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Review

Coupling pathogen recognition to innate immunity through glycan-dependent mechanisms

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ABSTRACT

Innate immune cells have evolved to sense microbial pathogens through pattern recognition receptors (PRRs), which interact with conserved pathogen-associated molecular patterns (PAMPs) to convey microbial information into immune cell signaling and activation events. PRRs also recognize endogenous damage-associated molecular patterns (DAMPs), including alarmins released during microbial invasion, initiation of autoimmune inflammation or tumor growth. In spite of the well-established role of Toll-like receptors (TLRs) in mediating these recognition events, compelling evidence supports a central function for lectin–glycan interactions in promoting microbial sensing and evoking immune responses. Here we discuss the role of glycans and lectins (particularly galectins) in mediating microbial recognition and initiation of innate immune responses. Both microbes and host cells are sources of glycan-containing information which is, at least in part, decoded by endogenous glycan-binding proteins or lectins, including C-type lectins, siglecs and galectins. Although C-type lectins and siglecs can recognize microbial glycans when expressed on the cell surface of innate immune cells, galectins mainly function as soluble mediators that bridge microbial or host glycans to amplify or attenuate immune responses. Galectins are widely expressed in host cells and play important roles during different steps of infection such as pathogen recognition, invasion and resolution. In addition, recent studies report the presence of conserved 'galectin-like' domains in certain pathogens including helminths and protistan parasites, suggesting that they could also serve as potential virulence factors that influence the outcome and course of infection. Understanding the role of lectin–glycan interactions and the relevance of PRR or PAMP glycosylation in microbial recognition might contribute to the design of novel prophylactic and therapeutic strategies.

Contents

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1. Introduction

The innate immune system has evolved to specifically recognize and eradicate potentially dangerous microorganisms. This intricate cell network, which includes monocytes, macrophages, mast cells, polymorphonuclear neutrophils and dendritic cells (DCs), is in charge of sensing pathogen-associated molecular patterns (PAMPs) through germ line-encoded receptors, called pattern recognition receptors (PRRs) to elicit immune cell signaling [1,2]. Additionally, PRRs can also recognize endogenous ligands termed 'damage-associated molecular patterns (DAMPs)', which are released by host cells into extracellular spaces upon tissue damage incited by microbial insults, acute inflammation or tumor growth [3,4]. While PAMPs include lipids, lipoproteins, proteins and nucleic acids derived from a wide range of microbes such as bacteria, viruses, parasites and fungi [5], the list of DAMPs is getting longer and include heat shock proteins, high mobility group protein-1 (HMGB1); interleukin-1(IL-1)α, defensins and annexins [4]. In addition, as host cells as well as virtually all bacteria, parasites, fungi and viruses, bear cell surface glycan structures, it has been proposed that these molecules may serve to display information critical for host–microbe communication. This information is, at least in part, decoded by endogenous glycan-binding proteins or lectins [5], which act as receptors that could be secreted or expressed on the cell surface of immune cells. Lectin families, including C-type lectins, siglecs and galectins, contain one or more carbohydrate-recognition domains (CRDs) responsible for sugar recognition. The glycan-binding specificity and cell type-specific expression of these receptors are well documented. Whereas galectins are secreted, most C-type lectins and all known siglecs are membrane-bound proteins [5]. Although galectins are synthesized and stored in the cytoplasmic compartment, they are either passively released by dying cells or actively secreted by inflammatory activated cells upon pathogen-driven tissue damage, through still poorly understood non-classical pathways. Due to the similarities with other DAMPs or alarmins, including cytosolic localization, release upon tissue damage and non-classical externalization, galectins have been postulated to act as potential DAMPs [4]. This attractive hypothesis merits careful consideration using *in vitro* and *in vivo* approaches.

According to subcellular compartmentalization, PRRs have been categorized in cytosolic, transmembrane and secreted PRRs [5,6]. Some lectins play essential roles as recognition molecules either by acting as transmembrane PRRs (C-type lectin receptors) or following secretion by innate immune cells. The group of secreted PRRs includes collectins, ficolins, pentraxins and galectins which are secreted from damaged tissues upon infection [4]. Accumulating evidence indicates that some members of the galectin family can bind to microbial glycans that are specific to pathogens or shared with host cells and such recognition can trigger innate immune responses, to either promote pathogen clearance or establish chronic infections [4]. The transmembrane PRRs include the Toll-like receptor (TLR) and the C-type lectin receptor (CLR) families. In mammals TLRs are either expressed on the plasma membrane or in endosomal/lysosomal compartments [6]. Cell-surface TLRs recognize PAMPs that are accessible on the cell surface, such as lipopolysaccharides (LPS) of Gram-negative bacteria (TLR4), lipoteichoic acids of Gram-positive bacteria and bacterial lipoproteins (TLR1/TLR2 and TLR2/TLR6) and flagellin (TLR5), whereas endosomal TLRs detect mainly nucleic acids, such as double-stranded RNA (dsRNA) (TLR3), single-stranded RNA (ssRNA) (TLR7), and dsDNA (TLR9). Expression of TLRs is cell-type specific, allowing assignment of different responsibilities to various cell types [5]. On the other hand, a variety of CLRs expressed by immune cells can distinguish among several pathogens and elicit signaling pathways to initiate immune responses. These include DC-SIGN that recognizes mannose and fucose-containing glycans, MGL which is specific for terminal GalNAc structures and Dectin-1 and -2 which recognize β-glucans and mannans respectively on fungal cell walls [5]. In this review we highlight recent advances on the role of lectin–glycan interactions in coupling pathogen recognition to innate immune responses. Particularly we discuss the roles of galectins, especially host-derived galectin-1 (Gal-1) and 'galectin-like' proteins present in microorganisms, in the control of microbial infection. Finally, to integrate TLR signaling and glycan recognition, we also discuss the relevance of glycosylation in TLR structure, trafficking and function.

