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Galectin-1 receptors in different cell types

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Summary

Galectins are a family of animal lectins defined by two properties: shared amino acid sequences in their carbohydrate-recognizing domain, and β-galactoside affinity. A wide variety of biological phenomena are related to galectins, i.e., development, differentiation, morphogenesis, tumor metastasis, apoptosis, RNA splicing, and immunoregulatory function. In this review, we will focus on galectin-1 receptors, and some of the mechanisms by which this lectin affects different cell types. Several galectin-1 receptors are discussed such as CD45, CD7, CD43, CD2, CD3, CD4, CD107, CEA, actin, extracellular matrix proteins such as laminin and fibronectin, glycosaminoglycans, integrins, a β-lactosamine glycolipid, GM1 ganglioside, polypeptide HBGp82, glycoprotein 90 K/MAC-2BP, CA125 cancer antigen, and pre-B cell receptor.

Abbreviations: CA125 – cancer antigen CA125; CD – circular dichroism; CEA – carcinoembryonic antigen; CHO – Chinese hamster ovary; C2GnT – core 2 β-1,6-*N*-acetylglucosaminyltransferase; CRD – carbohydrate-recognizing domain; CR3 – complement receptor 3; ECM – extracellular matrix; ER – endoplasmic reticulum; ERK – extracellular signal-regulated kinase; GAG – glycosaminoglycans; GM1 – GM1 ganglioside; GNTV – β-1,6-*N*-acetylglucosaminyl-transferase V; HBGp82 – human brain galectin-1-binding polypeptide of 82 kD; hsp – heat shock protein; 90 K/MAC-2BP – glycoprotein of 90 kD or Mac-2-binding protein; LacNAc – *N*-acetyl-lactosamine; LAMP-1/2 – lysosome-associated membrane proteins 1/2; LNnT – lacto-*N*-neotetraosyl ceramide; mAb – monoclonal antibody; MAPK – mitogen-activated protein kinase; MEK – mitogen-activated protein kinase kinase; MEKK – mitogen-activated protein kinase kinase kinase; MW – molecular weight; NMR – nuclear magnetic resonance; PL – poly-*N*-acetyl-lactosamine; pre-BCR – pre-B cell receptor; PTP – protein tyrosine phosphatase; RCA – *Ricinus communis* agglutinin; SLC – surrogate light chain; SMC – smooth muscle cells; SNA – *Sambucus nigra* agglutinin; ST6Gal I – sialyltransferase specific for SAα2,6Gal; TCR – T cell receptor; TDG – thiodigalactoside.

Galectin-1: a ubiquitous galectin

Galectins are a family of lectins previously known as S-type or S-Lac lectins, and they are defined by two properties: shared amino acid sequences in their carbohydrate-recognizing domain (CRD), and β-galactoside affinity [1–3]. Galectins are atypical secreted proteins, since cDNA that includes the entire coding length has not revealed a recognizable secretion signal. Intracellularly, the galectin message is found on free cytoplasmic ribosomes and the protein is present in the cytosol and nucleus, rather than in the membrane-bound compartments usually involved in protein secretion [4]. Other particular features characterize galectins such as an

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acetylated NH_2-terminus, absence of glycosylation, and a requirement for reducing conditions for carbohydrate-binding activity. To date, 14 mammalian members of the galectin family have been sequenced and they are well characterized in different species [5–8]. Galectins can be classified according to their CRD features. Galectin-1, -2, -5, -7, -10, -11, -13, and -14 are composed of one CRD ('prototype galectins'). Galectin-4, -6, -8, -9, and -12 have two non-identical CRDs in tandem with a short linker sequence ('tandem-repeat type galectins'). Galectin-3 has an exceptional structure, with one CRD, an extended repetitive domain consisting of glycine/proline repeats, and a short N-terminal end ('chimera type galectin') [9] (Figure 1).

Many galectins have been found in non-mammalian vertebrates [i.e. 10–14], in invertebrates [i.e. 15–17], in plants such as *Agrocybe cylindracea* and the mushroom *Coprinus cinereus*, and even in viruses [3]. Galectins must have evolved before the split of plants, animals and fungi because there are many galectin-like sequences in plant genomes, including at least seven in the genome of *Arabidopsis thaliana*. Novel galectin family members are being rapidly discovered by screening for related sequences in DNA data banks generated by various mass sequencing projects [3].

Galectin-1 is a non-covalent homodimeric galectin, with a 14 kD monomer which contains one CRD (prototype galectin). Galectin-1 preferentially recognizes Galβ1,4GlcNAc (LacNAc) sequences that can be present on N- or O-linked glycans. We have examined the unfolding process of galectin-1 in the presence of a denaturing agent, guanidine hydrochloride, using fluorescence and far-UV circular dichroism spectroscopy. Our results show that, in the absence of a ligand, unfolding transitions are biphasic, showing the existence of at least one stable intermediate which belongs to the molten globule type; in the presence of the ligand, the data fit a two-state model, including only the native and the unfolded states. The unfolding is fully reversible, as shown by the recovery of hemagglutinating activity, fluorescence emission, and CD spectra of the native state upon dialysis of the unfolded galectin-1. The protective effect of the ligand is also evidenced by the higher concentration of denaturing agent required for complete unfolding, indicative of greater stability toward denaturation [18].

Galectin-1 is present both extracellularly and intracellularly. It is also found at the cell surface

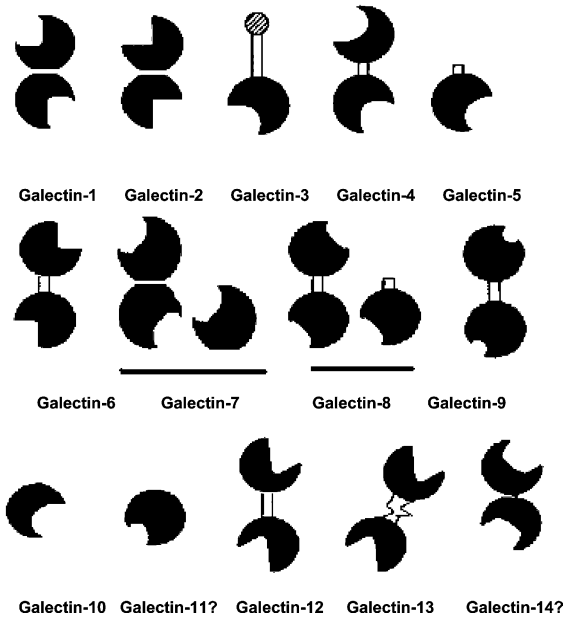

Figure 1. Schematic representation of domains and quarternary structures of galectins 1–14. Carbohydrate-binding domains are represented by black areas. The N-terminal domain of galectin-3 is striped. Linker or homologous regions in galectin-3, -4, -5, -6, -8, -9, -12 are white. Prototype galectins (one CRD): galectin-1, -2, -5, -7, -10, -11, -13, and -14. Tandem-repeat galectins (two CRDs): galectin-4, -6, -8, -9, and -12. Chimera galectin (one CRD, a repetitive domain, and a short N-terminal domain): galectin-3. Homodimers of prototype galectin-1, -2, and -7 are constituted by noncovalent bonds. Unlike all other prototype galectins, galectin-13 homodimer is formed by disulphide bonds ≈. The native quaternary structures of prototype galectin-11 and -14 have not yet been elucidated.

because secreted galectin-1 binds to lactosamines on glycoconjugates expressed by the same cell or by neighboring cells. In mammals, large amounts of galectin-1 have been described from different organs, i.e. placenta, lung, hepatoma, brain, heart, spleen, lymph nodes, and prostate. Galectin-1 is found in a variety of cell types, including fibroblasts, 3T3 cells, Chinese hamster ovary cells (CHO), thymic epithelial cells, endothelial cells, dendritic cells, macrophages, T and B cells, and bone marrow stromal cells [1, 3, 17].

