

Regulatory Roles of Galectins in the Immune Response

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Key Words

Glycobiology · Lectins · Galectins · Immune response

Abstract

Galectins are a family of animal lectins with affinity for β -galactosides. They are differentially expressed by various immune cells and their expression levels appear to be dependent on cell differentiation and activation. They can interact with cell-surface and extracellular matrix glycoconjugates (glycoproteins and glycolipids), through lectin-carbohydrate interactions. Through this action, they can promote cell growth, affect cell survival, modulate cell adhesions, and induce cell migration. They appear to do so by binding to different glycoconjugates decorated by suitable saccharides, rather than through specific receptors. Galectins do not have a classical signal peptide and are often localized in intracellular compartments, including the nucleus. Intracellularly, they can regulate cell growth and survival by interacting with cytoplasmic and nuclear proteins, through protein-protein interactions, thereby affecting intracellular signaling pathways. Current research indicates that galectins play important roles in the immune response through regulating the homeostasis and functions of the immune cells.

Galectins are a family of animal lectins defined by their recognition of β -galactose and the presence of consensus amino-acid sequences [1]. They exhibit a high level of evolutionary conservation, and family members are found in nematodes, insects, and mammals [2]. In mammals, fifteen galectins have been studied and reported to date, but additional identifiable homologues are present in the published database (reviewed in [3]). All galectins contain conserved carbohydrate-recognition domains (CRDs) of about 130 amino acids responsible for carbohydrate binding. The family can be subdivided into one-CRD (galectin-1, -2, -3, -5, -7, -10, -11, -13, -14, and -15) and two-CRD types (galectin-4, -6, -8, -9, and -12), the latter composed of two distinct but homologous CRDs in a single polypeptide chain, separated by a linker of up to 70 amino acids. Among the one-CRD type, galectin-3 is unique in that it contains a long N-terminal part (about 120 amino acids) connected to the CRD (fig. 1). One-CRD galectins can exist as dimers [4]; two-CRD galectins have two carbohydrate-binding sites; and galectin-3 forms pentamers upon binding to multivalent carbohydrates [5]. Thus, galectins can have bivalent or multivalent interactions with glycans. Although galactose is necessary, the affinity of galectins for galactose alone is low, and higher affinity interactions require the presence of other saccharides attached to galactose [6]. Galectins recognize galactose-containing oligosaccharides with high specificity [7].

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Type	Structure	Member
One-CRD		1, 2, 5, 7, 10, 11, 13, 14, 15
Chimeric		3
Two-CRD		4, 6, 8, 9, 12

Fig. 1. Galectins are a family of animal lectins containing conserved carbohydrate-recognition domains (CRDs) that are responsible for carbohydrate binding. They can be subdivided into three groups: those containing one CRD (galectins-1, -2, -5, -7, -10, -11, -13, -14, and -15); those containing two distinct CRDs in tandem, connected by a linker (galectins-4, -6, -8, -9, and -12); and galectin-3, which consists of unusual tandem repeats of proline- and glycine-rich short stretches fused onto the CRD, and is known as a chimeric galectin. Different isoforms of two-CRD type galectins exist, which vary in the length of the linker. Rectangular boxes represent CRD, while small circles in galectin-3 represent short repeated peptide sequences.

Galectins do not have a signal sequence and have other features characteristic of intracellular proteins; consistent with these, they reside intracellularly (reviewed in [8]). However, they are secreted by cells, likely through a non-classical secretory pathway, and present in the extracellular space [9]. Extracellularly, galectins are capable of binding to glycoproteins and glycolipids that contain suitable galactose-bearing oligosaccharides. A number of different glycoproteins and glycolipids on the surface of different cell types have been identified as ligands of galectins, as described below. Intracellularly, galectins are detected in both the cytoplasm and the nucleus and to shuttle between these two compartments [10]. Some members are found to be engaged in basic cellular functions, such as pre-mRNA splicing [11–13], as well as regulation of cellular homeostasis, as described below. Galectins appear to regulate these processes by interacting with intracellular proteins [8, 13].

Expression of Galectins in Immune Cells

Some galectins exhibit a wide tissue distribution, while others have a higher level of tissue specificity [14]. Among the immune cells, galectins in general appear to be preferentially expressed in myeloid cells constitutively and not expressed or expressed at lower levels in resting lym-

phoid cells, but are inducible in these cells. The known expression pattern of different galectins in immune cells is summarized in table 1. In cattle, a serial analysis of gene expression (SAGE) identified galectin-1 to be expressed in gamma/delta T cells; it is more highly expressed in the CD8⁺ than the CD8⁺ subset [15]. In mice, this lectin is expressed in CD8⁺ effector T cells induced by LCMV infection [15], as well as in both CD4⁺ and CD8⁺ T cells following activation with concanavalin A (Con A) and peptide antigens, respectively [16]. This lectin is also expressed in T cells following an allogenic response [17]. Galectin-1 is expressed in a low amount in purified B cells from normal mice, but at a higher level in activated B cells isolated from *Trypanosoma cruzi*-infected mice [18]. In both cases, its level is increased after the cells are stimulated by lipopolysaccharide (LPS) or by cross-linkage of their cell surface B cell receptor (BCR) or CD40 [18]. Finally, galectin-1 is expressed by macrophages from *Trypanosoma cruzi*-infected mice and in the J774 macrophage cell line cultured in the presence of the parasites [19].

Galectin-3 is not expressed in resting murine T cells, but is expressed in CD4⁺ and CD8⁺ T cells after they are activated by anti-CD3 antibody or mitogen (Con A) and then cultured in the presence of cytokines, IL-2, IL-4, and IL-7 [20]. Galectin-3 is not expressed in resting B cells, but is found in B cells treated with mitogenic stimuli, such as LPS and BCR cross-linkage, or activation/differentiation stimuli, such as IL-4 and CD40 cross-linkage. Galectin-3 is detectable in B cells from *Trypanosoma cruzi*-infected mice and its level is further increased significantly when the cells are treated with IL-4 [21]. Finally, infection and transformation of T cells with human T lymphotropic virus-1 (HTLV-1) [22] or human immunodeficiency virus (HIV) type 1 [23] result in significantly increased levels of galectin-3. This is related to the upregulation of the galectin-3 promoter by the HTLV-1 Tax transactivator protein [22] and the HIV-1 Tat protein [24], respectively.