2. Host–microbe interactions governed by protein–carbohydrate interactions

Cell surface lectins, including CLRs and siglecs, are crucial for recognition of specific glycans on PAMPs and initiation of immune responses [7,8]. In this regard, lectins are responsible of conveying information encoded by pathogen glycans to cellular responses such as immune signaling, antigen processing and presentation, cytokine production, activation and differentiation [9]. However, lectins are also encoded in the genome of diverse species including viruses, bacteria, fungi, protozoan parasites and helminths [7–11]. Thus, the complexity of host–pathogen interactions is amplified by microbial or host glycosyltransferases and glycosidases which act sequentially to modify glycan structures on the surface of host cells [5,12]. Clear examples are represented by pathogen sialidases which dramatically alter cell surface glycosylation to influence microbial invasion or shift immune responses. These range from neuraminidases expressed by viruses such as influenza virus to sialidases from bacteria including *Streptococcus pneumoniae* [13,14] and the transialidase from the protozoan parasite *Trypanosoma cruzi* [14].

The molecular mechanisms underlying lectin-mediated glycan recognition are still poorly understood. Recent studies showed that epitope density, number of *N*- and *O*-glycans, as well as the nature of glycoprotein receptors play central roles in lectin binding, cross-linking activity and effector functions [15]. Several authors suggested a different mechanism for the enhanced avidity and cross-linking activity of lectins [16]. Interestingly, a lectin molecule can "bind and jump" from one terminal oligosaccharide to another before its complete dissociation from the glycoprotein backbone [16,17]. Under this scenario, the affinity of a lectin increases with the number of glycan epitopes and spacing of the epitopes (density) in a given glycoprotein [15]. A second mechanism proposes that a lectin possesses multiple CRDs in a common direction, thereby enabling simultaneous interactions between multivalent glycans and clustered epitopes. [18]. This effect, which may lead to the formation of particular arrays of multivalent lectins and glycans, termed 'lattices' [15], suggests the potential role of galectins as soluble PRRs capable of bringing microbes close to the interface of host cells and modulating innate immune signaling.

3. Galectins: the 'sweet' art of coupling extracellular signals to intracellular responses

Galectins constitute a family of evolutionarily conserved lectins which share sequence similarities on the carbohydrate recognition domain (CRD), affinity for β-galactosides and absence of a signal peptide required for classical secretion [19,20]. Yet, galectins are soluble proteins that are externalized through an atypical secretory pathway and function mainly outside the cells to recognize microbial glycans and mediate cellular communication. Although galectins are in general soluble non-glycosylated proteins, members with transmembrane domains have also been described [21,22]. Interestingly, galectins were initially identified by their ability to mediate cellular processes such as embryogenesis and tissue architecture [23], although studies performed in the past

decade were critical at identifying the pivotal roles of galectins in cancer, immunity and inflammation [24,25].

Not surprisingly, 'galectin-like' structures are found in both mammalian and non-mammalian species. Mammalian galectins can be classified as '*proto-type' galectins* which are composed of at least one CRD that can dimerize, (ej., Gal-1, -2, -5, -7, -10, -13, -14, -15), '*chimera-type' galectins* (Gal-3) which share a carboxy-terminal CRD linked to an amino-terminal peptide and '*tandem repeat' galectins* (Gal-4, -6, -8, -9 and -12) which have two CRDs linked by a functional linker peptide [19,20,26]. In general, galectins recognize terminal *N*-acetyllactosamine (LacNAc) sequences on cell surface glycoproteins. This structure can be presented in both *N*- and *O*-glycans as multiple repetitive units of poly-LacNAc [27]. However, recent evidence underscored glycan-binding preferences among different members of the galectin family (extensively revised in [25]), which could account for divergences in functional activity of each individual galectin. *N*-glycan branching, multiplicity of LacNAc residues and modification of terminal saccharides (sialylation or fucosylation) are critical factors that contribute to variations in glycan recognition [27–29]. Regarding cellular distribution, some galectins such as Gal-1 and Gal-3 are present in virtually all immune cells although some other members of the family, including Gal-7, Gal-10 and Gal-12 have a more restricted localization. While galectins can act intracellularly by interacting with cell signaling cascades, most of them function extracellularly through direct engagement of specific cell surface glycoconjugates either by forming multivalent 'lattices' or by traditional ligand–receptor interactions [5,24,30–33]. Given the existence of several review articles on the structure and function of different members of the galectin family, in the next section, we will focus our attention on biochemical and functional features of Gal-1 which make this lectin a highly attractive candidate for modulating host–pathogen interactions, eliciting signaling processes and promoting or resolving tissue inflammation during microbial invasion.