Pleiotropic biological functions of galectin-1 in different cell types

A wide variety of biological phenomena have been shown to be related to galectin-1, i.e. cell adhesion, proliferation, apoptosis, T cell receptor counter-

stimulation, immunomodulatory effects, cell cycle arrest, pre-B cell signaling, RNA splicing, and promotion of H-Ras membrane anchorage [19–22] (Table 1). Galectin-1 is involved in cell–cell and cell–matrix adhesion in processes such as tumor invasion and metastasis, inflammation, and organ development. Galectin-1 binds to poly-*N*-acetyllactosamine (PL) chains from extracellular cell matrix proteins such as laminin and fibronectin, modulating cell adhesion positively or negatively [26, 39, 43–45]. Galectin-1 also affects the interaction of tumor cells with endothelial cells, which is critical in invasion and metastasis [19]. Accumulation of galectin-1 is observed at the contact sites between breast tumor cells and the endothelium: galectin-1 localizes on tumor cells and galectin-3 preferentially localizes on endothelial cells, suggesting different roles of these lectins in adhesion. Increased galectin-1 expression has been reported in many types of human cancer such as those arising from the thyroid, endometrium, head and neck, thymus, bladder, pancreas, and colon, and in cholangiocarcinoma, and glioma [46, 47].

In the immune system, galectin-1 is expressed in the thymus, spleen, lymph nodes, bone marrow, liver, and immune-privileged sites [35, 48]. Galectin-1 is also involved in adhesion of lymphoblastoid cells to thymic epithelial cells by binding to carbohydrates on thymocyte surface glycoproteins such as CD43 and CD45. Galectin-1 from human

Table 1. Galectin-1 biological functions.

Function	Cell type	Receptor	Reference
Cell adhesion (↑ or ↓)*	Myoblasts	Laminin, $\alpha_7\beta_1$integrin	[23, 24]
	Colon cancer	CEA	[25]
	Human melanoma	Laminin	[26]
	Human ovary carcinoma	Laminin, fibronectin	[27]
	Macrophages	CR3	[28]
	T cells	CD43, CD45	[29, 30]
	CHO cells	Lamp-1, -2	[31]
	Tumoral cells to endothelial cells	Various	[19]
Apoptosis	Immature thymocytes, activated peripheral T cell, B cell, prostate and breast cancer cells	CD45, CD43, CD7, others	[29, 32–34, 35, 36]
Inflammation (↓)	Activated macrophages, antigen-stimulated T cells, activated B cells, alloreactive T cells	?	[22, 32]
Modulation of autoimmune diseases (↓)	Collagen-induced arthritis, experimental colitis, autoimmune encephalomyelitis, autoimmune myasthenia gravis	?	[21, 22, 32]
TCR-counter-stimulation	T cell	CD3, CD4	[37, 38]
Proliferation (↑ or ↓)*	Smooth muscle cells	?	[39]
	Ovary carcinoma cells	?	[27]
	Arterial endothelial cells	?	[40]
	Hepatic stellate cells	?	[41]
	Thymocytes, activated T-cells, leukemia T-cells	?	[22, 37]
Migration	Hepatic stellate cells	?	[41]
Cell cycle arrest (S/G_2)	T cell, breast cancer cells, neuroblastoma cells	?	[32]
Pre-B cell receptor signaling	B cell	λ-like	[42]
Nuclear splicing of pre-mRNA	HeLa cells	Intracellular ligands (Gemin4)	[20]
Promotion of H-Ras membrane-anchorage	Rat-1 cells	Intracellular ligands (H-Ras)	[20]

thymic epithelial cells binds to core 2-O-glycans on immature cortical thymocytes and induces cell apoptosis during thymocyte maturation. Immature cortical thymocytes bind more galectin-1 than mature medullar thymocytes do [49]. The apoptotic effect is dose dependent, carbohydrate-specific, and Fas-, steroid- and CD3-independent. In addition, galectin-1 expressed on endothelial cells can trigger apoptosis of adherent T cells in a carbohydrate-dependent manner [34, 50].

Galectin-1 has been proposed as a TCR counterstimulator that antagonizes TCR-induced IL-2 production and proliferation. Galectin-1 does not equally antagonize all TCR-induced functions because it does not interfere with antigen-induced CD69 expression, IFN-γ production or apoptosis of T cells. In fact, galectin-1 T cell stimulation induces partial phosphorylation of the TCR-ζ chain (pp21ζ) relative to complete ζ chain phosphorylation (high pp23/pp21 ζ ratios). Chung et al. proposed that galectin-1 limits the extent of membrane reorganization and sustained TCR signal transduction by cross-linking lipid raft (GM1, CD4), raft translocatable (CD3), and non-raft (CD43, CD45) microdomain constituents. Such receptor cross-linking events might limit the size of lipid raft clusters at the TCR contact site and protein tyrosine phosphorylation: functions relying on partial TCR signal transduction are permitted, whereas complete TCR signals are inhibited by galectin-1. Thus, three operational galectin-1 functions in TCR antagonism are proposed: (1) limiting reorganization of the TCR contact site; (2) preventing sustained TCR signal transduction; and (3) skewing TCR functional responses from those promoting T activation to those promoting T cell inactivation and tolerance induction [37, 38].

In inflammation, activated macrophages, antigen-stimulated T cells, activated B cells, and alloreactive T cells produce high levels of galectin-1 to kill effector T cells after an immune response. Immune privileged tissues such as retina, placenta, testis, and ovary overexpress galectin-1 which might ensure the rapid elimination of inflammatory T cells by the galectin-1 apoptotic pathway to protect the integrity and function of these vulnerable tissues [21, 22].

Galectin-1 also modulates proliferation of normal and malignant cells, depending on the cell type: growth inhibition may be observed at high galectin-1 concentrations whereas lower concentrations enhance cell proliferation [51]. In human ovary carcinoma cells, low concentrations of galectin-1 do not show any effect, but higher concentrations decrease cell proliferation [27]. Galectin-1 increases serum-induced DNA synthesis in human SMC cultured on ECM [39]. In rat pulmonary arterial endothelial cells, galectin-1 also promotes proliferation [40]. Galectin-1 also induces proliferation of hepatic stellate cells but not isolated hepatocytes through signaling pathways that activate the mitogen-activated protein kinases MEK1/2-ERK1/2 [41]. Different signaling pathways are induced by galectin-1: i.e., galectin-1 triggers tyrosine phosphorylation of phospholipase Cγ1 [52], which produces inositol-1,4,5-triphosphate and intracellular calcium increase, as well as induction of ERK activation [38, 41], and dephosphorylation plus nuclear translocation of transcription factors AP1 [53] and NFAT [89] (Figure 2).