In rat bone marrow, galectin-3 is detectable in myelopoietic cells, but not in lymphopoietic cells [25]. In myeloid cells, however, its expression is still dependent on cell differentiation. For example, in human bone marrow, galectin-3 is expressed at a low level in CD34⁺ early myeloid cells, but at a higher level in more mature myeloid cells [26]. Murine Langerhans cells were found to be stained positively for galectin-3 [27], and human Langerhans cells isolated from the skin were found to display this lectin on the cell surface [28]. However, galectin-3 mRNA was not detectable in the latter, suggesting that

Table 1. Expression of galectins in immune cells (reported in the literature)

Galectin	Cell type	Subset/treatment	Animal species
Galectin-1	T cell	gamma/delta CD8+/induced by LCMV infection	cattle mouse
	B cell	CD4+ and CD8+/activated by Con A or antigen induced by parasite infection in the host; increased by treatment with LPS or cross-linkage of BCR or CD40	mouse mouse
	macrophage	cell line and cells from parasite infected animal	mouse
Galectin-3	T cell	CD4+ and CD8+/activated by anti-CD3 or Con A induced by HTLV-1 and HIV infection	mouse human
	B cell	induced by LPS, IL-4, or cross-linkage of BCR or CD40 induced by parasite infection in the host	mouse mouse
	myeloid	increased upon maturation	human
	Langerhans cell	cell surface and Birbeck granules (acquired from other cells?)	human
	dendritic cell	induced by parasite infection	mouse
	monocyte/ macrophage	primary cells and cell lines/induced upon cell differentiation	human and mouse
	mast cell		human and mouse
	neutrophil eosinophil		human human
Galectin-9	T cell	cell line (Jurkat)/upregulated by PMA	human
	PBMC	from allergic patients/induced by allergen	human
	eosinophil		human
Galectin-10	eosinophil		human
	basophil		human
Galectin-12	PBMC		human
	neutrophil		human
	myeloid cell lines		human

the lectin protein might be acquired from the neighboring epidermal keratinocytes, which secrete the lectin. Another study showed that Langerhans cells contain galectin-3 in the Birbeck granules [29]. In splenic dendritic cells, expression of galectin-3 is highly induced following a *Trypanosoma cruzi* infection [30]. Up-regulated expression of galectin-3 in dendritic cells is also demonstrated in a dendritic cell line, D2SC-1, following infection by the parasites [30].

Galectin-3 is previously known as Mac-2, a cell surface antigen identified by a monoclonal antibody generated against mouse peritoneal macrophages [31], and is considered as a marker for mature macrophages [32]. This marker was subsequently studied extensively in primary macrophages as well as monocyte and macrophage cell lines [33]. Interestingly, some of these cell lines have comparable amounts of total galectin-3, but differ in the amount of the protein on the cell surface as well as the amount of the protein secreted [33]. In humans, the ga-

lectin-3 level is increased dramatically as monocytes differentiate into macrophages [34]. In mice, its level is also markedly upregulated as peritoneal macrophages are cultured in vitro [35]. In addition, galectin-3 protein and mRNA levels are inducible in the human promyelocytic HL-60 cells by phorbol 12-myristate 13-acetate (PMA) [36, 37]. The level of galectin-3 in THP-1 cells is increased after treatment with PMA and additional studies indicated that the induction is dependent on the PKC-Ras-Raf-MKK1-MKK3-p38 pathway [38]. These authors further demonstrated that galectin-3 expression in murine resident peritoneal macrophages is increased when the cells are loaded with acetylated low-density lipoprotein (LDL) or oxidized LDL [38], which are lipoproteins known to activate components of the MAPK signaling pathway. Finally, galectin-3 is expressed in mast cells [39], neutrophils [40], and eosinophils [41].

In myeloid cells, galectin-3 can reside in the subcellular compartments. For example, a proteomic analysis

identified galectin-3 as part of the protein component of the exosomes in dendritic cells [42], which are small (50–90 nm diameter) vesicles secreted by a number of cell types and implicated in antigen-presenting and immunostimulating functions. By a similar approach, this lectin was detected as part of the phagosomes in macrophages [43].

Galectin-9 is constitutively expressed in the human lymphoma cell line Jurkat, and is further upregulated by PMA [44]. Its mRNA is induced in human peripheral blood mononuclear cells from allergic patients following stimulation by relevant allergens [45]. Finally, galectin-9 is expressed in eosinophils, with its level being higher in those isolated from allergic patients than from normal individuals [46]. Galectin-10, also known as Charcot-Leyden crystal, is specifically expressed by eosinophils and basophils [47]. Galectin-12 mRNA is detected in the peripheral blood mononuclear and polynuclear cells, the B-cell line Wil-2, as well as myeloid cell lines, U937, HL-60, and KU-812; it is also expressed at a low level in the human T cell line HuT-78 [48].

Thus, different immune cell types express a number of different galectins simultaneously, but they have different galectin expression profiles. This is best demonstrated by studying the human promyelocytic cell line HL-60 driven to differentiate into neutrophils, macrophages, or eosinophils, by different stimuli [49]. Some galectins are not expressed in any cell type (galectin-4 and -7), while others (galectin-1 and -8) remain expressed at similar levels in all differentiated cells. Galectin-3 is markedly upregulated in macrophages; galectin-9 is expressed at a high level in promyelocytes as well as neutrophils; galectin-10 is highly upregulated in eosinophils. Additional studies are clearly needed to establish the galectin expression profiles of different immune cells and their subsets, both at the resting states and when activated by different stimuli.

Regulation of the Homeostasis of the Immune Cells

The roles of galectins in different physiological and pathological processes are gradually being unraveled. In the immune system, they have been shown to exhibit a wide array of functions, from the regulation of cell differentiation, growth and survival to modulation of cell adhesion, activation, and chemoattraction (table 2). Many of these are demonstrated by using galectins exogenously added to the cells, while others are demonstrated

by using functional inhibitors, suppressing gene expression, or ectopically expressing the proteins.

