Chapter 7
Galectins in Host–Pathogen Interactions: Structural, Functional and Evolutionary Aspects

Gerardo R. Vasta

Abstract Galectins are a family of ß-galactoside-binding lectins characterized by a unique sequence motif in the carbohydrate recognition domain, and evolutionary and structural conservation from fungi to invertebrates and vertebrates, including mammals. Their biological roles, initially understood as limited to recognition of endogenous ("self") carbohydrate ligands in embryogenesis and early development, dramatically expanded in later years by the discovery of their roles in tissue repair, cancer, adipogenesis, and regulation of immune homeostasis. In recent years, however, evidence has also accumulated to support the notion that galectins can bind ("non-self") glycans on the surface of potentially pathogenic microbes, and function as recognition and effector factors in innate immunity. Thus, this evidence has established a new paradigm by which galectins can function not only as pattern recognition receptors but also as effector factors, by binding to the microbial surface and inhibiting adhesion and/or entry into the host cell, directly killing the potential pathogen by disrupting its surface structures, or by promoting phagocytosis, encapsulation, autophagy, and pathogen clearance from circulation. Strikingly, some viruses, bacteria, and protistan parasites take advantage of the aforementioned recognition roles of the vector/host galectins, for successful attachment and invasion. These recent findings suggest that galectin-mediated innate immune recognition and effector mechanisms, which throughout evolution have remained effective for preventing or fighting viral, bacterial, and parasitic infection, have been "subverted" by certain pathogens by unique evolutionary adaptations of their surface glycome to gain host entry, and the acquisition of effective mechanisms to evade the host's immune responses.

Keywords Galectin · Infection · Recognition · Effector · Subversion of galectin function

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7.1 Introduction

Based on unique sequence motifs in the carbohydrate recognition domain (CRD) and their structural fold, lectins from protistans, fungi, invertebrates, and vertebrates have been classified into families that include galectins (formerly S-type lectins), C-type, F-type, X-type, R-type, P-type, and others (reviewed in Vasta and Ahmed 2008). Rigorous structural and phylogenetic analyses of individual lectin families among extant animal species have yielded critical information about their evolutionary history and biological role(s). For example, C- and F-type lectins and galectins are of ubiquitous taxonomic distribution, and highly diversified from the functional standpoint (Zelensky and Gready 2005; Vasta et al. 2012). However, while C- and F-type lectins are structurally heterogeneous lectin families (Vasta et al. 2017), galectins are generally considered as structurally and evolutionary conserved (Cooper 2002; Cummings et al. 2017).

Galectins constitute a family of ß-galactoside-binding proteins characterized by a canonical sequence motif in their CRDs, that are widely distributed in eukaryotic taxa, including fungi, sponges, and both invertebrates and vertebrates (Cooper 2002). Most galectins are non-glycosylated soluble proteins, although exceptions with transmembrane domains have been reported (Lipkowitz et al. 2004). Galectins are synthesized in the cytosol, and can be translocated into the nucleus where they can form part of the spliceosome (Cho and Cummings 1995a, b; Tsay et al. 1999) (Fig. 7.1a). Moreover, although galectins lack a typical secretion signal peptide, they can be secreted into the extracellular space by direct translocation across the plasma membrane (Cleves et al. 1996).

Although galectins have been evolutionarily conserved from a structural standpoint (Houzelstein et al. 2004), the galectin repertoire in any given mammalian species is diversified, and constituted by multiple galectin types, subtypes, and isoforms (Vasta and Ahmed 2008; Cooper 2002). The distinct domain organization of mammalian galectins has led to their classification into three major types: "proto", "chimera", and "tandem-repeat" (TR) (Hirabayashi and Kasai 1993) (Fig. 7.1b). The peptide subunits of the proto-type galectins contain a single CRD and can form non-covalently linked homodimers, with a dimerization equilibrium Kd of 7 μM (Cho and Cummings 1995a, b). The chimera galectins also house a single CRD, but display an N-terminal domain rich in proline and glycine that enables the formation of oligomers. Finally, TR galectins display two CRDs that are bridged by a functional linker peptide that can range from 5 to over 50 amino acids in length. Within each galectin type, up to 15 distinct galectin subtypes have been identified, numbered following the order of their discovery. Among these, galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15 are proto-type, galectin-3 is a chimera-type, and galectin-4, -6, -8, -9, and -12 are TR-type (Hirabayashi and Kasai 1993). In invertebrate species, galectins may have unique structural features, including multiple CRDs *per* polypeptide, such as the 4-CRD galectins described in clams, oysters, and snails (Tasumi and Vasta 2007; Feng et al. 2013, 2015a, b; Kurz et al.
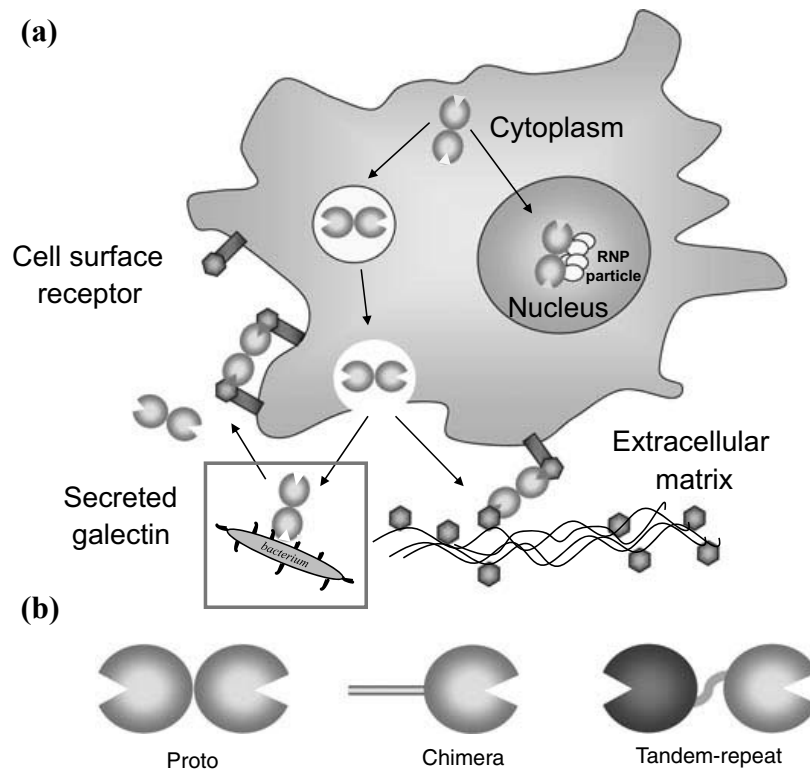
(a)

(b)

Fig. 7.1 Expression, secretion, and functions of galectins, and galectin types. **a** Galectins are synthesized in the cytosol, and can be translocated into the nucleus and interact with ribonucleo-protein (RNP) particles. Galectins can be transported and secreted to the extracellular space, where they interact with glycans in the extracellular matrix, bridge cell surface receptors, or recognize microbial glycans (red box). **b** Galectins are classified into three main types: proto, chimera, and tandem-repeat types. Proto-type galectins contain one carbohydrate-recognition domain (CRD) per subunit and are usually homodimers of non-covalently linked subunits (galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15). In contrast, chimera-type galectins are monomeric with a carboxy-terminal CRD, joined to an amino-terminal peptide that contains a collagen-like sequence rich in proline and glycine, and can oligomerize as trimers (galectin3). In the tandem-repeat galectins, two CRDs are covalently joined by a linker peptide (galectin-4, -6, -8, -9, and -12)

2013; Vasta et al. 2015), or the shrimp galectin MjGal that resembles a chimera-type galectin, although the CRD is located at the N-terminal end (Shi et al. 2014).

In addition to the three major galectin types, other galectin-related proteins that do not follow the canonical structure of the typical family members have been described. Among these, two notable exceptions have been reported to display transmembrane domains (Lipkowitz et al. 2004; Gorski et al. 2002). In addition, soluble galectin-like proteins, such as the eye lens galectin-related inter-fiber protein (GRIFIN), the galectin-related protein (GRP) (previously HSPC159; hematopoietic stem cell precursor), and the Charcot-Leyden crystals (galectin-10), have been identified, and their structural and functional aspects are characterized to various levels of detail (Su et al.

2018; Zhou et al. 2008; Ogden et al. 1998). Mammalian GRIFIN lacks carbohydrate-binding activity (Ogden et al. 1998), but the fish (Ahmed and Vasta 2008) and chicken (García Caballero et al. 2016) equivalents actively recognize ß-galactosides. Other unusual galectins are the sheep protein ovgal11 (galectin-11; Preston et al. 2015), and the proto-type galectin-13 ("pregnancy protein 13") which in solution forms a dimer stabilized by a disulfide bridge between Cys136 and Cys138 (Than et al. 1999, 2004).