In this review, we will discuss receptors for galectin-1, and some of the mechanisms by which this lectin affects different cell types. Galectin-1 recognizes *N*-acetyl-lactosamine (LacNAc) as a minimal oligosaccharide structure which is present in different glycoproteins and glycolipids with multiple and complex sugar structures. Therefore, the way a particular cell type responds to galectin-1 is also regulated by variations in the activity of glycosyl-transferases and/or-glycosidases. At present, several receptors have been found which mediate carbohydrate-protein (sugar–lectin) or protein–protein (protein–lectin) interactions. These receptors include extracellular cell matrix (ECM) proteins (laminin, fibronectin, thrombospondin, vitronectin, osteopontin), glycosaminoglycans (GAG) such as chondroitin sulfate B, integrins, CD45, CD43, CD7, CD2, CD3, CD4, CD107, CEA, actin, a β-lactosamine glycolipid, ganglioside GM1, polypeptide HBGp82, glycoprotein 90 K/MAC-2BP, CA125 cancer antigen, and pre-B cell receptor (Table 2).

Galectin-1 receptors

Carbohydrate–protein interactions

Extracellular matrix receptors

Laminin, fibronectin, thrombospondin, vitronectin, and glycosaminoglycans galectin-1 can modulate cell-ECM interactions in different bio-

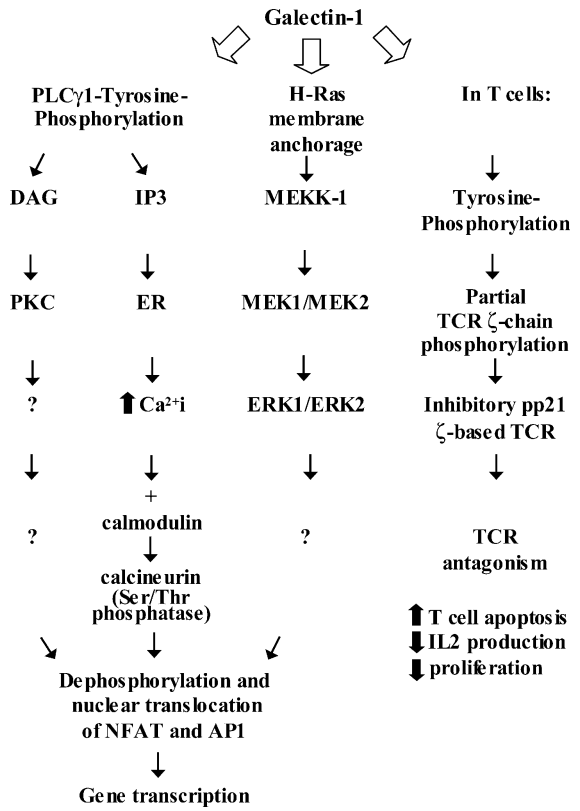

Figure 2. Schematic representation of signaling pathways triggered by galectin-1. Galectin-1 induces tyrosine phosphorylation of phospholipase Cγ1 (PLCγ1), which produces second messengers such as inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). As a result, IP_3 releases calcium from intracellular stores as the endoplasmic reticulum (ER), increasing intracytoplasmic free calcium ($Ca^{2+}i$). Calcium influx from the extracellular medium is also increased. DAG stimulates protein kinase C (PKC). Calcium/calmodulin activates calcineurin, a Ser/Thr phosphatase, which causes dephosphorylation and nuclear translocation of transcription factors NFAT and AP1. Galectin-1 also induces Ras activation and mitogen-activated protein kinase (MAPK) cascades: MAP kinase kinase kinase-1 (MEKK-1) probably activates MAP kinase kinase-1/-2 (MEK-1/-2) which phosphorylates extracellular signal-regulated kinases-1/-2 (ERK-1/-2). Different pathways probably end up in dephosphorylation and nuclear translocation of transcription factors such as NFAT and AP1, to produce expression of specific genes. Besides these pathways, in T cells galectin-1 induces tyrosine phosphorylation of the T cell receptor (TCR) ζ-chain, which is only partially phosphorylated, and antagonizes downstream TCR signals and functions such as interleukin 2 (IL2) production, and proliferation. ↑: increase; ↓: decrease.

logical systems. However, whether extracellular galectin-1 promotes cell attachment or detachment in the context of the ECM is controversial. It seems that the pro- or anti-adhesive effect varies according to the cell type, cell activation status, and, more importantly, the relative cell-surface expression of particular laminin and fibronectin receptors. Laminin and fibronectin are two ECM proteins proposed as the main receptors for galectin-1 [26, 45]. Laminin is a large glycoprotein and a major component of the basement membrane in all types of tissues. Many biological phenomena such as cellular adhesion, spreading, proliferation, and differentiation involve interactions between cells and laminin. Laminin is composed by three chains, the A chain (400 kDa), B1 chain (210 kDa), and B2 chain (200 kDa). It binds to certain receptors on cell surfaces, including a 67 kDa receptor, integrins, some other high-molecular-weight laminin-binding proteins, gangliosides, sulfatides, type IV collagen, entactin/nidogen, and heparan sulfate proteoglycan. Laminin shows Asn-linked oligosaccharides containing the repeating disaccharide (3Galβ1,4GlcNAcβ1)n or PL sequence. Galectin-1 binds strongly to A and B subunits of laminin; it also binds to asialofetuin, and weakly to fetuin, in a lactose- and LacNAc-inhibitable fashion [44].

Galectin-1 also modulates human melanoma cell adhesion to laminin. Attachment assays have shown that recombinant galectin-1 increases melanoma cell attachment to laminin in a dose-dependent manner. Anti-galectin-1 antibodies decrease galectin-1 induced attachment. Local increases or decreases of galectin-1 expression may play a critical role during attachment and detachment of cancer cells throughout the cancer progression process [26]. A preferential accumulation of galectin-1 has been demonstrated in the stroma associated with prostate carcinoma cells and in the stroma adjacent to ovary carcinoma cells. Immunohistochemical studies have been performed on co-expression of galectin-1, laminin, and fibronectin in ovary carcinoma cell-associated stroma: most of the samples that were positive for galectin-1 were also positive for laminin and fibronectin. Galectin-1-mediated attachment of ovary carcinoma cells to laminin and fibronectin was analyzed in vitro: galectin-1 induced a dose-dependent increase in adhesion to laminin or fibronectin. Thus, galectin-1 which has accumulated at the border between cancer cells and host stroma might modulate interactions between laminin and glycoconjugates located on the cell surface, and hence affect invasion [27].

Table 2. Galectin-1 receptors.

Receptor	Localization	Reference
Integrins	Skeletal and smooth muscle cells	[23, 24, 39]
Laminin	Human melanoma cells, myoblasts, human ovary carcinoma cells, T cells	[23, 24, 26, 27, 45, 55, 56]
Fibronectin	Human placenta, human ovary carcinoma cells, T cells	[27, 43, 56]
CD45	T cell lines	[34]
CD43	T cell lines	[25]
CD7	T cell lines	[36, 57]
CD2/CD3	Jurkat T cells	[52]
CD4	T cell lines	[29, 58]
CD107a/b (LAMPS 1/2)	Human ovarian carcinoma cells	[25, 59]
1B2 glycolipid	Olfatory axon in olfatory bulb	[60]
Glycoprotein 90K/MAC-2BP	Human breast cancer cells	[61]
CEA	Human colon carcinoma cells	[25]
GM1	Human neuroblastoma cells	[62, 63]
CA125	Human ovarian cancer cells	[64]
HBGp82	Human brain	[65]
Actin	Human brain, MOLT-4 T cells	[29, 66]
Gastrointestinal mucin	Mucin granules from mucous cells	[19]
Pre-B cell receptor (λ-like)	Human and mouse B cell lines	[42]

Other studies have also confirmed the role of laminin as a galectin-1 receptor [99, 13]. Human T cell adhesion to ECM and to individual ECM components such as laminin, fibronectin and collagen IV has also been tested. Galectin-1 substantially decreases attachment of IL-2-activated T cell to whole ECM and intact laminin or fibronectin in a dose-dependent manner. Thiodigalactoside (TDG), a potent galectin inhibitor, partially abolishes galectin-1-mediated inhibition of T cell attachment [56].