Regulation of Cell Differentiation

The correlation between galectin expression and cell differentiation as noted above suggests that these proteins might play a role in cell differentiation. In the immune cells, data are emerging that some galectins regulate B cell differentiation. It is known that during B cell development Ig μ chain is associated with a surrogate light chain forming a functional pre-B cell receptor (pre-BCR), which interacts with the stromal cells. In the effort to elucidate pre-BCR-stromal cell interactions, Gauthier et al. [50] affinity purified proteins in the stromal cells that bound to pre-BCR and identified galectin-1 as a pre-BCR-interacting protein. This involves a protein-protein interaction (as the lectin activity is not involved) and the surrogate light chain is what galectin-1 binds to. In the mean time, pre-BCR-bound galectin-1 is anchored to glycoproteins expressed on the stromal cells, and pre-BCR-galectin-1-glycoprotein complexes are localized at the contact zone between pre-B cells and stromal cells. The complex formation is accompanied by the initiation of intracellular tyrosine kinase activation and signal transduction from the pre-BCR [50]. The signaling is inhibited by lactose, an effective inhibitor of galectins, suggesting that galectin-1 is involved in the process. Thus, galectin-1 produced by stromal cells is able to bind to pre-B cells and localize at the contact zone between these cells, triggering transmembrane signal transduction and possibly B cell differentiation.

B cells are known to differentiate into memory cells upon activation by IL-4. By using the antisense strategy to inhibit galectin-3 expression in B cells, Acosta-Rodriguez et al. [21] showed that those cells with down-regulated expression of galectin-3 tend to differentiate into plasma cells rather than memory cells under the same conditions. The transcription factor Blimp-1 is known to be down-regulated by IL-4. In cells with suppressed galectin-3 expression, IL-4 treatment did not result in Blimp-1 down-regulation [21]. The authors concluded that galectin-3 contributes to IL-4-induced down-regulation of Blimp-1, thereby driving the development of memory cells.

Regulation of Cell Growth

Because of their capacity for bivalent or multivalent binding to carbohydrates, galectins can form ordered arrays of complexes upon binding to multivalent glycoproteins on the cell surfaces [4]. This can trigger a cascade of

Table 2. Functions of galectins in immune cells demonstrated in vitro

Galectin	Function	Remark
Galectin-1	mediates B cell differentiation	endogenous (antisense)
	inhibits mitogen-induced T cell growth	exogenous
	induces apoptosis in activated T cells, T cell lines, thymocytes	exogenous
	induces apoptosis in B cell line	exogenous
	increases phagocytosis of neutrophils by phagocytes	exogenous
	mediates adhesion of T lymphoblastoid cells to thymic epithelial cells	endogenous (inhibitor)
	mediates adhesion of lymphocytes to dendritic cells	exogenous
	induces oxidative burst in neutrophils	exogenous
Galectin-2	inhibits IL-12 and nitric oxide production by macrophages	exogenous
Galectin-2	induces apoptosis in T cell line	exogenous
	mediates lymphotoxin-alpha secretion by macrophages	endogenous (siRNA)
Galectin-3	induces B cell differentiation	endogenous (antisense)
	mediates Con A/cytokine-induced T cell growth	endogenous (antisense)
	promotes growth of T cell line	endogenous (gene transfection)
		exogenous
	promotes G-CSF-driven growth of myeloid cells	exogenous
	inhibits GM-CSF-driven growth of bone marrow cells	exogenous
	induces apoptosis in T cell line (extracellular)	exogenous
	inhibits apoptosis in T cell line (intracellular)	endogenous (gene transfection)
	inhibits apoptosis in B cell line	endogenous (gene transfection)
	inhibits apoptosis in macrophages	endogenous (gene knock out)
	inhibits adhesion of thymocytes to thymic nurse cells	exogenous
	promotes adhesion of neutrophils to ECM and endothelial cells	exogenous
	induces IL-2 production by T cell line	exogenous
	induces oxidative burst in neutrophils	exogenous
	induces mast cell degranulation	exogenous
Galectin-4	induces oxidative burst and IL-1 production in monocytes	exogenous
	induces migration of monocytes/macrophages	exogenous
	potentiates phagocytosis	endogenous (gene knock out)
Galectin-4	stimulates IL-6 production by T cells	exogenous
Galectin-8	induces adhesion of neutrophils to ECM	exogenous
Galectin-9	suppress apoptosis in eosinophils from eosinophilic patients	exogenous
	enhances apoptosis in eosinophils from healthy subjects	
	induces apoptosis in B cell line, myeloid cell line	exogenous
	induces apoptosis in T cell line	exogenous
	mediates adhesion of eosinophils to fibroblast line	endogenous (inhibitor)
	induces migration of eosinophils	exogenous

Exogenous: demonstrated by using galectins (recombinant proteins in most cases) added exogenously to cells; endogenous: demonstrated by inhibiting galectin functions (using appropriate saccharide inhibitors or neutralizing antibodies), by inhibiting galectin expression (using the antisense or siRNA strategy, or using cells from knock-out mice) or by ectopically expressing galectins (using the gene transfection approach).

transmembrane signaling events. The effects of galectin-1 on regulation of growth of various cell types have been extensively documented (reviewed in [51]). With regard to its effects on immune cells, galectin-1 appears to be a negative growth regulator of T cells. Chicken galectin-1

inhibits the growth of Con A-stimulated rat spleen mononuclear cells [52]. Similarly, human galectin-1 inhibits the IL-2-induced proliferation of phytohemagglutinin (PHA)-activated T lymphocytes, as well as the IL-2-independent proliferation of T lymphoma cells [53]. Galectin

tin-1 was also shown to inhibit antigen-induced proliferation of naive and antigen-sensitized CD8+ T cells [16].

The effects of galectin-3 on the growth of different cell types have also been extensively documented (reviewed in [54, 55]). However, relatively little information has been accumulated regarding its effects on immune cells. Human recombinant galectin-3 was shown to promote granulocyte-colony-stimulating factor (G-CSF)-driven proliferation of CD34+ early myeloid cells in vitro [26]. On the other hand, rat recombinant galectin-3 was found to suppress GM-CSF-induced proliferation of rat bone marrow cells [25]. Thus, extracellular galectin-3 can function as a positive or a negative growth factor depending on the target cells.