7.2   The Galectin CRD: Structure and Carbohydrate-Binding Properties

The structure of galectin-1 (Gal1) in complex with *N*-acetyl-lactosamine (LacNAc) enabled the identification of the specific amino acid residues of the CRD that interact directly or indirectly, through water molecules and hydroxyl groups on the disaccharide, as well as the nature of the interactions (hydrogen bonds, electrostatic, and van der Waals) (Liao et al. 1994). The 135 amino acids-long polypeptide subunit of Gal1 folds into a β-sandwich that comprises two anti-parallel β-sheets of five and six strands each (S1–S6 and F1–F5). In the Gal1 dimer, the subunits are related by a two-fold rotational axis perpendicular to the plane of the β-sheets. The single carbohydrate recognition cleft is formed by three continuous concave strands (S4–S6) in which H^{44}, B^{46}, R^{48}, H^{52}, B^{61}, W^{68}, E^{71}, and R^{73} establish direct interactions with LacNAc, and determine the carbohydrate specificity of galectin-1 (Liao et al. 1994). The non-reducing terminal galactose ring is maintained in place by a hydrophobic interaction with W^{68}, while additional water-mediated interactions between H^{52}, D^{54}, and R^{73} and the nitrogen of the *N*-acetyl group determine the higher affinity for LacNAc over lactose. The dissociation constants of bovine Gal1 for lactose, LacNAc, and thiodigalactoside (TDG) measured by microcalorimetry are in the range of 10^{-5} M, with two binding sites per Gal1 dimer (Schwarz et al. 1998). The crystal structures of additional galectin types (human galectin-2, -3, and -7) in complex with mono- or disaccharides, or biantennary oligosaccharides were later resolved (Seetharaman et al. 1998; Caldararu et al. 2019; Si et al. 2016; Ramaswamy et al. 2015). More recently, the structures of the individual N- and C-terminal CRDs of TR galectins, such as galectins-4, -8, and -9, were resolved by either crystallization or NMR spectroscopy (reviewed in Di Lella et al. 2011).

In addition to the primary binding cleft in the galectin CRD described above, additional carbohydrate-binding areas (extended binding site) can enhance affinity for larger or more complex glycans. In the galectin-3 (Gal3) CRD, for example, the carbohydrate-binding site is shaped as a cleft open at both ends, exposing the GlcNAc of the LacNAc to the solvent (Seetharaman et al. 1998). This extended binding site in Gal3 results in increased affinity for polylactosamines and ABH blood group oligosaccharides [Fucα1, 2; GalNAcα1,3(Fucα1,2); and Galα1,3(Fucα1,2)] (Seetharaman et al. 1998). For TR galectins, the two CRDs are structurally similar

but show either different affinities for the same ligand such as galectin-4 (Gal4), or different fold and specificities altogether, such as galectin-8 (Krejcirikova et al. 2011; Ideo et al. 2003, 2011). For galectins from invertebrates, such as the *Caenorhabditis elegans* 16-kDa galectin and the oyster (*Crassostrea virginica*) galectins CvGal1 and CvGal2, their binding specificity for blood group oligosaccharides is determined by a shorter loop 4 in the primary binding cleft in the galectin CRD (Ahmed et al. 2002; Feng et al. 2013; Vasta et al. 2015).

The dimerization of proto-type galectins such as Gal1 is critical for their function in mediating interactions between cells, or cells and glycans in the extracellular matrix (ECM) (Gabius 1997). The binding of galectins to multivalent glycans on the cell surface may promote lattice formation, as supported by the structure of Gal1 complexed with a biantennary glycan, in which the ligand is cross-linked between two Gal1 dimers (Bourne et al. 1994). Similar interactions via the N terminus domain leading to the formation of oligomers (trimers and pentamers) have been proposed for Gal3 (Morris et al. 2004; Fortuna-Costa et al. 2014). For the vertebrate TR galectins and the multi-CRD galectins from invertebrates, the carbohydrate specificity of the CRDs present in the polypeptide is similar but not identical (Carlsson et al. 2007; Nagae et al. 2009; Vasta et al. 2015; Houzelstein et al. 2004; Krejcirikova et al. 2011). This is supported by the capacity of TR galectins to cross-link cells with different synthetic glycoconjugates (Tomizawa et al. 2005; Ideo et al. 2011). The multivalency of galectins attained by the presence of multiple distinct CRDs in a single subunit polypeptide as described above for the TR galectins, or by oligomerization of the galectin subunits, such as in the proto or chimera types, enables cross-linking of two or more cells, and adhesion of cells to glycosylated surfaces, as well as the formation of lattices at the cell surface that are critical for signaling or receptor endocytosis (Nabi et al. 2015; Kutzner et al. 2019; Garner and Baum 2008; Rabinovich et al. 2007a, b) (Fig. 7.2a).

The compact β-sandwich structure of the galectin CRD in the presence of bound ligand determines the resistance of Gal1 to protease activity (Liao et al. 1994), while the resistance to oxidative inactivation in the extracellular environment can be rationalized by changes in the dimerization equilibrium which are determined by the oxidation state of cysteine sulfhydryl groups (Stowell et al. 2009). Six key cysteine residues, some of which are located on the surface of the molecule on the face opposite to the CRD, are potentially susceptible to oxidation (DiLella et al. 2010; Liao et al. 1994; Lobsanov et al. 1993). Upon secretion to the oxidative extracellular environment, some of these cysteines establish intramolecular disulfide bonds, causing conformational changes that hinder dimerization (Lopez-Lucendo et al. 2004). Therefore, the oxidation state of cysteine sulfhydryl groups, the presence of carbohydrate ligand, and the dimerization equilibrium play critical roles in a dynamic interplay in which specific binding to glycan ligands enhances dimerization of Gal1, and reduces its sensitivity to oxidative inactivation (Stowell et al. 2009).

Fig. 7.2 Recognition of "self" and "non-self" glycans by galectins. **a** In the extracellular space, galectins form multivalent oligomers that cross-link cell surface glycoproteins and glycolipids, form microdomains and lattices, and activate signaling pathways. **b** Proto, chimera, and tandem-repeat galectins can function as pattern recognition receptors (PRRs) and establish trans-interactions with the host cell surface and microbial glycans
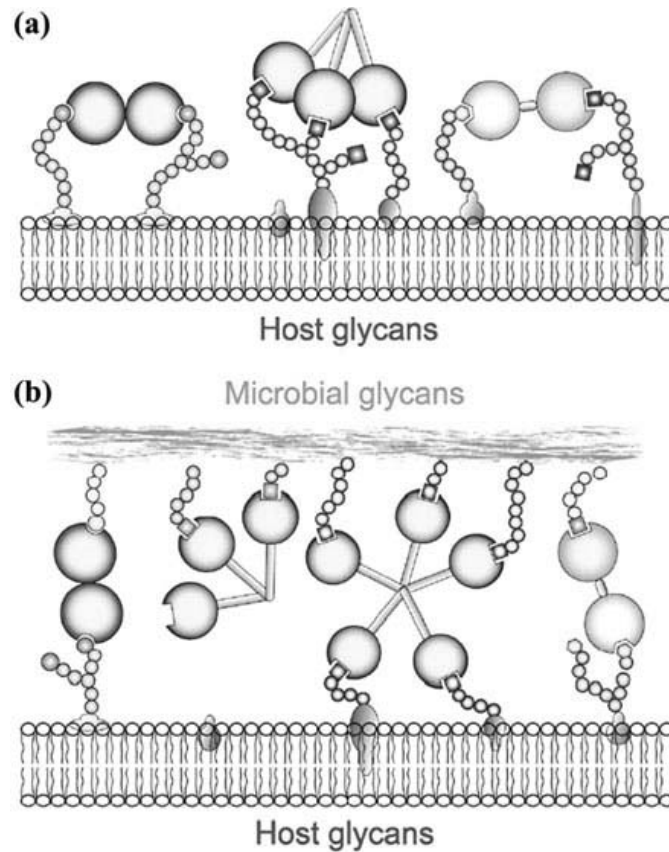
7.3 Functional Aspects: Recognition of Endogenous ("Self") Glycans