Tissue fibronectin has also been proposed as an endogenous receptor for galectin-1. In human placenta extracts, two major galectin-1 receptors were obtained by affinity chromatography and identified as fibronectin and laminin. Fibronectin, laminin, and galectin-1 colocalize in the extracellular matrix of placental tissue. In cell attachment assays, tissue fibronectin and laminin have been shown to function as endogenous receptors for galectin-1, and their binding is inhibited by lactose [43]. Other ECM proteins such as thrombospondin and vitronectin and, to a lower extent, osteopontin can also bind to galectin-1 [39]. Galectin-1 binds to several ECM in a dose-dependent and β-galactoside-dependent manner, in the following order: laminin > cellular fibronectin > thrombospondin > plasma fibronectin > vitronectin > osteopontin. This binding is reduced by half in the presence of lactose for all proteins except vitronectin (see below). Moreover, galectin-1 interacts with GAG chains from ECM. For example, heparan sulfate and chondroitin sulfate reduce the binding of galectin-1 to ECM proteins [67]. Interactions between galectin-1 and chondroitin sulfate proteoglycans have also been described [64]. Chondroitin sulfate B contains galactose-like residues and shows significant β-galactoside-dependent binding to galectin-1 in the solid phase, compared to chondroitin sulfate A and C and heparan sulfate, which do not bind to galectin-1.

Vitronectin can also bind to galectin-1. Interactions between vitronectin and galectin-1 seem to depend on vitronectin conformation: vitronectin exists either as a folded inactive monomer or an unfolded multimer able to interact with ECM components. Vitronectin shows significant binding to galectin-1 in the presence of lactose, probably because lactose induces unfolding of vitronectin. Heparan sulfate is also known to enable unfolding of vitronectin, and, as expected, the binding of vitronectin to immobilized galectin-1 in the solid phase is increased by heparan sulfate. Moreover, galectin-1 bound to the ECM reduces the incorporation of

vitronectin and chondroitin sulfate B to the ECM in a β-galactoside dependent manner. Thus, ECM-bound galectin-1 can decrease incorporation of its receptors into the ECM, which suggests a role for galectin-1 in ECM assembly and tissue matrix remodeling [67].

Cell surface receptors

Integrins

The $\alpha_7\beta_1$ integrin is the predominant laminin-binding integrin on differentiating skeletal muscle cells [68]. The expression of the α_7 is developmentally regulated during skeletal muscle differentiation and has been used to identify cells at distinct stages of the myogenic lineage. The addition of purified recombinant galectin-1 to myogenic cells plated on laminin inhibits myoblasts spreading and fusion, suggesting that galectin-1 regulates muscle cell interactions with extracellular matrix [55]. Gu et al. have demonstrated that $\alpha_7\beta_1$ integrin binds to fibronectin and to galectin-1. The $\alpha_7\beta_1$ integrin binds to fibronectin by the fibronectin peptide RGD in a divalent cation-dependent fashion; galectin-1 recognizes specific oligosaccharides on the $\alpha_7\beta_1$ integrin [8], and binds to them in a lactose-inhibitable fashion. Galectin-1 binds to both laminin and to $\alpha_7\beta_1$ integrin, and it can effectively inhibit the association between laminin and $\alpha_7\beta_1$. TDG blocks galectin-1-mediated inhibition of $\alpha_7\beta_1$ binding to laminin. Galectin-1 interactions with laminin and $\alpha_7\beta_1$ integrin regulate myogenesis by inhibiting the association of laminin with integrin: both galectin-1 concentrations relative to those of $\alpha_7\beta_1$ integrin and laminin, and the glycosylation state of $\alpha_7\beta_1$, laminin, and fibronectin can modify galectin-1 effects [24].

Relative to other integrins, galectin-1 from mouse macrophage cell lines has been found specifically associated with complement receptor 3 (CR3) (CD11b/CD18, $\alpha_M\beta_2$ integrin). Galectin-1 binds to CR3 via its CRD, and can be immunoprecipitated from cell lysates with anti-CR3 mAbs in a lactose-inhibitable fashion. Moreover, CR3 and galectin-1 colocalize on the cell surface of macrophages as determined by confocal microscopy, although some galectin-1 molecules have been found dispersed onto the cell surface [28]. In this case, the fine interaction of α_M or β_2 subunits of the integrin with galectin-1 has not been determined.

Evidence for a direct binding of galectin-1 to the β_1 subunit of integrin from human vascular SMC has been provided by chemical cross-linking experiments. Galectin-1 binding to cultured vascular SMC is 30- to 40-fold higher than to ECM produced by these cells [39]. Galectin-1 enhances expression of β_1 integrin on the cell surface, as shown by flow cytometry. Moreover, galectin-1 influences the cell binding of an antibody which recognizes only an active form of β_1 integrin. The galectin-1 effect on β_1 integrin may be caused by a conformational change in β_1 integrin after galectin-1 binding, since clustering of β_1 integrin is unlikely because galectin-1 does not cross-link β_1 [46].

CD45

CD45 is a family of integral membrane tyrosine phosphatases (PTP) expressed on cells of hemopoietic origin. Human CD45 molecules described vary in molecular weight (MW) from 180 to 220 kDa, accounted for by alternative splicing of a single precursor mRNA [70]. Lymphocytes express predominantly higher MW isoforms (CD45RA), whereas the majority of thymocytes express the lowest MW structure (CD45RO) [71]. Additional heterogeneity is accounted for by differences in glycosylation of the protein backbone. Studies of both *N*-linked and O-linked sugar chains revealed that they contain large numbers of PL units [72]. Several reports have identified CD45 as a receptor for galectin-1 [29, 50, 73], although other glycoproteins such as CD43, CD7, CD2, CD3, and CD4 also function as specific T cell surface receptors [29, 52].

The role of CD45 in T cell apoptosis mediated by galectin-1 is controversial [74], because CD45 expression is not absolutely required for galectin-1-induced T cells. Walzel et al. demonstrated that CD45 is involved in galectin-1-induced apoptosis in $CD45^+$ Jurkat T cells and this activity is inhibited by asialofetuin and lactose. Neither induction of apoptosis by galectin-1 nor sugar inhibition of this induction was observed in $CD45^-$ cells [30]. Other reports on galectin-1-induced T cell apoptosis also demonstrated that a $CD45^-$ cell line is resistant to galectin-1, suggesting that CD45 is essential for galectin-1-induced T cell apoptosis [50].