The effects of galectin-1 and -3 described above were all demonstrated by using exogenously added proteins on cultured cells. The mechanisms by which galectins regulate cell growth when added exogenously to the cells are largely unknown. It is possible that these lectins recognize cell surface receptors related to cell growth and containing suitable saccharide side chains. In this regard, galectin-3 has indeed been found to bind to epidermal growth factor receptor, as well as transforming growth factor- β receptor, on an epithelial tumor cell line [56]. In this report, galectin-3 was noted to impede the endocytosis of the receptors, but its effect on cell growth was not described.

There is a study on the effect of down-regulating galectin-3 expression in T cells on cell proliferation. Mouse splenic T cells treated with galectin-3-specific antisense oligonucleotides, thus having a reduced level of this protein, were noted to have a decreased growth rate compared to control cells treated with sense oligonucleotides, when cultured in the presence of Con A and IL-2 [20]. The results suggest that galectin-3 is a positive regulator of T cell growth. It remains to be determined whether in the described experimental conditions galectin-3 is secreted by the cells and thus controls the cell growth by targeting the cell surface receptors, or functions inside the cell by targeting intracellular signaling pathways.

Regulation of Apoptosis (Demonstrated by Using Exogenously Added Galectins)

Galectins can induce apoptosis thus influencing cellular homeostasis independently of their effects on cell proliferation. A large number of studies have demonstrated that galectin-1 induces apoptosis in activated human T cells and T leukemia cell lines [57–60], including activated T cells developed following an allogenic T-cell response [17]. Furthermore, both latently HIV-1-infected

T cell lines and peripheral CD4+ and CD8+ T cells from AIDS patients demonstrated increased susceptibility to galectin-1-induced cell death, compared to cells from uninfected donors [61]. All the above-described effects were demonstrated by using exogenously added galectin-1 and relatively high concentrations ($\sim 10 \mu M$) were required. However, it has been recently shown that galectin-1 presented by the extracellular matrix is significantly more effective in inducing apoptosis in T cell lines [62]. In addition, by using an antisense strategy, evidence has been provided that galectin-1 secreted by murine melanoma cells can induce apoptosis of activated T cells targeting the tumor cells [63].

The effects of galectin-1 are dependent on N-glycans expressed on the cell surfaces [64]. Similarly, the increased susceptibility of HIV-infected T cells to this lectin is related to altered glycosylation of cell surface glycoproteins, resulting in a better recognition of the cells by the lectin [61]. Galectin-1 binds to a restricted set of T cell surface glycoproteins, including CD45, CD43, and CD7 [65]. It causes a redistribution of some of these proteins into segregated microdomains and, interestingly, some of them aggregate with the apoptotic blebs, while others aggregate at sites away from these blebs. Other investigators also noted that galectin-1 bound to CD45 (a tyrosine phosphatase) and suppressed its phosphatase activity [66]. The relevance of CD45 in mediating galectin-1's action is further supported by the fact that CD45+ Jurkat T cells are more sensitive to apoptosis induced by galectin-1 than CD45- cells [66]. However, galectin-1 also induces apoptosis in a CD45-deficient Jurkat cell variant, suggesting that apoptosis can be induced through other receptors [67]. Additional studies demonstrated that glycosylation of CD45 on T cell lines by core 2 β -1,6-N-acetylglucosaminyltransferase is critical for the galectin-1 susceptibility [68].

The signal transduction events induced by galectin-1 leading to apoptosis have been investigated and the studies revealed the involvement of various known apoptotic pathways. Galectin-1-induced apoptosis is accompanied by activation of extracellular signal-regulated kinase-2 (ERK-2) [60], and induction of the transcription factor AP-1 [69]. It is associated with a down-regulation of the anti-apoptotic protein Bcl-2, thus a predominance of the proapoptotic protein Bax [17, 70], and activation of caspases [17]. Finally, galectin-1 can up-regulate the expression of both the α - and β -chains of the interferon- γ (IFN- γ) receptor on activated T lymphocytes, thus rendering the cells sensitive to IFN- γ -induced apoptosis [53].

More recently, it was documented that galectin-1 triggers translocation of endonuclease G from the mitochondria to the nucleus in human T cell lines. However, cytochrome c release from the mitochondria, commonly associated with apoptosis, was not detected. In addition, galectin-1 treatment did not result in caspase activation, and apoptosis was not blocked by caspase inhibitors [71]. Another study found that galectin-1 induced the exposure of phosphatidylserine on the cell surface of a T cell line, MOLT-4, which is an early event of apoptosis that causes the cell to become recognizable by annexin-V. However, there was no detectable DNA fragmentation, which is also commonly associated with apoptosis [72]. Therefore, it appears that in different cell types, galectin-1 may effect a different end point in the apoptotic pathway and may or may not cause apoptosis.

Galectin-2 [73], galectin-3 [74], and galectin-9 [75, 76] can also induce T cell apoptosis. Galectin-2's effect involves activation of caspases-3 and -9, cytochrome c release, disruption of the mitochondrial membrane potential, as well as DNA fragmentation. There was also an increase in the ratio of the pro-apoptotic and anti-apoptotic proteins, Bax and Bcl-2 [73]. Galectin-3 induces apoptosis of human T leukemia cell lines, human peripheral blood mononuclear cells, and activated mouse T cells [74]. Interestingly, among a number of T cell lines tested, those not containing galectin-3, such as Jurkat, CEM, and MOLT-4 cells, are significantly more sensitive to apoptosis induced by exogenous galectin-3, compared with those expressing galectin-3, such as SKW6.4 and H9 cells [74]. This is consistent with galectin-3's anti-apoptotic activity described below. Galectin-3 was shown to bind to CD7 and CD29 (a $\beta 1$ integrin) on the cell surface, as neutralizing antibodies to these antigens inhibited galectin-3-induced apoptosis [74], suggesting that these antigens mediate apoptosis induced by this lectin. Galectin-3 causes activation of mitochondrial apoptosis events, including cytochrome c release and caspase-3 activation, but not caspase-8 activation [74]. Galectin-9's apoptotic induction effect was demonstrated with Jurkat cells as well as with human peripheral blood T cells. Both CD4+ and CD8+ cells are susceptible to apoptosis induced by this lectin [76]. Apoptosis in these cells are more pronounced if they are also activated with anti-CD3 antibody. Using inhibitors for various caspases, it was concluded that galectin-9 induces apoptosis via the caspase-1 pathway while caspase-8, -9, and -10 are not involved [76].