While proto and chimera type galectin subunits possess a single CRD, they can form oligomeric structures that can interact with and cross-link multivalent ligands, either soluble glycoproteins or glycolipids, and complex glycans on the cell surface or ECM with increased avidity (Dam and Brewer 2008; Cho and Cummings 1995a, b). TR galectins possess two CRDs in a single polypeptide, and can function similarly. The density and presentation of the cell surface glycans modulates affinity of the CRD–ligand interaction via negative cooperativity and thus, multivalent galectins can cross-link them into lattices that induce their clustering into lipid raft microdomains (Dam and Brewer 2008; Cho and Cummings 1995a, b; Nabi et al. 2015; Kutzner et al. 2019). Therefore, galectin–ligand interactions can modulate cell function by inducing reorganization or association of cell surface components, regulating turnover of endocytic receptors, and activating or attenuating signaling pathways (Dam and Brewer 2008; Garner and Baum 2008; Rabinovich et al. 2007a, b). Moreover, because the various galectin types and subtypes exhibit differences in carbohydrate specificity and affinity, and bind a broad range of glycans that display the requisite topologies, the galectin repertoire is endowed of substantial diversity in recognition properties. This, together with the galectins' unique tissue-specific expression, distribution, and local

concentrations, supports extensive functional diversification (Cooper 2002; Vasta and Ahmed 2008; Vasta 2009). Accordingly, the biological function of a particular galectin may vary among cells, tissues, and fluids depending on their concentration, the redox properties of the intra- or extracellular environments, and the availability and multivalent presentation of carbohydrate ligands at the cell surface or ECM (Vasta and Ahmed 2008; Vasta 2009).

The observation that galectins in chicken muscle were developmentally regulated suggested that their biological roles were related to embryogenesis and early development. The finding that these galectins preferentially recognized polylactosamines present on the myoblast surface and the ECM led to hypothesize that galectins promote myoblast fusion (reviewed in Cummings et al. 2017). Subsequent studies on murine Gal1 and Gal3 revealed key roles in the development of notochord, skeletal muscle, and central nervous system (Colnot et al. 1997, 2001; Georgiadis et al. 2007). More recently, the increasing availability of null mice for selected galectins enabled analyses of developmental phenotypes, and the elucidation of the unique functions of the multiple galectin types and subtypes in the complex mammalian galectin repertoires. In the past few years, genetically tractable models such as *Drosophila, C. elegans,* and zebrafish (*Danio rerio*) have become useful systems for addressing the biological roles of galectins (Pace et al. 2002; Ahmed et al. 2002; Vasta et al. 2004; Nemoto-Sasaki et al. 2008; Feng et al. 2015a, b; Nita-Lazar et al. 2016). For example, anti-sense knockdown approaches in zebrafish revealed key roles of galectins in early differentiation and development of the myotome (Ahmed et al. 2009a, b), and retinal repair and regeneration (Craig et al. 2010; Eastlake et al. 2017).

The multiple roles of galectins in cancer have been addressed with increasing interest over the past two decades (Reviewed in Méndez-Huergo et al. 2017). Melanoma, prostate, and ovarian cancer may overexpress galectin-1, -3, -7, -8 and -9, and in some cases their expression profiles can be associated with malignancy stage or metastatic potential (Blidner et al. 2015; Hill et al. 2010). Expression of Gal1 in the vascular endothelium promotes tumor angiogenesis, by a mechanism that involves binding of Gal1 to complex *N*-glycans on VEGF receptor 2 (VEGFR2) and activation of VEGF-like signaling (Croci et al. 2014). In the early stages of prostate adenocarcinoma, Gal3 expression can be silenced by promoter methylation (Ahmed et al. 2009a, b; Ahmed and Al Sadek 2015), but in later stages, together with its preferred ligand on the cell surface—the Thomsen-Friedenreich disaccharide (Galβ1,3GalNAc)—Gal3 has key roles in tumor angiogenesis, tumor-endothelial cell adhesion, metastasis, and evasion of immune surveillance by killing of activated T cells (Guha et al. 2013). Since the 1990s, the roles of galectins as regulators of both innate and adaptive immune homeostasis have firmly established and characterized in detail (Di Lella et al. 2011). Galectins are ubiquitously expressed and distributed in mammalian tissues, including most cells of the innate (dendritic cells, macrophages, mast cells, natural killer cells, gamma/delta T cells, and B-1 cells) and adaptive (activated B and T cells) immune system, and as in other cell types (Stowell et al. 2008; Rabinovich et al. 2007a, b). Immune challenge by viruses, bacteria, and eukaryotic parasites, however, can significantly alter their expression and secretion (Rabinovich et al. 2012;

Stowell et al. 2008). Endogenous glycans recognized by galectins on the surface of immune cells and other cell types include β-integrins, CD45, GM1, CD44, Tim3, MUC1, podoplanin, CD166, ABH-type oligosaccharides CD43, CD45, CD7, CD71, CD44, TIM3, CTLA4, MUC1, MUC16, and MerTK (Rabinovich and Toscano 2009; Guzman-Aranguez et al. 2009; Hirabayashi et al. 2002; Wu et al. 2002; Krzeminski et al. 2011; Zhu et al. 2005). Galectins can function as pro- or anti-inflammatory factors in innate immune responses. For example, Gal1 can block or attenuate signaling that promote leukocyte infiltration, migration, and recruitment (Stowell et al. 2008). Gal3 expression in epithelia, macrophages, and dendritic cells is upregulated during inflammation, and can promote macrophage recruitment and anti-microbial activity (Liu et al. 2012; Toledo et al. 2014). Galectin-9 (Gal9) functions as a chemoat-tractant for eosinophils and further promotes their activation, oxidative activity, and degranulation (Hirashima et al. 2002).

The functions of galectins as modulators of lymphocyte development and adaptive immune responses have been the focus of intense research in the past few years (Rabinovich et al. 2012; Liu et al. 2012). In the bone marrow and thymic compartments lymphocyte precursors interact with stromal cells via Gal1, which is critical to their development, selection, and migration to the periphery (Rossi et al. 2006). Additionally, Gal1 can either drive apoptosis or enhance proliferation of T-cells, depending on the microenvironment in which the exposure takes place, as well as the T cell developmental stage and activation status. For Gal3, however, pro- or anti-apoptotic effects on T-cells are determined by whether the exposure is extracellular or intracellular, respectively (Hsu and Liu 2008). Galectins can also modulate cytokine synthesis and secretion by T cells, and determine the Th1/Th2 balance of the immune (Rabinovich et al. 2012; Liu et al. 2012; Hsu and Liu 2008). More recently, it was shown that Gal1 can induce tolerogenic phenotypes in dendritic cells leading to expansion of regulatory T cells that can promote feto-maternal tolerance and suppress autoimmune neuroinflammation (Blois et al. 2007, 2019). Thus, it has become firmly established that galectins can modulate immune homeostasis with either beneficial or detrimental effects on pathological conditions that result from depressed or exacerbated immune function, such as cancer, inflammation, allergy, and autoimmune disorders (Rabinovich et al. 2012; Liu et al. 2012; Hsu and Liu 2008).

In recent years, associations of type 2 diabetes, obesity, and inflammation with adipogenic roles of galectin-12 (Gal12) and Gal3 have been identified and characterized in detail (Yang et al. 2011a, b, c; Hsu et al. 2018; Pejnovic et al. 2013; Pang et al. 2013). Gal12 was shown to function as a negative regulator of lipolysis and insulin sensitivity (Yang et al. 2011a, b, c; Hsu et al. 2018). In contrast, Gal3 null mice exhibit increased adiposity, systemic inflammation, dysregulated glucose metabolism, diabetes-associated kidney damage, and diet-induced atherogenesis (Pejnovic et al. 2013; Pang et al. 2013).