Indeed, galectin-1-induced T cell apoptosis is regulated by the expression of specific glycosyltransferase enzymes such as core 2 β-1,6-*N*-

acetylglucosaminyltransferase (C2GnT), which creates a core 2 branch on O-glycans allowing the addition of lactosamine sequences [32]. Core 2 O-glycans have been described on both CD45 and CD43, two of the most abundant and highly glycosylated T cell surface glycoproteins. Nguyen et al. studied two murine T cell lines, $T200^-$ and $CD45^-$ (derived from a parental cell line BW5147), that lack CD45 and C2GnT, but are susceptible to galectin-1-induced T cell apoptosis. Therefore, CD45 expression is not absolutely required for galectin-1-induced T cells. Because the parental BW5147 cell line, which is $CD45^+$ $C2GnT^-$, is resistant to galectin-1-induced apoptosis as compared to cell lines $T200^-$ and $CD45^-$, it is suggested that CD45 actually inhibits galectin-1-induced apoptosis. The inhibitory effect of CD45 may involve tyrosine-phosphatase domains of CD45 because a phosphatase inhibitor restores sensitivity of BW5147 cells to galectin-1-induced apoptosis, but it has no effect on $T200^-$ cells that lack CD45. Expression of core 2 O-glycans on cell-surface glycoproteins of C2GnT-transfected $CD45^+$ cells dramatically increases cell susceptibility to galectin-1. No enhancement in galectin-1 susceptibility of $CD45^-$ cells expressing C2GnT was observed, compared with $CD45^-$ cells transfected only with vector, indicating that addition of core O-glycans to other galectin-1 receptors such as CD7 or CD43 does not affect this susceptibility. These data suggest that, in the absence of core 2 O-glycans, CD45 expression interferes with the proapoptotic galectin-1 signal delivered through other cell-surface receptors. It is important to note that C2GnT expression is not absolutely required for binding of soluble CD45 to galectin-1, as CD45 from BW5147 cells that do not express C2GnT bind to a galectin-1 affinity matrix. In this case, galectin-1 may bind to other CD45 glycans that bear lactosamine sequences. However, expression of C2GnT is not absolutely essential for galectin-1-induced T cell apoptosis because $T200^-$ and $CD45^-$ cells, which express neither CD45 nor C2GnT, are susceptible to galectin-1 [34].

Confocal microscopy showed clustering of surface CD45 in adherent T cells which express C2GnT: PhaR2.1 cells bound to thymic stromal cells have irregular membrane contours with CD45 localized unevenly on membrane blebs and colocalization of CD45 and galectin-1 [29]. Taken together, a model of segregation of CD45 has been proposed by Baum. After galectin-1 binding, CD7 and CD43 colocalize in clusters that are physically separate from CD3 and CD45 which localize to apoptotic membrane blebs. Segregation of CD45 has been proposed to block access of CD45 phosphatase domains, an effect that may be mimicked by phosphatase inhibitors (Figure 3) [32, 34]. Relative to PTP activity of CD45, interactions between CD45 and galectin-1 inhibit the PTP activity of CD45. CD45 dephosphorylates regulatory phosphotyrosyl residues at the C-terminus of src-family tyrosine kinases, in order for these kinases to function [75, 76]. When tyrosine phosphorylation of a src-tyrosine kinase called Lyn was evaluated before and after galectin-1 treatment, Lyn kinase activity was abolished with galectin-1 treatment [77].

N-glycans on CD45 are also essential for galectin-1-induced apoptosis of T cells, and SA6-Gal I sialyltransferase preferentially sialylates terminal galactose residues on N-glycans with the sequence SAα2,6Gal. ST6Gal I has been expressed in galectin-1-susceptible cells. Transfected clones express ST6Gal I and its product, α2,6-linked sialic acid, as demonstrated by binding of the plant lectin SNA which recognizes SAα2,6Gal. Galectin-1 binding to transfected cells is markedly reduced compared with the level of binding observed for control cells transfected with vector alone. Moreover, expression of ST6Gal I reduces susceptibility to galectin-1-induced T cell apoptosis. CD45, CD43, and CD7 have been examined for increased sialylation of N-glycans by SNA lectin binding, and only CD45 has shown increased binding. In summary, addition of SAα2,6Gal sequences to CD45 may be a mechanism to tune immune regulation by galectin-1 and to prevent galectin-1-induced T cell apoptosis of specific populations, i.e. those expressing high levels of SAα2,6Gal sequences such as mature medullary thymocytes. Addition of α2,6-linked sialic acid might mask LacNAc sequences, inhibiting galectin-1 binding to CD45, or might increase the negative charge, preventing close packing of CD45 [78]. Sialylation of CD45 also regulates homodimerization of CD45, which down-modulates CD45 PTP activity [79]. A branching enzyme for N-glycans, β-1,6-*N*-acetylglucosaminyl-transferase V (GNTV) also participates in regulating galectin-1-induced T cell apoptosis. GNTV is expressed throughout thymic maturation of T cells, but it is up-regulated with activation. However, GNTV is not essential for

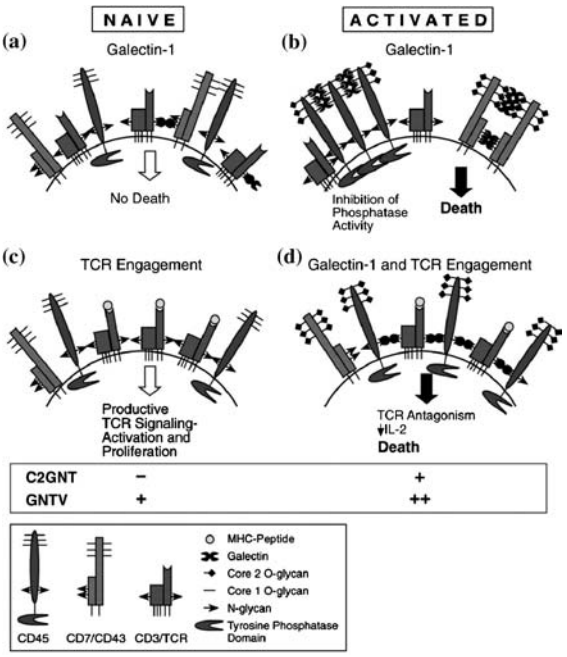

Figure 3. A detailed model of galectin-1 signaling events in peripheral T cells. (a) On naive T cells, galectin-1 binding does not result in the clustering of CD3, CD7, CD43, or CD45 or in cell apoptosis because the appropriate complement of glycosyltransferases is not expressed [core 2 β-1,6-*N*-acetylglucosaminyltransferase (C2GnT) is absent]. (b) On activated T cells expressing the required glycosyltransferases such as C2GnT, galectin-1 binding results in the segregation of CD45, CD3, CD7, and CD43 into discrete membrane microdomains to initiate the apoptosis cascade. Galectin-1 clustering of CD45 on activated T cells also results in inhibition of tyrosine phosphatase activity. (c) On naive T cells, TCR engagement results in TCR clustering, proliferation, and activation. (d) On activated T cells, concurrent TCR engagement and galectin-1 binding decreases TCR clustering, resulting in diminished TCR signaling and apoptosis. Taken from [32], Hernandez J.D. and Baum L.G.; Ah, sweet mystery of death! Galectins and control of cell fate. Glycobiology 12: 127–136, 2002, by permission of Oxford University Press.

apoptosis, because a GNTV$^-$ murine T cell line has been shown to be susceptible to galectin-1-induced apoptosis [32].