Induction of apoptosis by galectins in other immune cells has also been studied. Galectin-1 induces apoptosis in B cells; this is demonstrated by using BL36 cells, a

Burkitt lymphoma cell line [77]. Galectin-1 was found to bind to CD45 on this cell line and trigger the latter's phosphatase activity [77]. Human galectin-1 induces phosphatidylserine exposure on the human promyelocytic HL-60 cells, and fMet-Leu-Phe-activated, but not resting, human neutrophils. However, like in the case of MOLT-4 cells described above, DNA fragmentation was not detected in these cells [72]. Interestingly, the treatment significantly promotes phagocytosis of these cells by activated mouse macrophages, apparently due to the recognition of phosphatidylserine on the cell surface by phagocytes. Galectin-9 has been shown to significantly suppress apoptosis in eosinophils from eosinophilic patients, but enhance apoptosis in those from healthy subjects [46]. Furthermore, it suppresses dexamethasone-induced apoptosis of eosinophils from healthy subjects, but enhances apoptosis induced by anti-Fas antibody in eosinophils from both eosinophilic patients and healthy subjects [46]. Galectin-9 also induces apoptosis in a human B cell line (BALL-1), a monocytic cell line (THP-1), and a promyelocytic cell line (HL-60) [76]. All of these effects were demonstrated by using exogenously added proteins and whether endogenously produced galectins induce or suppress apoptosis in an autocrine or paracrine fashion remains to be determined.

Regulation of Apoptosis (Studies Using the Gene Transfection Approach)

Galectins have also been found to affect apoptosis by using the gene transfection approach, in which cells were transfected with galectin cDNA or anti-sense DNA or oligonucleotides. Additional studies provided substantial evidence that galectins can control apoptosis by functioning intracellularly, without encountering cell surface glycoconjugates. The most extensively documented example is the anti-apoptotic activity of galectin-3. This has been demonstrated with a range of cell types exposed to diverse apoptotic stimuli (reviewed in [8]). Most studies were performed by using different types of tumor cells, and information on immune cells is relatively scarce. The human T lymphoma Jurkat cell line transfected with galectin-3 cDNA is more resistant to apoptosis induced by anti-Fas antibody and staurosporine [78]. Similarly, another human T cell line CEM transfected with galectin-3 cDNA is more resistant to apoptosis induced by galectin-1 [71]. Furthermore, galectin-3-transfected CEM cells were found to be more resistant to C(2)-ceramide-induced apoptosis [74]. Human B lymphoma cells transfected with galectin-3 cDNA became more resistant to apoptosis induced by anti-Fas antibody [79]. Conversely, a galec-

tin-3-positive B cell line transfected with a plasmid containing cDNA coding for amino-terminal truncated galectin-3 (that functions in a dominant negative fashion) exhibited increased sensitivity to anti-Fas-induced apoptosis [79]. There are two types of cells depending on the signaling response initiated by the engagement of one of the death receptor family members, Fas (CD95). In type I cells the response involves the recruitment of Fas-associated proteins as well as caspase-8 to Fas, resulting in the formation of death-inducing signaling complex (DISC). In type II cells, the DISC formation is inefficient. Fukumori et al. [80] noted that among different T cell lines, galectin-3 is strictly expressed by type I cells and that ectopic expression of galectin-3 in type II cells converted these cells to type I cells. Finally, the anti-apoptotic activity of galectin-3 has been confirmed by studying transgenic mice deficient in this protein. Thus, macrophages from galectin-3-deficient mice are more sensitive to apoptosis induced by the combination of lipopolysaccharide and IFN- γ [81].

The mechanism of the anti-apoptotic activity by galectin-3 is not fully understood, but probably involves its engagement in the intracellular apoptosis-regulation pathways. Current evidence suggests that the activity involves translocation of galectin-3 either from the cytosol or the nucleus to the mitochondria following exposure to apoptotic stimuli [82, 83]. In addition, galectin-3 expression inhibited the loss of mitochondrial membrane potential, which is commonly associated with apoptosis [83]. Therefore, galectin-3 may exert its anti-apoptotic activity by interacting with other apoptosis regulators that function in the mitochondria. With regard to the structural requirements, galectin-3's anti-apoptotic activity is apparently dependent on phosphorylation on serine residue at the sixth amino acid position [84], which is also necessary for the protein's export out of the nucleus when the cells are exposed to apoptotic stimuli [85]. In addition, galectin-3's activity is dependent on the Asp-Trp-Gly-Arg (NWGR) motif in the C-terminal part of the molecule [86]. This motif is also found in the Bcl-2 family members, which are well-characterized regulators of apoptosis, and is essential for their apoptosis-regulation functions [87]. This motif is present in galectin-3 proteins from a number of different animal species [78] and is involved in the carbohydrate binding [88].

Galectin-3 has been found to interact with a number of proteins implicated in apoptosis regulation. First, Bcl-2 in cell lysates can be pulled down by galectin-3-conjugated Sepharose 4B [78]; however, whether the interaction is direct or indirect remains to be conclusively estab-

lished. Another study found that galectin-3 is complexed with Fas receptor (CD95) and is thus possibly involved in mediating apoptosis-signaling pathways initiated by engagement of Fas [80]. Finally, synexin, a Ca^{2+} - and phospholipid-binding protein, has been identified as a galectin-3-interacting protein, and necessary for the translocation of galectin-3 to the perinuclear membranes in cells treated with apoptotic stimuli [82]. Current evidence indicates that the mechanisms by which galectin-3 inhibits apoptosis are complex and dependent upon galectin-3 localization. Specifically, it appears that galectin-3 localized in the cytosol protects the cell from apoptosis, but that localized in the nucleus has an opposite effect [89].