7.4 Role(s) of Galectins in Infection: Recognition of Exogenous ("Non-self") Glycans and Effector Functions

The recent finding that galectins can bind exogenous ("non-self") glycans on the surface of viruses, bacteria, protistan parasites, and fungi has led to a new paradigm about their potential roles in innate immunity as pattern recognition receptors (PRRs) (reviewed in Vasta 2009) (Fig. 7.2b). For example, Gal1 can bind to complex-type N-linked oligosaccharide on the HIV-1 gp120 envelope glycoprotein and to *Trichomonas vaginalis* lipophosphoglycan, whereas Gal3 recognizes both terminal and internal *N*-acetyllactosamine units in lipopolysaccharides from meningococcus (*Neisseria meningitidis*), gonococcus (*N. gonorrhoeae*), *Haemophilus influenzae*, and *Helicobacter pylori*, polysaccharide type XIV from pneumococcus (*Streptococcus pneumoniae*), LacdiNAc from *Schistosoma mansoni*, and oligomannans from *Candida albicans*. The lipophosphoglycan from *Leishmania major* is recognized by both Gal3 and Gal9, while Gal4 recognizes *Escherichia coli* strains that display blood group B oligosaccharides. The substantial diversity of the galectin repertoire(s), including the presence of isoforms, and unique specificity of each galectin subtype toward glycan ligands, suggests a broad recognition capacity for non-self carbohydrate moieties. In addition to the TR galectins that have at least two CRDs (up to four CRDs in invertebrate galectins; Vasta et al. 2015), the proto- and chimera-type galectins can form oligomers with two or more CRDs per molecule. Thus, all three galectin types are endowed with multivalent binding properties that enable not only the formation of lattices at the cell surface but also the capacity to cross-link cells. These properties enable galectins to participate in direct recognition of pathogens and parasites, as well as effector factors in downstream processes that lead to modulation of innate and adaptive immune responses. As will be discussed below, binding of host galectins to surface glycans either on the surface of pathogens and parasites or to their receptors on the host cell surface can lead to various outcomes beneficial to the host, including hindering or blocking their attachment to or entry into the host cells, direct killing of the pathogen, opsonization followed by phagocytosis and intracellular killing, encapsulation, or granuloma formation. In most cases, these galectin-mediated innate immune defense mechanisms take place simultaneously or subsequently upon challenge of any given pathogen. Furthermore, as multiple lectin families and other innate immune receptors such as TLRs are present in any single species, cooperative/synergic defense functions of galectins with other receptors have been described (Esteban et al. 2011; Jouault et al. 2006).

It should be noted that as invertebrates lack the adaptive immune response typical of vertebrates characterized by immunoglobulins and B and T cells, their galectins function as recognition and effector molecules as part of innate immune responses, exerting defense roles through the various mechanisms described below. In vertebrates, however, in addition to innate immune responses similar to those operative in invertebrates, several galectin-mediated key immunoregulatory functions (discussed in Sect. 7.3) triggered by the infectious challenge further contribute to maintain

immune homeostasis and to potentially develop an efficient and long-lasting adaptive immunity against the potential pathogen. The recognition of pathogens and parasites by galectins from both invertebrate and vertebrate hosts, however, can also lead to contrasting outcomes, either by promoting effective host defense mechanisms as discussed above, or by facilitating pathogen attachment, entry and infection of the host. In this regard, whether the recognition of pathogens and parasites by host galectins is beneficial to the host, or constitutes a pathogen's host attachment and entry strategies, can be interpreted as the outcome of the host–pathogen co-evolutionary process (Vasta 2009).

   1. Blocking of pathogen attachment to the host cell surface: Gal1 can bind to the envelope glycoprotein of influenza A virus (IAV) and reduce infection severity, possibly by hindering viral attachment to the cell surface sialylated glycan receptors (Yang et al. 2011a, b, c) (Fig. 7.3a). However, the detailed mechanisms involved have not been fully elucidated yet (reviewed in Machala et al. 2019). Similarly, Hattori et al. reported that Gal9 bound to the influenza A virus (PR8/H1N1 strain) and blocked virus attachment to the host cells in a lactose-specific manner (Hattori et al. 2013). Furthermore, in the experimentally IAV-infected mice, Gal9 expression was upregulated (Hattori et al. 2013), an observation consistent with the elevated levels of Gal9 observed in patients with IAV infection (Katoh et al. 2014). In contrast, Gal3 can function as an anti-viral galectin not by direct interactions as reported by
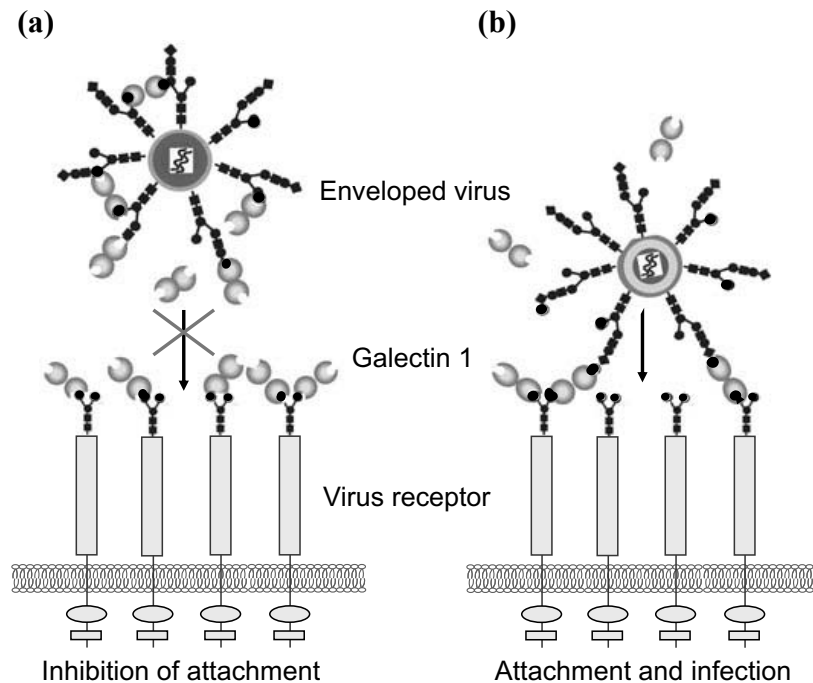

Fig. 7.3 Galectins can inhibit or facilitate attachment of enveloped viruses to the host cell surface. **a** Gal1 can block the attachment of viruses such as dengue by binding to and "coating" the envelope oligosaccharides (influenza A and IHNV), or by hindering access to the viral receptors (IHNV and dengue) on the host cell surface. **b** Gal1 can also cross-link the viral envelope to the host cell surface receptors facilitating attachment (Nipah and HIV)

Gal1 and Gal9, but by activating signaling via the JAK-STAT pathway, leading to an enhanced innate immune response (Jeon et al. 2010). A recent study reported that Gal1 can directly interact with dengue virus (DENV), a mosquito-transmitted enveloped RNA virus that can cause hemorrhagic fever. Gal1 directly binds to DENV and inhibits in vitro viral adhesion and internalization into host cells (Toledo et al. 2014). Prior exposure of the cells to dimeric Gal1, however, resulted in inhibition of viral attachment and infection that was greater than exposure of the virus alone. The role of Gal1 was also examined in vivo using Gal1 knockout mice, and demonstrated that the expression of endogenous Gal1 contributes to resistance against DENV infection (Toledo et al. 2014). During infection by Nipah virus (NiV) Gal1 can cross-link the N glycans displayed in the NiV envelope glycoproteins and reduce cell–cell fusion, thereby attenuating the pathophysiologic effects of NiV infection (Levroney et al. 2005). However, the beneficial effects of Gal1 in NiV infection are conditioned by the timing of the virus–galectin interaction, as during early stages of the viral exposure, Gal1 can enhance viral attachment and entry (Garner et al. 2015).

During the past few years we have used the zebrafish model to examine the roles of galectins in viral adhesion and entry by the infectious hematopoietic necrosis virus (IHNV), which is responsible for significant losses in both farmed and wild salmon and trout populations (Nita-Lazar et al. 2016). IHNV enters the host through the epithelial cells of the skin, the gills, and the gut, but the viral adhesion and entry mechanisms are not fully understood (Harmache et al. 2006). These epithelial cells express all three galectin types. They are secreted to the extracellular space, and are abundant in the mucus that coats the fish external surfaces. Results of the study showed that the zebrafish galectins Drgal1-L2 and Drgal3-L1 interact directly with the glycosylated envelope of IHNV significantly reducing viral attachment (Nita-Lazar et al. 2016). The structure of the complex of Drgal1-L2 with *N*-acetyl-D-lactosamine at 2.0 Å resolution together with models of Drgal3-L1 and the ectodomain of the IHNV glycoprotein provided insight into the mechanisms by which the binding of these galectins to the IHNV glycoprotein hinders the viral attachment (Ghosh et al. 2019). The IHNV envelope in glycoprotein is arranged in a honeycomb-like (hexagonal) arrangement of spikes, decorated by N-linked biantennary oligosaccharides that are also displayed by the host epithelial cells. Drgal1-L2 dimers can cross-link biantennary oligosaccharides from two spikes, thereby occluding two of the six co-receptor attachment sites on the surface of the virus, while the single C-terminal CRD of Drgal3-L1 oligomers can block three sites (Ghosh et al. 2019). Thus, the viral surface coverage by Drgal3-L1 is greater than that for Drgal1-L2 by a factor of 3/2 (Ghosh et al. 2019), which is consistent with the ratio of their inhibitory efficiency of 65%/40% determined in viral attachment experiments (Nita-Lazar et al. 2016). However, because the Drgal1-L2 and Drgal3-L1 secreted to the extracellular space also bind strongly to the fish epithelial cell surface, they can block IHNV cell surface receptors and hinder viral attachment via an alternative mechanism (Nita-Lazar et al. 2016). Furthermore, as the secreted galectins also bind strongly to the skin mucus glycans, a third defense mechanism mediated by these galectins would consist in cross-linking and immobilization of the virus in the mucus matrix, which is sloughed off periodically from the fish skin (Abernathy and Vasta, unpublished). A

similar mechanism has been proposed for galectin-4 (Gal4), which is expressed and secreted by gut epithelial cells. Once in the extracellular space, Gal4 would hinder attachment of *Bordetella pertussis* and *Helicobacter pylori* by binding to their gut epithelial cell surface receptors (Danielsen and Hansen 2006; Ideo et al. 2005).