Galectin-1 in the extracellular matrix can kill adherent T cells, while no T cell apoptosis has been observed when galectin-1 is secreted from cells into the surrounding media. Extracellular matrix glycoproteins may thus serve three roles mediating galectin-1-triggered T cell apoptosis. First, the extracellular matrix provides a rich source of saccharide ligands on glycoproteins such as laminin, fibronectin, and vitronectin, which can contribute to maintaining galectin-1-carbohydrate binding activity. Second, matrix glycoproteins can concentrate galectin-1 secreted by surrounding cells to increase the fraction of galectin-1 molecules that are homodimers. Third, matrix presents galectin-1 to the T cell surface in a two-dimensional array, rather than in a three-dimensional space as when T cells encounter soluble galectin-1, which facilitates interaction of galectin-1 with large T cell surface glycoproteins such as CD43 and CD45 [33].

CD43

As mentioned before, CD43 has also been identified as a galectin-1 ligand in T cells. Confocal microscopy studies have demonstrated that galectin-1 treatment promotes CD45 segregation, virtually excluding CD43. To verify apoptosis of cells undergoing receptor redistribution, annexin V binding was evaluated. Annexin V only localized in large patches of CD45 on the surface of apoptotic cells treated with galectin-1. Indeed, before galectin-1 induction, CD7 colocalized with CD43, and after addition of galectin-1, CD43 and CD7 were still associated and moved into larger aggregates. These observations indicate that CD43 and CD7 may act as a complex during the delivery of galectin-1 apoptotic signals [29, 50].

Altered glycosylation of CD43 and CD45 has been observed in HIV-1 infection of T cells: decreased sialylation and increased expression of core 2 O-glycans have been demonstrated. Therefore, HIV-1 infection results in accumulation of exposed lactosamine residues, oligosaccharides recognized by galectin-1 on CD43 and CD45, promoting galectin-1 binding, receptor cross-linking, and segregation, critical steps in triggering apoptosis [80].

CD7

CD7 appears to have immunomodulatory activity, although ligands for CD7 have been difficult to find [80]. Galectin-1 susceptible T cells, including human and murine immature thymocytes, activated T cells, and T cell lines express CD7. Human CD7 possesses two N-linked glycans that are clustered on either side of an IgG fold, and several O-linked glycans are arranged closely in a mucin-like domain [81]. Specific glycoforms of CD7 may be important for CD7 signaling in galectin-1-induced T cell apoptosis.

To determine whether CD7 is necessary for galectin-1-induced T cell apoptosis, human CD7

has been expressed in a $CD7^-$ HUT78 T cell line, which is not susceptible to galectin-1-induced apoptosis. CD7 expression renders HUT78 cells susceptible to galectin-1, which is determined by both cell loss and phosphatidylserine exposure. Indeed, CD7 is necessary for galectin-1-induced T cell apoptosis via a Ca^{2+}-independent pathway. CD7 is present on human immature thymocytes and is up-regulated on activated T cells. Therefore, $CD7^+$ T cells may bind galectin-1 expressed by stromal or dendritic cells in tissues where T cells die, such as the thymus during T cell development or peripheral lymphoid organs following an immune response [36].

Lack of CD7 expression has been proposed to produce resistance to galectin-1-induced T cell apoptosis. CD7 expression is lost in a number of T cell-mediated inflammatory diseases, as well as in T cell neoplasms. Sézary syndrome is defined as a peripheral T cell neoplasm with tumor cells that are typically $CD4^+CD45RO^+CD45RA^-$ and $CD7^-$ lymphocytes. In fact, $CD7^-$ Sézary cell lines show essentially no galectin-1-induced T cell apoptosis, although these cells express abundant galectin-1. Thus, loss of CD7 expression and aberrant glycosylation can both contribute to decreased galectin-1 sensitivity and resistance to galectin-1-induced T cell apoptosis. Moreover, production of galectin-1 by Sézary cells may contribute to apoptotic elimination of reactive tumor-infiltrating T cells [57]. Peripheral blood mononuclear T cells from patients with Sézary syndrome harbor a substantial number of $CD7^-$ T cells, while the number of $CD4^+CD7^+$ and $CD8^+CD7^+$ T cells is relatively low. Activated $CD69^+CD4^+CD7^+$ T cells from healthy donors enter apoptosis whereas activated $CD69^+$ $CD4^+CD7^-$ T cells do not. Cells of the population $CD7^-$ from the peripheral blood of all Sézary syndrome patients are resistant to galectin-1-induced apoptosis. Thus, resistance of $CD7^-$ T cells to galectin-1-induced apoptosis may contribute to the accumulation of $CD7^-$ Sézary T cells during progression of the disease [58, 82].

CD2 and CD3

CD2 and CD3 have also been identified as galectin-1 receptors. Biotinylated galectin-1 binds to CD2 immunoprecipitated from Jurkat T cell lysates, and asialofetuin markedly reduces lectin binding. Similarly, Jurkat T cell lysates loaded onto a galectin-1-agarose column and eluted with lactose show that CD2 can be recovered and recognized by anti-CD2 mAbs. CD3 from Jurkat T cells immunoprecipitated with anti-CD3 mAb is also recognized by biotinylated galectin-1, and this interaction is blocked by asialofetuin, which contains a triantennary oligosaccharide with three Galβ1-4GlcNAc sequences and terminal nonreducing galactose residues [83], but is not blocked by lactose. Regarding signaling pathways, ligation of CD2 and CD3 by galectin-1 induces early events in T cell activation comparable with those elicited by CD2 and CD3 mAbs. In T cells, TCR/CD3 signaling involving increased concentrations of intracellular Ca^{2+} results in cellular activation, a process marked by proliferation, production of IL-2 and expression of other immune functions such as cytotoxicity [52]. In fact, galectin-1 modulates TCR signals leading to T cell apoptosis, inhibition of IL-2 production, and proliferation inhibition [38].

CD4

Rappl et al. found that CD4 is a galectin-1 receptor in the peripheral blood T cells of patients with Sézary syndrome and other T cell leukemias. The role of CD4 as a galectin-1 ligand remains obscure [58]. Galectin-1-induced T cell apoptosis does not require CD2, CD3, or CD4. However, these receptors may be important for mediating other biological effects of galectin-1 on T cells [32].

Actin

Information about actin as a galectin-1 receptor is controversial because the interaction was initially described as a protein–protein recognition. Joubert et al. identified actin as a receptor for human brain galectin-1. Periodate-treated brain extracts were treated with galectin-1, and immunoblotted. Periodate treatment is known to inactivate the lectin-binding activity of glycoproteins, because it produces a complete oxidation of sugars. A periodate-treated sample that binds to galectin-1 exhibited a single band of 43 kDa, which was identified as actin by means of mAb. Therefore, interaction between galectin-1 and actin from extracts after periodate treatment seems to depend on noncarbohydrate recognition [66]. Actin has also been shown to bind to galectin-1 in extracts from MOLT-4 T cells, but in a carbohydrate-dependent manner. Membrane proteins were

subjected to galectin-1 affinity chromatography, and analyzed by immunoblotting. A 45 kDa band was identified as actin with mAbs [29]. Further studies are necessary to elucidate the true nature of the interactions between galectin-1 and actin.

The human brain galectin-1-binding polypeptide HBGp82

A galectin-1 receptor has been isolated from human brain. It is called human brain galectin-1-binding polypeptide of 82 kDa (HBGp82) and is partially homologous to heat shock protein of 90 kDa (hsp90). After affinity chromatography, a 440 kDa protein was resolved in four polypeptides, although only the 82 kDa peptide showed galectin-1-binding ability. *N*-linked oligosaccharides of HBGp82 represent ~18% of the apparent MW. Homologies have been found between tryptic peptide sequences of HBGp82 and heat shock protein hsp 90 kDa, but the latter has an additional peptide domain. Differences between the two polypeptides may only reflect differences in some posttranslational modifications, i.e. release of N-terminal peptide and enzymatic glycosylation. A plant lectin-binding profile of oligosaccharides on HBGp82 has shown that terminal galactose and sialic acid residues are present, as demonstrated by the binding of *Ricinus communis* (RCA) and SNA lectins [65].