Regulation of the Functions of Immune Cells

Modulation of Cell Adhesion

The bivalent or multivalent properties of galectins together with their ability to bind to cell surface glycoproteins make them suited for modulating cell-cell adhesion. In addition, galectins are known to bind to extracellular matrix proteins, including laminin [90–94], fibronectin [95], elastin [96] and hensin [97]. Therefore, they can affect adhesion between cells and extracellular matrices. Furthermore, galectins have been shown to recognize in a carbohydrate-dependent manner the most extensively studied family of cell adhesion molecules, integrins. For example, galectin-1 binds to $\alpha 7\beta 1$ [98], galectin-3 binds to $\alpha 1\beta 1$ [99], and galectin-8 binds to $\alpha 3\beta 1$ and $\alpha 6\beta 1$ [100] as well as αM [101]. Thus, galectins can affect cell adhesion through binding to integrins. Since cell adhesion is prominently involved in the immune and inflammatory responses, it is highly conceivable that galectins can affect various immune and inflammatory processes through modulating cell adhesion.

Galectin-1 and -3 can either potentiate or inhibit adhesion of different cell types to extracellular matrix proteins (reviewed in [90]). In the immune system, galectin-3 can inhibit thymocyte interactions with thymic microenvironmental cells in vitro, thus accelerating the release of thymocytes from thymic nurse cells [102]. The results suggest that endogenous galectin-3 expressed in the thymus functions as a de-adhesion molecules facilitating the exit of thymocytes from the thymus into the periphery. Galectin-3 can also promote adhesion of human neutrophils to laminin [94] as well as to a human endothelial cell line [103]. Human galectin-8 was found to induce adhesion of neutrophils to extracellular matrices [101].

The above-described effects were all demonstrated by using exogenously added galectins. However, there are data supporting that endogenous galectins are involved in cell adhesion. Baum et al. [104] demonstrated that galectin-1 expressed by thymic epithelial cells appears to mediate the adhesion of T lymphoblastoid cells to these cells, as the adhesion can be inhibited by this lectin's saccharide ligands and specific antibody. Similarly, Swarte et al. [105] provided evidence that galectin-3 is involved in the adhesion of L-selectin-activated lymphocytes to dendritic cells. Finally, Asakura et al. [106] demonstrated that endogenous galectin-9 mediated adhesion of eosinophils to the human fibroblast line HFL.

The overall effects of galectins on cell adhesion probably depend on which particular galectins are involved and which subsets of the galectin-interacting glycoconjugates are expressed in the immune cells, as well as whether these glycoconjugates are pertinent to cell adhesion [90]. It is to be noted that galectin-3 can regulate the expression of integrins [107] and induce endocytosis of $\beta 1$ integrins in certain cells [108]. Thus, galectins may also modulate cell adhesion through controlling the level of cell surface integrins.

Activation of Cells

The bivalent and multivalent properties, as well as the carbohydrate-binding activity of galectins also make these proteins capable of cross-linking cell-surface glycoconjugates. Since cross-linking of cell surface proteins is often the first step in transmembrane signal transduction, galectins are expected to induce cell activation. Galectin-1 was shown to induce a Ca^{2+} influx in the human Jurkat T cell line [109]. This activity is dependent on the presence of CD45 on the cell surface and involves ligation of CD2 and CD3 [110]. On the other hand, galectin-1 was also found to down-regulate IL-12 and nitric oxide production by mouse macrophages [19]. Galectin-1 can activate the respiratory burst in neutrophils isolated from skin blister fluid, but not peripheral blood neutrophils [111]. The response of neutrophils to galectin-1 was found to correlate with the mobilization of the intracellular granules resulting in the exposure of granule-associated glycoproteins on the cell surface; these proteins then serve as the receptors for galectin-1. Thus, galectin-1 can have both positive and negative effects on immune cells.

Galectin-3 can also be a positive or negative regulator of immune cell activation. It is known to activate human Jurkat T cells, as evidenced by IL-2 production [22, 112], and to mediate IgE production by B from patients with IgE-associated atopic eczema/dermatitis syndrome in-

duced by polymorphonuclear cells (PMN) from these patients [113]. On the other hand, it was shown to be associated with the T cell receptor (TCR) complex, in a fashion that is dependent on the glycosylation of TCR controlled by $\beta 1,6\text{N-acetylglucosaminyltransferase V}$ (Mgat5), an enzyme in the N-glycosylation pathway [114]. Pre-treatment of T cells with lactose to compete for galectin binding reduced TCR clustering as well as signaling through the receptor. Thus, it was concluded that galectin-3 has a potential role in restricting TCR-initiated signal transduction, due to its forming multivalent complexes with saccharides on TCR [114].

Galectin-3 can activate mast cells resulting in mediator release; both IgE-sensitized and nonsensitized cells can be affected [115, 116]. Interestingly, while galectin-3 is previously known to bind to IgE (hence the name IgE-binding protein), it also binds to IgE receptor (Fc ϵ RI) [115]. It is thus possible that galectin-3 activates mast cells through cross-linking Fc ϵ RI-bound IgE, Fc ϵ RI, or both.

Galectin-3 has also been shown to activate human neutrophils [117, 118]. We found that galectin-3 activated human peripheral blood neutrophils to produce superoxide anion, when the cells were first primed with cytochalasin-D [117]. Another group found that galectin-3, like galectin-1, activated oxidative burst in exudated human neutrophils obtained from skin blister fluid, but not peripheral blood neutrophils [118]. Galectin-3 can induce an oxidative response in peripheral blood neutrophils, when the cells are treated with stimuli such as fMLP that induce the expression on the cell surface glycoproteins that are normally associated with the intracellular organelles [118]. Subsequent studies revealed that treatment of neutrophils with LPS also led to the mobilization of granular proteins to the cell surface thus making the cells responsive to galectin-3 [119]. This group also concluded that CD66a and CD66b on neutrophils are recognized by galectin-3 [120]. The binding of neutrophils surface proteins by galectin-1 and -3 has been compared and these two lectins were noted to recognize different proteins [111]. Thus, it appears that they activate neutrophils through engaging two different sets of receptors.