In addition to blocking pathogen attachment to the host cell surface described above, galectins can inhibit interaction of the pathogen's virulence factors with host receptors. For example, the glycolipid-binding galectin Lec-8, which is strongly expressed in sections of the digestive tract of the nematode *Caenorhabditis elegans*, contributes to host defense against bacterial virulence by competitive binding to glycolipid receptors for the pore-forming toxin Cry5B from *Bacillus thuringiensis*, a nematocidal pathogen (Ideo et al. 2009). Interestingly, nematodes can also be susceptible to galectins from their fungal prey. For example, when the nematotoxic galectin CGL2 from the fungus *Coprinopsis cinerea* is ingested by *C. elegans*, it inhibits development and reproduction, and kills the nematode by specifically binding to a trisaccharide (Galβ1,4Fucα1,6GlcNAc) displayed on the nematode intestine, suggesting that fungal galectins constitute a defense mechanism against predator nematodes (Butschi et al. 2010). Similarly, the mammalian Gal2 can also suppress *C. elegans* development by binding to the Galβ1,4Fuc glycotope, a moiety that is also recognized on the parasitic nematodes of humans, such as *Ascaris*, *Nippostrongylus*, and *Brugia* spp., suggesting that it may contribute to anti-parasitic responses (Takeuchi et al. 2019). Gal11 may also contribute to host defense in ruminants against the gastrointestinal nematode parasite, *Haemonchus contortus* (Preston et al. 2015).

2. Direct killing of the pathogen: Some galectins have been reported to not only bind to, but also directly kill the pathogens, possibly by disrupting their cell surface integrity and their normal physiology. For example, the tandem repeat Gal4 and galectin-8 (Gal8), which are expressed in the human intestinal tract, can specifically recognize and kill *Escherichia coli* strains that display B-blood group oligosaccharides (BGB+ *E. coli*), while other *E. coli* strains or bacterial species are not affected (Stowell et al. 2010). The killing activity of both galectins is mediated by their C-terminal domains, and appears to be caused by compromising the integrity of the bacterial cell surface. Mutation of key residues in CRD revealed that the C-CRD mediates recognition of the BGB+ *E. coli* but does not affect its viability, while the N-CRD might be endowed with killing activity (Stowell et al. 2010).

More recently, Park et al. (2016) reported that recombinant Gal3 agglutinated *Helicobacter pylori* and displayed a potent bactericidal effect, as revealed by propidium iodide uptake and drastic morphological changes. The significance of this observation was buttressed by the higher bacterial loads in Gal3-deficient mice than in WT mice that had been experimentally infected with *H. pylori* via gastric tube, supporting the notion that Gal3 plays an important role in innate immunity to infection and gastric colonization by *H. pylori* (Park et al. 2016).

Although galectins can modulate phagocytosis and cytokine responses (IL-17, IL-23, TNFα, and others) to several fungal pathogens, including *Candida albicans* (Linden et al. 2013a, b), *Cryptococcus neoformans* (Almeida et al. 2017), *Histoplasma capsulatum* (Wu et al. 2013), and *Paracoccidioides brasiliensis* (Ruas et al.

2009), the galectin-mediated anti-fungal defense mechanisms may also include binding to and direct killing of the pathogen. The first two, *C. albicans* and *C. neoformans*, are particularly susceptible to direct recognition and fungicidal activity by galectins, although the mechanisms involved appear to be different. Gal3 recognizes and kills *Candida* species that display β1,2-linked oligomannans on the cell surface, but does not bind to *Candida* species or strains, or other fungal species such as *Saccharomyces cerevisiae* that lack these glycans. The binding of Gal3 to *C. albicans* oligomannans is intriguing, as it is well established that like other members of the galectin family, β-galactosyl moieties, particularly LacNAc, are the preferred ligands. Like for the bacteriocidal activity of Gal4 for *E. coli* described above (Stowell et al. 2010), changes in the *C. albicans* cell morphology upon exposure to Gal3 suggested damage to the cell membrane as the basis for the fungicidal activity, although the detailed mechanism has not been elucidated (Kohatsu et al. 2006). In contrast, exposure of *C. neoformans* to Gal3 causes lysis of the fungal extracellular vesicles which contain virulence factors and inhibits fungal growth (Almeida et al. 2017). The disruption of the extracellular vesicles by Gal3 prevents the efficient delivery of their contents to macrophages, and together with its fungistatic activity favors the host's anti-fungal immune response. The cell surface of *C. neoformans* appears to lack β-galactosides, or the β-oligomannosides recognized by Gal3 on *C. albicans*, and the ligand(s) recognized on the *C. neoformans* capsule or vesicles have not been identified so far (Almeida et al. 2017).

3. Opsonization, phagocytosis, encapsulation, and clearance of the pathogen: As mentioned above, galectins can bind to and promote phagocytosis of pathogens, which are killed by intracellular oxidative burst, and cleared from the internal milieu. An example that illustrates the opsonic role of galectins in host defense is the galectin MjGal from the kuruma shrimp, *Marsupenaeus japonicus* (Shi et al. 2014). This galectin is upregulated in circulating phagocytic cells (hemocytes) and hepatopancreas upon bacterial infection, and can bind to both gram-positive and gram-negative bacteria through the recognition of lipoteichoic acid or lipopolysaccharide, respectively. By also binding to the shrimp hemocyte surface, MjGal functions as an opsonin, cross-linking the potentially pathogenic bacteria to the hemocyte surface and promoting their phagocytosis and facilitating their clearance from circulation, as shown in vivo by RNA interference (Shi et al. 2014). There are several reports of galectins from both invertebrates and vertebrates which have been reported as opsonic, or at least promoting phagocytosis by more complex mechanisms that may involve additional receptors on the phagocytic cell surface. Gal3 can recognize surface glycans on the opportunistic fungal pathogens *Candida* spp. and not only exert direct fungicidal activity, as described above, but also promote phagocytosis by neutrophils (Linden et al. 2013a, b). In contrast, phagocytosis of *Candida* spp. by macrophages appears to require TLR2 (Jouault et al. 2006).

In addition to phagocytosis, a defense response to infectious challenge that is typical of invertebrates consists of encapsulation of potential pathogens with multiple layers of cells, particularly when the infectious particle is too large to be phagocytosed by a single cell. The encapsulated, immobilized pathogen can then be killed by diverse mechanisms, such as oxidative stress or melanization (Xia et al. 2018; Vazquez et al.

2009), which have been conserved in vertebrates from fish to mammals. Parasitic nematodes (*Cucullanus* spp.) in the abdominal cavity of the conger eel (*Conger myriaster*) are immobilized and encapsulated by layers of cells with the participation of congerins I and II, which are galectins that can bind to glycans on both the nematode surface as well as the encapsulating cells (Nakamura et al. 2012). In mammals, the formation of granulomas appears as an analogous defense mechanism in which galectins may play a key role for the immobilization of pathogen or parasites. Such is the case of recognition of LacdiNAc (GalNAcβ1,4-GlcNAc) of the eggs and parasite surface of the helminth *Schistosoma mansoni* by the host Gal3, and its potential role on the formation of liver granulomas (van den Berg et al. 2004). Gal3-null mice experimentally infected with *S. mansoni* showed reduced liver granulomas in both the acute and chronic phases, as compared with wild-type mice (Breuilh et al. 2007).