Cancer antigen CA125

CA125 is a mucin-like glycoprotein, and this gene has been cloned. Full-length CA125 is a type I transmembrane protein with a single membrane-spanning domain close to the C-terminus. The extracellular domain contains repeat structures that are likely to be heavily O-glycosylated [84]. Besides its putative nature as an integral membrane protein, soluble fragments of CA125 have been observed. Apparently, phosphorylation of the cytoplasmatic domain causes extracellular cleavage of the N-terminal domain, which results in the release of soluble fragments into the extracellular space [85, 86]. From immunological studies, the CA125 antigen is known to be present on the cell surface of ovarian cancer cells; however, it is also found in other carcinomas and in normal secretory tissues [87, 88].

CA125 was identified as a galectin-1 receptor by affinity chromatography followed by mass spectrometry of tryptic peptides. To analyze the role of O-glycosylation, cDNA encoding the C-terminal part of CA125 (CA125-C-TERM) was cloned and expressed in CHO cells, which were cultured in tunicamycin, a drug that inhibits N-glycosylation. CA125-C-TERM binding to galectin-1 was partially inhibited in the presence of tunicamycin, which suggests that recognition depends on O-linked-β-galactose-terminated oligosaccharide chains. Confocal microscopy shows that CA125-C-TERM enters the classical (i.e. ER/Golgi-dependent) secretory route as determined by colocalization of CA125-C-TERM and marker proteins of ER and Golgi. Therefore, CA125-C-TERM cell-surface expression is mediated by ER/Golgi-dependent secretory transport. CA125 expression by tumor cells concomitant with the increased cell-surface expression of galectin-1 remains correlative and, therefore, future studies are needed to elucidate whether CA125 expression has a direct impact on the non-classical export route of galectin-1 [64].

CD107a/b (LAMP-1 and LAMP-2)

N-glycosylated lysosome-associated membrane proteins 1 and 2 (LAMP-1 and LAMP-2), containing phospholipids, are also receptors for galectin-1. They are also expressed in plasma membrane, although at a lower level than in lysosomal membrane. Galectin-1 receptors from membrane extracts of CHO cells have been submitted to affinity columns with galectin-1 and immunoprecipitation with anti-LAMP-1, identifying LAMP-1 as a receptor. Similarly, in A121 human ovary carcinoma LAMP-1 has been identified as a galectin-1 receptor, which binds in a lactose-inhibitable fashion to the lectin. In immunofluorescence studies with polyclonal anti-LAMP antibodies, LAMP-1 and LAMP-2 were detected on the surface of ovary carcinoma cells [31, 59]. Glycoconjugate receptors were also isolated from KM12 colon carcinoma cells, in which the expression of galectin-1 was induced with sodium butyrate, an agent known as an inductor of cell differentiation. Immunoprecipitation of the eluate from Affi-gel-galectin-1 demonstrated that LAMP-1 and the carcinoembryonic antigen (CEA) are the main galectin-1 receptors [25].

CEA (carcinoembryonic antigen; CD66e)

CEA proteins are multifunctional and heavily glycosylated molecules expressed in different

epithelia, vessel endothelia, and hematopoietic cells. As mentioned above, CEA is one of the endogenous receptors for galectin-1 in KM12 human colon carcinoma cells [25]. CEA purified from colon carcinoma liver metastasis binds to galectin-1 in a carbohydrate-dependent manner. In fact, CEA has been shown to possess oligosaccharide chains containing LacNAc [89].

1B2 glycolipid

In previous studies, 1B2 glycolipid, expressed by nascent olfatory axons, has been described as a galectin-1 receptor [90–92]. In immunofluorescence studies, galectin-1, 1B2-glycolipid, and laminin were co-expressed within the outer layer of the olfatory bulb. The glycolipid was purified and submitted to binding studies that demonstrated that it interacts with galectin-1 in a lactose-inhibitable fashion. It is hypothesized that divalent galectin-1 may crosslink axonal surfaces to each other through 1B2 glycolipid to promote fasciculation and binding to laminin molecules [60].

GM1 ganglioside

Galectin-1 is a major receptor for the carbohydrate portion of ganglioside GM1 exposed on the surface of cultured human SK-N-MC neuroblastoma cells. Immunofluorescent monitoring of SK-N-MC cells clearly revealed the presence of galectin-1. When cells were exposed to a ganglioside sialidase inhibitor, a treatment which prevents the generation of GM1 ganglioside, a significant decrease of galectin-1 binding was detected [62]. Moreover, a GM1-albumin neoganglioprotein was synthesized and its specific binding to the cell surface was drastically reduced by galectin-1-specific antibodies. RT-PCR analysis performed in SK-N-MC neuroblastoma cells showed galectin-1 expression but no evidence of any marked up-regulation during cell growth [93].

A combined approach using NMR spectroscopic methods and computational molecular modeling has been used to define contact sites and conformation of GM1 in complex with galectin-1. The pentasaccharide of ganglioside GM1 presents two building blocks, the disaccharide Galβ1-3GalNAc and the central trisaccharide Neu5Acα2-3Galβ1-4Glc: these two galactose moieties in central and terminal positions are potential binding sites for galectin-1. Laser photo-chemically-induced dynamic polarization shows that GM1 binding to galectin-1 involves interaction between Trp68 and a galactose residue. Herein, the disaccharide Galβ1-3GalNAc, a weak galectin-1 inhibitor in other systems, constitutes the target site, and GalNAc contributes to the interaction energy in an even stronger manner than the terminal Gal residue. Notably, this weakly inhibitory disaccharide turns into a suitable ligand for galectin-1. GM1 disaccharide Galβ1-3GalNAc recognition by galectin-1, which otherwise prefers Galβ1-4GlcNAc determinants, teaches an instructive lesson about the versatile way the sugar code is read by a natural receptor [63].

Glycoprotein 90 K/MAC-2BP

Glycoprotein 90 K was originally described as a tumor-secreted antigen in the culture medium of human breast cancer cells. cDNA cloning revealed that each subunit contains a number of cysteines and N-glycosylation sites and that its N-terminal domain shares significant sequence similarity with an extracellular domain of the macrophage scavenger receptor [94]. Galectin-1 binds to 90 K in a dose-dependent fashion, and the binding is blocked by lactose, suggesting that the lectin recognizes carbohydrate moieties on the 90 K molecule. Glycoprotein 90 K has stimulatory activity on natural killer cells and lymphokine-activated killer cells, and this activity appears to be mediated through the induction of cytokines. Levels of 90 K in various cancer cells correlate inversely with their tumorigenicity, and tumors with 90 K-overexpression through gene transfection exhibit reduced growth rates, which may also be explained by the immunostimulatory effect of 90 K. Galectin-1 induces cell aggregation of the human melanoma cell line A375, which is blocked by a mAb against 90 K and by lactose. Thus, galectin-1 induces cell aggregation, at least partly, through binding to 90 K in a carbohydrate-dependent manner. There are two possible mechanisms by which 90 K mediates galectin-1 aggregation: (i) galectin-1 binds to 90 K displayed on the cell surface and, because of its bivalent nature, can bind to 90 K on different cells, resulting in homotypic cell adhesion; (ii) galectin-1 binds to 90 K secreted by melanoma cells and another cell-surface glycoconjugate simultaneously. Therefore, homotypic cell adhesion may be proposed with 90 K functioning as the aggregation factor and galectin-1 bridging 90 K and cell surface