Finally, galectin-3 can trigger human peripheral blood monocytes to produce superoxide anion [34] and potentiate their LPS-induced IL-1 production [121]. Galectin-3 can also have suppressive effects on myeloid cells. One example is its inhibition of IL-5 production by human eosinophils, as well as an eosinophilic cell line, human peripheral blood mononuclear cells (PBMC), and an antigen-specific T cell line; the effects appear to be related

to a down-regulation of the IL-5 mRNA level [122]. Subsequent studies established that galectin-3 binds to Fc γ RII and, by using PBMC from Fc γ RII-deficient mice, the authors concluded that galectin-3 down-regulates IL-5 production through engaging Fc γ RII [123].

Much less is known about the effects of other galectins. Galectin-4 was shown to stimulate IL-6 production by CD4⁺ T cells in a fashion that is independent of MHC class II and induced by a PKC θ -associated pathway through the immunological synapse [124]. Galectin-8 was found to induce superoxide production in neutrophils, to an extent comparable to that induced by the potent stimulus fMLP [101].

In most of the above-described studies, the effect of one galectin on one cell type was analyzed. In one paper, comparative analyses of effects of a number of galectins on different cell types were reported [125]. The authors found that galectin-1 and -3 as well as CG-16 (a chicken galectin), but not galectin-7, caused aggregation of neutrophils, thymocytes and platelets. On the other hand, galectin-3, and -7 as well as CG-16, but not galectin-1, enhanced detergent (SDS)-induced hemolysis of human erythrocytes. These results demonstrate the specificity of galectins for different cell surface glycoconjugates.

Galectins can also control cellular activity by functioning intracellularly. Galectin-2 was shown to bind to lymphotoxin- α and regulate the latter's secretion by macrophages [126]. Macrophages from galectin-3-deficient mice were found to be defective in phagocytic activity and galectin-3 was found to be concentrated in the phagocytic cups and phagosomes in wild-type macrophages engulfing opsonized erythrocytes [127]. The results suggest that intracellular galectin-3 participates in the process of phagocytosis; however, the mechanism for this remains to be elucidated.

Chemoattraction

Migration of various immune cells induced by chemoattractants, such as chemokines, is essential in the development of immune and inflammatory responses. Galectins have been shown to be able to contribute to regulation of the migration of immune cells. Human recombinant galectin-1 was found to inhibit chemotaxis as well as trans-endothelial migration of PMN in vitro and inhibit interleukin-1 β -induced PMN recruitment into the mouse peritoneal cavity [128]. Intravital microscopy analysis demonstrated that it suppresses the leukocyte flux, but not their rolling velocity [128].

Galectin-3 on the other hand was found to induce human monocytes and macrophages as well as alveolar mac-

rophages to migrate both in vitro and in vivo [129]. Both the N-terminal and C-terminal domains are involved in this activity. The activity is mediated at least in part through a pertussis toxin (PTX)-sensitive (G protein-coupled) pathway, which is known to be engaged by various chemokines [129]. However, there is so far no evidence for the involvement of chemokine receptors in galectin-3's chemoattractant activity. Galectin-9 has been found to be a potent eosinophil chemoattractant [45]. The activity is dependent on its carbohydrate-binding activity and requires both CRDs [130].

As mentioned above, galectins have been found to bind to integrins. Since integrins play an important role in controlling cell migration [131], it is possible that galectins regulate cell motility and invasion by involving activation of integrins. In fact, galectin-8 has been reported to ligate integrins and trigger integrin-mediated signaling cascades, resulting in cytoskeletal changes and cell spreading in different cell types [100]. However, its effects on immune cells have not yet been reported.

Recognition of Pathogens

The effect of some infectious processes on galectin expression and the possible roles of galectins in the response of the host to infections have been described in previous sections. In addition, galectins can recognize glycans expressed by certain pathogens. Galectin-3 was found to bind to two sites in LPS from *Klebsiella pneumoniae*. One involves its C-terminal lectin domain and a β -galactoside-containing polysaccharide chain in LPS. The other involves its N-terminal part and the lipid A/inner core region in LPS [132]. LPS from *Salmonella minnesota* R7 devoid of β -galactosides binds only to the N-terminal part of galectin-3. Another study showed that LPS from *Pseudomonas aeruginosa* bound to galectin-3 apparently by involving the outer core portion of the former [133]. Galectin-3 was also found to bind to lipophosphoglycans of *Leishmania major*, through polygalactose epitopes [134]. The authors subsequently reported that galectin-9 can also recognize *L. major* by binding to polygalactosyl epitopes [135].

Galectin-3 can bind to neoglycoconjugates carrying GalNAc β 1-4GlcNAc (LacdiNAc, LDN), which is the most common component of N-linked glycans in invertebrates, such as helminths. Thus, galectin-3 was found to bind to *Schistosoma mansoni* soluble egg antigens in a fashion that is inhibitable by a monoclonal antibody against the LDN glycan, suggesting that LDN glycans are involved. Moreover, immunocytochemistry demonstrated a colocalization of galectin-3 and LDN glycans on the

parasite eggshells. Finally, galectin-3 can mediate phagocytosis of LDN-coated particles by macrophages [136]. Galectin-3 was found to accumulate in *Mycobacterium*-containing phagosomes in macrophages during the course of infection. The protein resides primarily on the cytosolic face of the phagosomal membrane and it binds to the glycolipid component of *Mycobacteria* [137].

In vivo studies have begun to reveal the role of galectins in innate immunity against infection. In a *Streptococcal pneumonia* mouse model, Sato et al. [103] found accumulation of galectin-3 in the alveolar space in the infected lungs, correlating well with the onset of neutrophil extravasation. As mentioned earlier, these authors also demonstrated in vitro that galectin-3 can promote neutrophil adhesion to endothelial cells. They thus proposed that galectin-3 plays an important role in the extravasation of neutrophils in the lung infection by streptococcal pneumonia. Beatty et al. [137] observed that galectin-3-deficient mice had a reduced capacity to clear late *Mycobacterium tuberculosis* infection compared to wild-type mice [137], suggesting the contribution of galectin-3 in clearance of *Mycobacteria*.