The recognition and clearance of both intra- and extracellular pathogens and parasites, however, can be accomplished by alternative mechanisms that lead to autophagy, and Gal8 has been shown to function as a key participant in this process. Epithelial cells infected with *Salmonella typhi*, *Listeria monocytogenes*, or *Shigella flexneri* exhibit damage to cytoplasmic endosomes and lysosomes (Thurston et al. 2012). It has been proposed that the damaged vacuolar membranes signal to recruit Gal8, which by binding to the exposed glycans activates autophagy (Thurston et al. 2012). A recent report describes a related anti-microbial mechanism by which *Helicobacter pylori* infection causes lysosomal damage in epithelial cells of the gastric epithelium (Li et al. 2019). Lysosome damage exposes membrane luminal *O*-glycans that are recognized by and induce the aggregation of cytoplasmic Gal8, which in turn increases autophagy activity in the *H. pylori*-infected cells (Li et al. 2019).

7.5 "Subversion" of the Galectins' Defense Functions by Pathogens and Parasites

In recent years, mounting experimental evidence has shown that some pathogens and parasites can "subvert" the defense roles of galectins from the host or invertebrate vector, and use galectin-mediated recognition to attach to, or to gain entry to their cells (Vasta 2009). These galectin-mediated interactions are clearly beneficial for the pathogen or parasite and can take place by various mechanisms. One fairly prevalent mechanism consists of the direct cross-linking of the pathogen to the host or vector cells by recognition of similar or different glycans on the cell surfaces, either by multivalent TR galectin or oligomeric proto- or chimera-type galectins. Another mechanism that can result as a downstream from the first consists of the downregulation of the host innate and adaptive immune response by galectin overexpression, secretion, or binding to host cell surfaces upon infectious challenge.

The participation of galectin interactions in the infection mechanisms of HIV (human immunodeficiency virus) has been widely reported (Ouellet et al. 2005;

Mercier et al. 2008). Gal1, which is abundant in organs that represent major reservoirs for HIV-1, such as the thymus and lymph nodes, promotes infection by HIV-1 by cross-linking the LacNAc moieties on the viral glycoprotein gp120 to its cellular glycoprotein receptor CD4 on T cells facilitating viral attachment, increasing viral residence time on the cell surface, and infection efficiency (Sato et al. 2012) (Fig. 7.3b). Additionally, Gal1 would enhance the uptake of the virus by macrophages acting as a soluble scavenger receptor (Sato et al. 2012). In contrast, Gal3, which is upregulated by the HIV Tat protein in several human cell lines, has no effect on HIV-1 attachment or entry (Fogel et al. 1999), but may exert anti-viral activity by inducing apoptosis of HIV-infected cells (Xue et al. 2017). Gal9 can also enhance HIV entry into T cells but via an indirect mechanism based on changes in the redox status of the cell surface (Bi et al. 2011). It is noteworthy that DC-SIGN, a C-type lectin, also facilitates HIV entry into dendritic cells (Sato et al. 2012), illustrating the diversity of protein–carbohydrate interactions that may participate in HIV infection (Ouellet et al. 2005; Mercier et al. 2008). By a mechanism similar to HIV, Gal1 can enhance and stabilize attachment of human T-cell lymphotropic virus (HTLV) to human T cells (Gauthier et al. 2008). Gal1 can also interact with capsid proteins of enteroviruses and promote infection of epithelial cells, although the mechanism(s) involved are not clear (Lee et al. 2015). In contrast, Gal3 would increase cell survival and inhibit apoptosis of the enterovirus-infected cells, facilitating release of the mature viral progeny (Huang et al. 2016). As indicated above for the Nipah virus, if Gal1 is present during the initial phase of virus exposure, it can enhance NiV attachment to the endothelial cell surface by bridging glycans on the viral envelope to host cell glycoproteins (Garner et al. 2015). A similar mechanism has been proposed for the interaction of Gal3 with the herpes simplex virus type 1 (HSV-1). Gal3 enhances HSV-1 attachment to and infection of human corneal keratinocytes, which can be ameliorated by the presence of MUC16, a soluble mucin that is secreted by the corneal cells and is strongly bound by Gal3 (Woodward et al. 2013). In the zebrafish-IHNV infection model described above, different members of the galectin repertoire can display opposite functions: while the proto-type galectin Drgal1-L2 and the chimera-type galectin DrGal3-L1 can inhibit viral adhesion to epithelial cells (Nita-Lazar et al. 2016), the TR galectin DrGal9-L1 can significantly promote viral adhesion and infection (Mancini and Vasta, unpublished).

Galectins can also promote the attachment of bacterial pathogens and facilitate infection, as shown in a murine model for influenza A infection and pneumococcal pneumonia revealed. Neuraminidases from both influenza A virus (IAV) and *Streptococcus pneumoniae* significantly desialylate the airway epithelial surface and modulate expression and release of Gal1 and Gal3 to the bronchoalveolar space (Nita-Lazar et al. 2015a). Studies on the human airway epithelial cell line A549 supported the observations made in the mouse model, and revealed that both Gal1 and Gal3 bind strongly to IAV and to *S. pneumoniae*. Furthermore, exposure of A549 cells to viral neuraminidase or influenza infection significantly increased galectin-mediated *S. pneumoniae* adhesion to the cell surface (Nita-Lazar et al. 2015a). Thus, these observations suggest that upon influenza infection, pneumococcal attachment

to the airway epithelial surface is enhanced by the activity of both viral and pneumo-coccal neuraminidases and the secreted host galectins, and possibly contributes to the greater susceptibility of influenza patients to secondary pneumonia (Nita-Lazar et al. 2015a). In addition, the study revealed that the binding of Gal1 and Gal3 to the epithelial cell surface downregulates the expression of SOCS1 and RIG1, and acti-vation of ERK, AKT, or JAK/STAT1 signaling pathways, leading to overexpression and release of pro-inflammatory cytokines (Nita-Lazar et al. 2015b). These results suggest that upon influenza infection, the binding of secreted Gal3 to the desialy-lated airway epithelia can severely dysregulate the immune response, leading to the frequently observed "cytokine storm" (Nita-Lazar et al. 2015b).

More recently, the role of Gal1 in infections by *Chlamydia trachomatis*, a highly prevalent sexually transmitted bacterium worldwide, was investigated in detail, revealing that Gal1 enhanced *C. trachomatis* attachment to cervical epithelial cells through recognition of bacterial glycoproteins and N-glycosylated host cell recep-tors, particularly platelet-derived growth factor receptor (PDGFR)β and β1/αVβ3 integrins (Lujan et al. 2018). Bacterial entry was facilitated by exposure to Gal1, mainly in its dimeric form, which favored interactions among *C. trachomatis*, and between the bacteria and host cells. In vivo studies in mice lacking Gal1 or complex branched *N*-glycans supported the in vitro results (Lujan et al. 2018).

Interactions of galectins with eukaryotic parasites can also promote their attach-ment and entry into epithelial or phagocytic cells, the latter by opsonic effect. The protozoan parasite *Trichomonas vaginalis* is the causative agent of a sexually trans-mitted human infection, which can effectively colonize cervical epithelial cells, pla-centa, endometrial and decidual tissue, as well as prostate (Okumura et al. 2008). Gal1 was identified as the receptor for *T. vaginalis* on epithelial cells: the parasite exhibits abundant lipophosphoglycan (LPG) with galactosyl moieties that are rec-ognized by Gal1 expressed by the epithelial cells, facilitating parasite attachment to the cervix linings (Okumura et al. 2008).