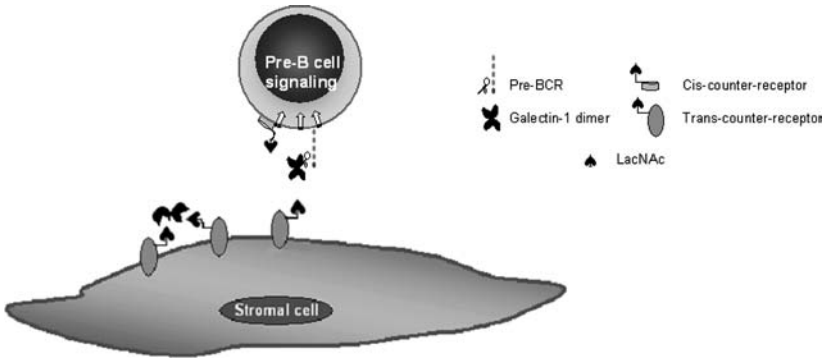

Figure 4. Model for the molecular organization of interactions between pre-B cells and bone marrow stromal cells (adapted from [42]). Galectin-1 secreted by bone marrow stromal cells is captured by cis- and trans-counter-receptors and pre-B cell receptor (pre-BCR). Galectin-1 binds to the λ-like chain of pre-BCR, in a carbohydrate-independent manner (protein-protein interaction). Galectin-1 is a supramolecular organizer of a 3D lattice that clusters together counter-receptors and pre-BCR. Interactions result in pre-BCR polarization and triggering of pre-BCR intracellular signaling. LacNAc: *N*-acetyl-lactosamine. λ-like chain of pre-BCR: ρ.

glycoconjugates, positively contributing to cancer metastasis. However, galectin-1-90 K interactions might also have a suppressive effect on tumor progression, as mentioned above [61].

Gastrointestinal mucin

Apical cell surface and secretory granules of mucous cells from stomach fundic glands were intensely stained by galectin-1 in histochemical studies of the gastrointestinal tract. The receptor implicated in galectin-1 binding is probably a mucin characteristic of granules from gastric glands and gastrointestinal ECM. Enterocytes microvilli from the duodenum to the ileum were also labeled by galectin-1 [19]. Mucin oligosaccharide chains specifically involved in galectin-1-binding have not been studied.

Protein–protein interactions

Pre-B cell receptor (Pre-BCR)

Galectin-1 from bone marrow stromal cells has been identified as a pre-B cell receptor (pre-BCR) ligand. Galectin-1 binding to pre-B cells leads to formation of a pre-BCR/galectin-1 lattice, polarized at the contact zone between pre-B and stromal cells, which results in pre-BCR triggering. During B cell differentiation, the Igμ chain synthesized by developing pre-BII cells must associate with surrogate light chain (SLC) made of λ-like (λ5 in mice) and VpreB to form a functional pre-BCR. Different recombinant pre-BCR have been constructed and expressed, such as scSLC (λ-like linked to VpreB by a linker peptide), two Fab-like (VH-CH1μ and scSLC), and a conventional Fab (VH-CH1μ and Igκ). Binding of Fab-like, conventional Fab, and different SLC recombinant proteins to a bone marrow-derived murine MS5.1 cell line was analyzed by flow cytometry: Fab-like and SLC proteins did bind to MS5.1 cell. Conventional Fab did not bind to stromal cells, suggesting that the SCL is the critical component in pre-BCR/stromal cell interactions. Stromal cell-derived SLC-binding proteins were identified by affinity chromatography which eluted a band of 14.5 kDa identified as galectin-1 by mass spectrometry. By surface plasmon resonance, nonglycosylated scSLC and λ-like were shown to interact efficiently with galectin-1, while VpreB failed to bind to immobilized galectin-1. Preincubation of scSLC with antiserum against the NH_2-terminal peptide of λ-like blocked the binding of scSLC to immobilized galectin-1, suggesting that the NH_2-terminal extra loop peptide of λ-like is involved in the binding. Galectin-1 was detected at the surface of stromal cells, and total inhibition of anti-galectin-1 and SLC binding to them was observed by lactose treatment. Lactose had no effect on direct scSLC-galectin-1 interactions, indicating that this binding takes place in a galectin-1 CRD-independent manner. Therefore, galectin-1 is anchored at the cell surface of stromal cells by glycosylated receptors, while SLC binding to the stromal cell surface is caused by the presence of membrane-associated galectin-1 in both stromal and pre-B cells. SLC-galectin-1 interactions were

analyzed by confocal microscopy after pre-B/stromal cell cocultures: galectin-1 and pre-BCR colocalized and were polarized at the contact area between the two cells, with a molecular surface organization presenting the characteristic of a synapse. Moreover, the relocalization process was inhibited by scSLC and TDG. In summary, a model has been proposed in which galectin-1 secreted by stromal cells is captured by pre-BCR and counter-receptors on B cells (cis-counter-receptors) and on stromal cells (trans-counter-receptors). Therefore, galectin-1 is a supramolecular organizer of a tridimensional lattice that clusters together glycoconjugate receptors and pre-BCR [42] (Figure 4).

Concluding remarks

Galectin-1 can be characterized as a truly matricellular protein which serves as an adapter between cells and ECM modulating cell-cell and cell-ECM adhesion, migration, proliferation, and apoptosis. Sometimes, 'pro- or anti-effects' of galectin-1 (i.e. adhesive/anti-adhesive; proliferative/anti-proliferative) are observed and these depend on the cell type, cell activation status, relative cell surface expression and glycosylation of particular receptors, and the monomer-dimer equilibrium of galectin-1. The existence of so many galectin-1 receptors in different cell types and the ECM is striking. The occurrence of at least 13 β-1,3(4)-galactosyltransferases gives reason to expect a fine-tuned regulation of synthesis and diversity of distinct determinants. Functional redundancy between galectin-1 receptors, most of them carrying LacNAc, may insure cross-talking and intracellular signaling in events that are essential for a defined biological function. Functional redundancy between galectins-1 and -3 has also been proposed because in one study, homozygous transgenic mice carrying a null mutation in the gene encoding for galectin-1 did not show apparent damage in development [95]. In general, some functions assigned to galectin-1 might be complemented by a redundant relative, i.e. galectin-3 or -7.

Understanding cross-linking interactions of galectin-1 with specific receptors, and signal transduction mechanisms might help to explain galectin-1 effects which might have therapeutic implications. For instance, galectin-1 may contribute to the immune privilege of tumors by modulating survival of effector T cells: tumor cells may impair T cell effector functions by secretion of galectin-1, tilting the balance towards an immunosuppressive environment at the tumor site [96]. When murine melanoma cells transfected with galectin-1 antisense cDNA are injected in mice, enhanced tumor rejection is observed as compared with mice injected with mock transfectants. Therefore, a potential molecular target for manipulation of T cell tolerance and apoptosis with profound implications for cancer immunotherapy might be possible in the near future.

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