Galectins as Autoantigens

Galectins can also participate in immune and inflammatory responses by functioning as autoantigens. A number of reports demonstrated the presence of autoantibodies to different galectins in normal individuals or selected patient populations. Autoantibodies to galectin-1 were found in sera from patients with multiple sclerosis and the titers were significantly higher than in sera from healthy controls [138]. Another study showed that there was a marked increase in the level and frequency of IgE antibodies against galectin-1 in sera from patients with the acute stage of Chagas' disease [139]. In addition, a high frequency of autoantibodies to galectin-3 was found in patients with systemic lupus erythematosus and polymyositis/dermatomyositis compared to healthy individuals [140]. Finally, a substantial higher percentage of sera from Crohn's disease patients contains anti-galectin-3 autoantibodies [141].

Autoantibodies to galectins have also been shown to be associated with neoplasm. A subject with newly diagnosed adenocarcinoma of the colon was found to have a significantly elevated level of IgG anti-galectin-3 [142]. Similarly, the occurrence of IgG antibodies to galectin-3 was noted in a high percentage of the sera from patients with pharynx/larynx squamous cell carcinoma [143]. However, in the latter studies, similar autoantibodies were also found in healthy donors and there was no sta-

tistically significant difference between the two populations.

Biological Effects Demonstrated in vivo

The role of galectin-1 in the inflammatory response is supported by several in vivo studies. Offner et al. [144] demonstrated that recombinant human galectin-1 prevents experimental autoimmune encephalomyelitis (EAE) in mice. Rabinovich et al. [145] demonstrated that injections of recombinant galectin-1 or transfected fibroblasts secreting galectin-1 abrogated clinical and histopathological manifestations of collagen-induced arthritis in mice, a model of rheumatoid arthritis. Inhibitory effects of galectin-1 on Con A-induced hepatitis have also been reported [146]. Finally, galectin-1 treatment suppresses murine models of graft versus host disease [147] and experimental colitis [148], as well as acute inflammation induced by bee venom phospholipase A2 in the hind paws of rats [149]. Thus, studies in multiple model systems have established that administered galectin-1 has anti-inflammatory activity. In addition, in a murine model of melanoma, blockade of galectin-1 results in an enhanced tumor-specific T cell-mediated response and consequently tumor rejection, suggesting that galectin-1 has a role in tumor immune escape through its proapoptotic activity on T cells [63]. Galectin-4 on the other hand appears to have proinflammatory activity, possibly by stimulating CD4⁺ T cells, and the administration of this protein was shown to exacerbate chronic colitis and delay the recovery from acute intestinal injury [124].

Studies of galectin-3-deficient (gal3^{-/-}) mice have provided significant support for the proinflammatory role of this lectin. Colnot et al. [150] found that gal3^{-/-} mice exhibited a significantly reduced number of granulocytes compared to wild-type (wt) animals in peritoneal cavity after thioglycollate injection. Our laboratory also provided evidence for reduced peritoneal inflammation in gal3^{-/-} mice [81]. When peritoneal inflammation was induced with thioglycollate broth, gal3^{-/-} mice consistently developed less macrophage infiltrations than wt mice. Our laboratory also obtained evidence for the proinflammatory role of galectin-3 in a murine asthma model. OVA-sensitized gal3^{-/-} mice developed fewer eosinophils and significantly less airway hyperresponsiveness, compared to similarly treated wt mice following airway OVA challenge [151]. However, another group demonstrated that intranasal delivery of plasmids containing galectin-3 cDNA into rats resulted in a reduced eosinophil infiltra-

tion and airway hyperresponsiveness in response to airway antigen challenge [152]. Thus, it appears while the endogenous protein have a potentiative role, application of pharmacological concentrations of a galectin-3-expression construct to the airways can have suppressive effects on airway responses.

Conclusions

The information summarized in this review suggests that galectins have a wide variety of functions and play important roles in regulation of the immune and inflammatory responses. They can function extracellularly by affecting cell growth and death, activating or inhibiting cellular responses, promoting or inhibiting cell adhesion, and influencing cell migration. They can also exert these activities by functioning inside the cells.

The extracellular functions have been mostly demonstrated by using recombinant galectins added to cells and high concentrations are often required to demonstrate the activities. However, as mentioned above, galectins may be more effective when presented by the extracellular matrices. In addition, some galectins are known to be secreted by cultured cells (reviewed in [9]) and readily detectable in the extracellular environment, especially under inflammatory conditions. For example, galectin-3 was detected in tears from patients with ocular diseases [153]. Its expression is upregulated in the synovial tissues in patients with rheumatoid arthritis and detectable in synovial fluid from these patients [154]. Galectin-3 is also detected in bronchoalveolar lavage fluid obtained from mice with inflamed airways [151] and mice infected with *Streptococcus pneumoniae* [103].

It is to be noted that there is evidence that galectin-3 is cleaved by matrix metalloproteases, and thus its biological activities may be controlled by these enzymes present in the microenvironment [155]. In addition, it is known that galectin-1 can be oxidized resulting in an abolishment of carbohydrate-binding activity [156], suggesting that many of the activities of galectin-1 that are dependent on the protein's lectin properties may eventually be lost as the protein diffuses away from the cell source and become oxidized in the oxidative extracellular environment.

One unique property of galectins is that each of them does not appear to have a specific receptor, like cytokines or chemokines. When searching for glycoproteins that a galectin binds to, several are often identified. This suggests that galectins function by binding to a set of glyco-

proteins that bear the appropriate oligosaccharides. Nevertheless, consistent with the specificity of galectins for oligosaccharides, different galectins appear to bind to different sets of glycoproteins.

Regarding the intracellular functions, they have been mostly inferred from studying cells ectopically expressing certain galectins by gene transfection. While the functions demonstrated could be due to the lectin being secreted by the cells and functioning extracellularly, in many cases the conclusion is supported by the demonstration of intracellular localization of the protein. Additional studies using the small interference RNA (siRNA) approach or different cell populations from galectin-specific knock-out mice will be useful to provide new insights into the functions of various galectins. In addition, continued studies of the existing and newly developed knock-out mice in vivo will help definitely establish the roles of different galectins in the immune and inflammatory responses.

Acknowledgements

The author thanks members of his research group who contributed to the work cited in this review. The work has been supported by grants from the National Institutes of Health (RO1AI20958, RO1AI39620, and PO1 AI50498).

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