Taking advantage of galectin functions as opsonins, the protozoan parasite *Perkin-sus marinus*, a facultative intracellular parasite of the eastern oyster *Crassostrea virginica* is recognized via the oyster's 4-CRD galectins CvGal1 and CvGal2 that recognize and promote phagocytosis of the parasite by the circulating hemocytes (Fig. 7.4a). Both CvGal1 and CvGal2 recognize and bind to microalgae such as *Tetraselmis* spp., and potentially pathogenic bacterial species such as *Aeromonas* spp., *Carnobacterium* spp., *Streptococcus* spp., *Bacillus* spp., and *Vibrio* spp., sup-porting the notion that during filter-feeding, the oyster galectins contribute to feeding and anti-microbial defense in the gut lumen by promoting phagocytosis of both phy-toplankton and bacteria, killing by intracellular respiratory burst, and digesting them in the phagosome compartment by lysosomal enzymes. Therefore, *P. marinus* par-asites may have co-evolved with their host to subvert the defense and feeding roles of the oyster galectins. This would have taken place by adaptation of the parasite's glycocalyx to be competitively recognized by the hemocyte galectins over microal-gal food and microbial pathogens, and phagocytosed by the oyster hemocytes, where they inhibit respiratory burst and proliferate, eventually causing systemic infection
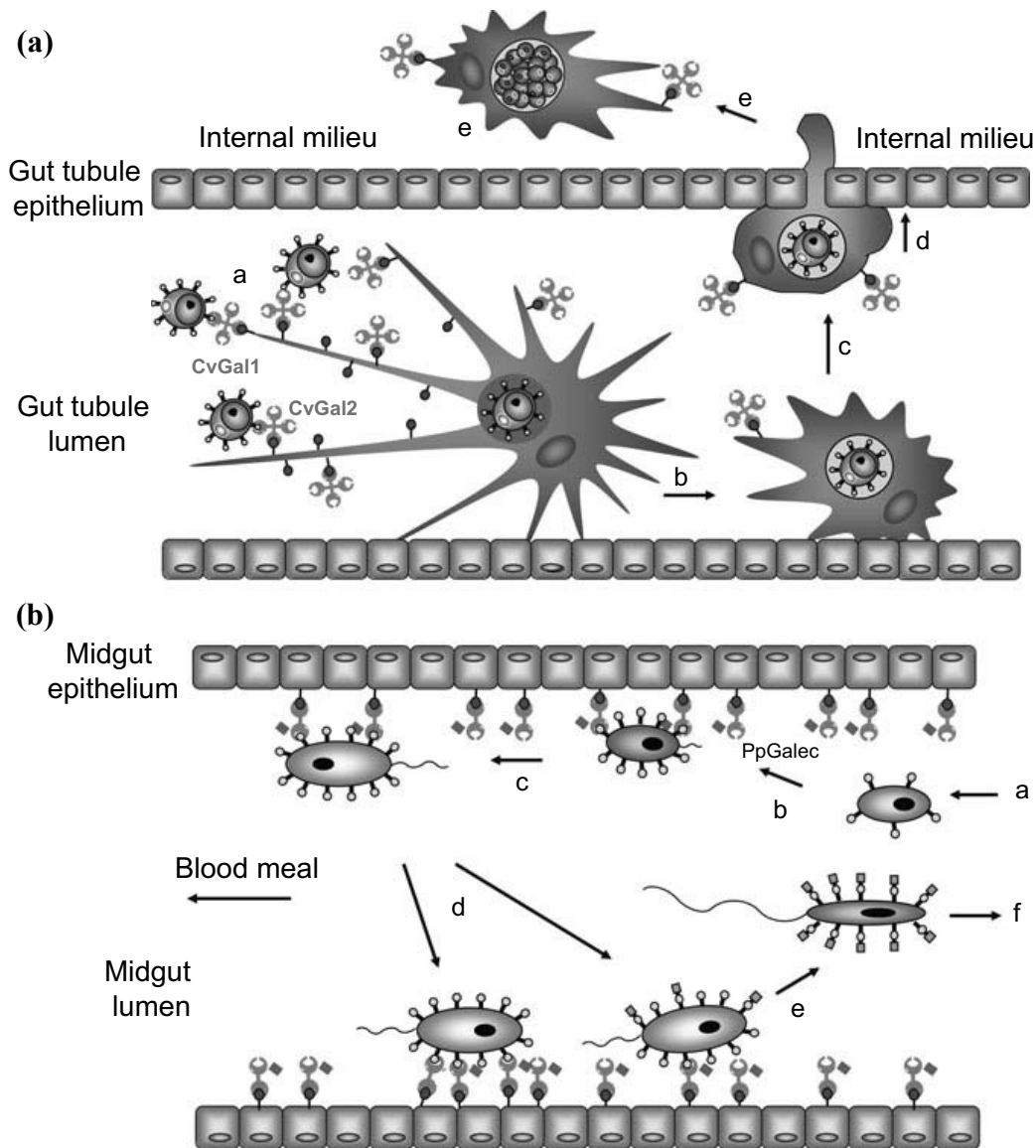
Fig. 7.4 Galectins from the host or vector can facilitate attachment of parasites. **a** The galectins CvGal1 and CvGal2 from the oyster (*Crassostrea virginica*) recognize and opsonize *Perkinsus marinus* trophozoites and promote their phagocytosis (a) by hemocytes (phagocytic cells in the oyster hemolymph and tissues). The phagocytosed *P. marinus* trophozoites avoid intracellular killing by inhibiting the hemocyte oxidative burst (b), and transmigrate through the gut epithelium (c, d) into the internal milieu, where it proliferates (e), causing systemic infection and eventually death of the oyster host. **b** The sandfly (*Phlebotomus papatasi*) TR galectin PpGalec recognizes and binds to poly-Gal(β1–3) side chains (light blue circles) on the lipophosphoglycan (LPG) of *Leishmania major* amastigotes (a) and facilitates attachment of the parasite to the midgut (b), preventing their excretion along with the digested blood meal. The amastigotes mature into promastigotes (c) and undergo numerous divisions (d) before differentiating into infective metacyclics (e, f). During metacyclogenesis, LPG can be capped with arabinose (pink squares), inhibiting recognition by PpGalec and allowing the free-swimming infective metacyclic promastigotes to detach from the sandfly midgut and migrate to the salivary glands for transmission to the mammalian host (Adapted from Vasta 2009)

and death of the oyster host (Tasumi and Vasta 2007; Feng et al. 2013, 2015a, b; Kurz et al. 2013; Vasta et al. 2015).

Eukaryotic parasites also take advantage of the binding properties of galectins not only from the host but also from invertebrate vectors to attach to their cell surfaces. These can be illustrated by interactions of *Leishmania* species with the midgut linings of their insect vectors prior to transmission to the vertebrate hosts (Fig. 7.4b). *Leishmania* amastigotes attach to the insect midgut epithelium via the sandfly galectin PpGalec that binds to the Gal(ß1-3) side chains on the *Leishmania* LPG, to prevent their excretion along with the digested bloodmeal (Kamhawi et al. 2004). During differentiation of the amastigotes into free-swimming infective metacyclics, modifications of their glycocalyx reduce binding by PpGalec, releasing flagellated metacyclics that migrate to the vector's salivary glands, and upon the sandfly's next feeding will infect a new vertebrate host (Kamhawi et al. 2004).

7.6 Conclusion

It is currently well established that by recognizing endogenous (self) cell surface and soluble glycans ligands, galectins participate in early development, tissue repair, regulate immune homeostasis, and contribute to trigger immune responses upon infectious challenge. As discussed throughout this review, as galectins can recognize exogenous (non-self) glycans present on the surface of virus, bacteria, and eukaryotic parasites, they are also considered bona fide pattern recognition receptors (PRRs). The capacity of galectins to recognize structural topologies ("patterns") by a galectin can be illustrated by the recognition with similar binding affinity of the disaccharides LacNAc and TDG by the proto-type galectin from the South American toad *Bufo arenarum* (Bianchet et al. 2000). The crystal structures of the *B. arenarum* galectin complexes with LacNAc and TDG allowed us to rationalize the structural basis of the PRR concept as it concerns the recognition of "self" and "non-self" carbohydrate moieties by galectins (Fig. 7.5). The structures revealed that the non-reducing terminal galactose, shared by both disaccharide ligands, shows identical interactions with the protein. The second moiety (GlcNAc in LacNAc and another galactose in TDG) establishes different contacts in the TDG and in the LacNAc complex, although the same number and quality of H-bonds are present in both cases (two direct and one water mediated to the protein) (Fig. 7.5b, c). This structural evidence reveals that the galectin is exquisitely specific, displaying a surprising structural plasticity to establish well-defined amino acid/sugar interactions for the two chemically distinct disaccharides, LacNAc and TDG, resulting in similar binding affinity for both (Bianchet et al. 2000).

