatic process has been suggested recently. Lahm and colleagues [109] conducted a comprehensive study by screening for the presence of galectins in a panel of 61 human tumor cell lines of different origin. The most abundant member found was galectin-8 with 59 positive cell lines. Positivity for galectins-2 and -4 was restricted to colorectal and neural tumors, and galectin-9 signal appeared to be restricted to colorectal carcinoma cell lines.

In particular, galectin-8 has been functionally associated with the metastatic process. Hadari and colleagues [62] have demonstrated recently that this protein modulates tumor-matrix interactions through an integrin-dependent mechanism. Conversely, galectin-7 has been found to be present in human basal and spinous-cell carcinoma and proposed to be an early transcriptional target of the tumor suppressor protein p53 [61]. It has been found that galectin-7 levels are increased after UVB radiation of epidermal keratinocytes, paralleling p53 stabilization [61]. Further studies are necessary in order to establish the precise role of each galectin within the tumorigenic and metastatic processes.

CONCLUSIONS AND FUTURE DIRECTIONS

This overview reports the latest advances in galectin research, particularly the role of these carbohydrate-binding proteins in the immune-inflammatory responses and in cell-adhesive events. Encouraging initial efforts suggest that galectins may have a profound effect in cell signaling and leukocyte function in vivo. Undoubtedly, a future challenge will be the identification of unique, glycoconjugate ligands for each galectin member and the investigation of individual functions. The current wealth of information emanating from the human genome project and from the proteomics initiatives should have to be integrated with the functional impact of these carbohydrate-binding proteins. Research should be aimed at identifying new strategies, based on galectin-sugar interactions, to manipulate autoimmune diseases, inflammatory processes, allergic reactions, microbial invasion, and tumor spreading, thus providing clues to solve the most challenging problems in human disease.

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