Based on the Medzhitov and Janeway model (Medzhitov and Janeway 2002), however, PRRs such as the mannose-binding lectin (MBL) recognize pathogens via highly conserved microbial surface molecules of wide distribution such as lipopolysaccharide or peptidoglycan (pathogen- or microbe-associated molecular patterns, PAMPs or MAMPs), which are absent in the host (Fig. 7.6a). Although
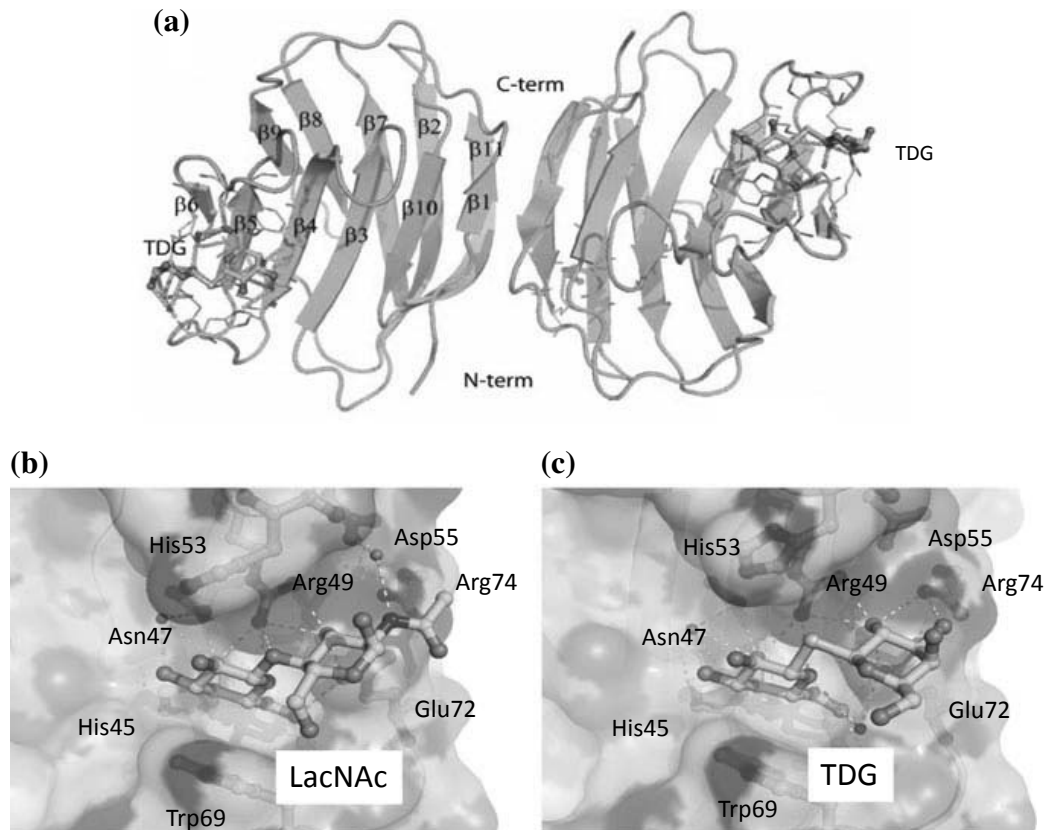
Fig. 7.5 Recognition of topologically similar disaccharides (LacNAc and TDG) by the *Bufo arenarum* proto-type galectin. **a** Structure of the dimer of *B. arenarum* galectin-thiodigalactoside (TDG) complex. Bound TDG disaccharides are shown on the binding clefts of both galectin subunits. **b** N-acetyllactosamine (LacNAc) bound to *B. arenarum* galectin (monomer B). The relevant CRD residues and the hydrogen bonds (dashed lines) to the sugar are shown. Carbon atoms are in white, oxygen in red; nitrogen in blue. **c** Thiodigalactoside (TDG) bound to *B. arenarum* galectin (monomer B). The relevant CRD residues and the hydrogen bonds (dashed lines) to the sugar are shown. Carbon atoms are in white, oxygen in red; sulfur in yellow; nitrogen in blue (Adapted from Bianchet et al. 2000)

this is true for some galectin ligands such as LacDiNAc in the helminth *Schistosoma mansoni*, the *Candida albicans* β1,2-linked oligomannans, and the *Perkinsus marinus* trophozoite surface glycans, most of the galectin carbohydrate ligands on the pathogen or parasite surface are identical or highly similar to those endogenous host ligands (Fig. 7.6b). Therefore, galectins do not rigorously fit the definition of PRRs, as they can recognize carbohydrate ligands that are displayed on both the host and the pathogen cell surface. Because TR galectins display in tandem-arrayed CRDs of similar but distinct specificity in a single polypeptide monomer, the binding and cross-linking of endogenous and exogenous glycans can be rationalized by the distinct properties of their binding sites. For other lectins such as the proto- and chimera-type galectins that display a single binding site per monomer, their capacity to recognize both endogenous and exogenous glycans through the same binding
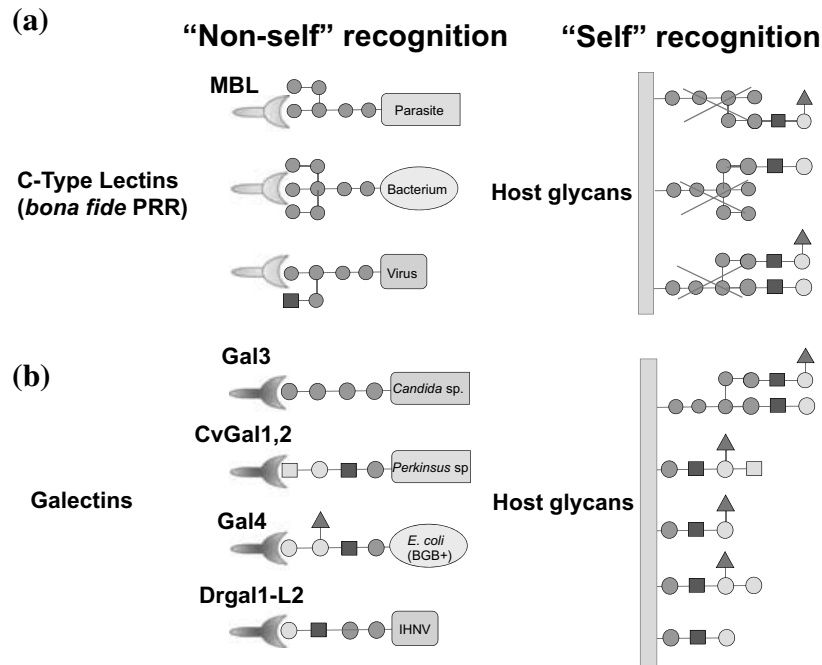
Fig. 7.6 Recognition of exogenous ("non-self") carbohydrate ligands on viruses, bacteria, parasites, and fungi by a *bona fide* pattern recognition receptor (PRR: mannose-binding lectin; MBL) and galectins: **a** The mannose-binding lectin (MBL) functions as a *bona fide* pattern recognition receptor (PRR) by recognizing oligosaccharides (microbe-associated molecular patterns, MAMPs, or pathogen-associated molecular patterns, PAMPs) on the surface of potential pathogens (parasites, bacteria, and viruses) that are either absent or unavailable for binding in the host cell surfaces. **b** Galectin-3 (Gal3) can function as true PRR by recognizing β1,2-linked oligomannans on the surface of *Candida albicans*, which are absent from the host, but can also recognize LacNAc and polylactosamines abundant on the host cell surface. Other galectins such as the oyster CvGal1 and CvGal2, Gal4 and the zebrafish Drgal1-L2 can recognize exogenous ("non-self") carbohydrate ligands on viruses, bacteria, parasites, and fungi that may be topologically similar (*Perkinsus marinus*, recognized by CvGal1 and CvGal2) or chemically identical (IHNV envelope oligosaccharides recognized by Drgal1-L2) to the host's endogenous ligands

site cannot be rigorously explained with the current evidence. This apparent paradox reveals our limited knowledge about the actual diversity in recognition of the host galectin repertoire and the structural and biophysical aspects of ligand binding preference (Dam and Brewer 2010). This lack of detailed information particularly concerns the diverse architectural display of the galectin ligands within the complex carbohydrate moieties of the host cell and the microbial surface, and how these features impact the affinity and avidity of the oligomeric galectins in the extracellular space. Furthermore, multiple factors pertaining to the local galectin concentrations and oligomerization, susceptibility to oxidative inactivation and proteolytic cleavage, and the biophysical properties of the microenvironment(s) in which the aforementioned interactions take place warrant further investigation (Vasta 2009).

From a functional point of view, recognition of glycans on the surface of virus, bacteria, and parasites by host galectins can lead to various and sometimes opposite outcomes that benefit either the host or the pathogen. For example, as discussed

above galectins can block pathogen attachment and infection, or directly kill or promote phagocytosis, intracellular killing, and clearance of the pathogen from the host, which are defense functions that seem to have arisen early in evolution. In contrast, galectins can also promote attachment of pathogens and parasites to the host cells, and facilitate infection. From an evolutionary standpoint, given that host galectins play key roles in early development, tissue repair, and regulation of immune homeostasis via recognition of "self" carbohydrate moieties, the substantial conservation of this lectin family from protistans, and fungi, to invertebrates and vertebrates supports the notion that strong functional constraints would prevent any dramatic evolutionary changes in galectin structure and carbohydrate specificity that would be detrimental to the host. Together with the well-recognized evolutionary plasticity of pathogens for colonization of host tissues, it seems plausible that pathogens would have rather evolved their surface glycosomes to mimic their hosts, and subvert the roles of host galectins by taking advantage of their carbohydrate-binding properties for attachment and entry into the host cells in a "Trojan horse" model (Vasta 2009).

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