4. Galectin-1: a multifunctional regulator

Gal-1 is a 'proto-type' member of the galectin family widely distributed in different tissues including innate and adaptive immune compartments and sites of tissue inflammation [34]. In addition, expression of this endogenous lectin is abundant in immune privileged sites such as placenta [35–37], testis [38,39] and retina [40,41] and is significantly altered (up- or down-regulated) during several pathological conditions, including cancer, infections, and autoimmunity [42–45]. This regulated secretion and preferential localization prompted many investigators to study the potential role for Gal-1 in the control of leukocyte trafficking, immune tolerance and homeostasis.

4.1. Gal-1 distribution and function on innate immune cells

In spite of significant advances in elucidating the role of Gal-1 within the T- and B-cell compartments, the effects of this protein among innate immune cells are poorly understood [45]. These effects are particularly interesting in terms of the potential role of lectin–glycan lattices at the interface of innate and adaptive immune responses. In this regard, *in vitro* studies showed that Gal-1 functions to limit neutrophil recruitment to TNF-treated human endothelia, a property that may underline its inhibitory effects in acute inflammation. In *in vivo* studies, leukocyte trafficking and transmigration were considerably increased in the cremasteric circulation of Gal-1 null mice challenged with IL-1β [46]. Moreover, Stowell and colleagues (2009) showed that Gal-1 induces phosphatidylserine exposure in human neutrophils independently of alterations in mitochondrial potential, caspase activation, or cell death, thus favoring phagocytic removal of neutrophils [47]. These results suggested a novel immunoregulatory role of Gal-1 in facilitating leukocyte turnover independently of apoptosis, a process that was named as '*preaparesis*' [48]. In addition, *in vitro* studies demonstrated that recombinant human Gal-1 inhibits neutrophil chemotaxis and transendothelial migration. *In vivo*, hrGal-1 limits IL-1β-induced neutrophil recruitment into the mouse peritoneal cavity [49]. These data showed previously unrecognized functions for Gal-1 in the control of leukocyte trafficking and extravasation.

In keeping with its anti-inflammatory functions, *in vivo* study in rats demonstrated that Gal-1 inhibits acute inflammation induced by bee venom phospholipase A2 (PLA2). Histopathological studies showed a clear reduction of the inflammatory process when Gal-1 was injected before PLA2. The anti-inflammatory effect was also assessed *in vitro*, showing that Gal-1 treatment reduced prostaglandin E2 secretion and arachidonic acid release from stimulated peritoneal macrophages [50]. Moreover, pretreatment of rat macrophages with Gal-1 induced a dose and time-dependent inhibition of lipopolysaccharide-induced nitric oxide (NO) production, while favored the balance toward activation of L-arginase, the alternative metabolic pathway of L-arginine. Inhibition of NO production was not the result of increased macrophage apoptosis because addition of this β-galactoside-binding protein to macrophages under the same experimental conditions did not affect the apoptotic threshold of these cells. [51]. On the other hand, *in vitro* exposure to Gal-1 differentially regulated constitutive and inducible FcγRI expression on human monocytes and FcγRI-dependent phagocytosis. In addition, Gal-1 inhibited IFN-γ-induced MHC class II (MHC-II) expression and MHC-II-dependent antigen presentation in a dose-dependent manner. These regulatory effects were also evident in mouse macrophages. Investigation of the mechanisms involved in these functions showed that Gal-1 does not affect survival of human monocytes, but rather influences FcγRI- and MHC-II-dependent functions through active mechanisms involving modulation of an ERK1/2-dependent pathway [52].

Recent findings from our laboratory identified an immunoregulatory circuit by which Gal-1 favors the differentiation of regulatory DCs, which promotes T cell tolerance through mechanisms involving IL-27 and IL-10 [53]. Using several experimental approaches, we found that DCs differentiated in a Gal-1-enriched microenvironment acquired IL-27-dependent regulatory function, promoted IL-10-mediated T cell tolerance and suppressed autoimmune neuroinflammation [54]. Consistent with these findings, at the fetomaternal interface Gal-1 favored the recruitment of a population of uterine innate DCs with a regulatory cell-surface phenotype [55].

In contrast to the considerable information on the role of Gal-3 in mast cell physiology [45], scarce information is available on the role of Gal-1 on this cell type [56]. In this regard, an indirect inhibitory role of Gal-1 on mast degranulation has been reported [51]. This inhibitory effect did not appear to be associated with an induction of cell death as recombinant Gal-1 did not trigger mast cell apoptosis [45,56].

Thus, several studies demonstrated that Gal-1 and other galectin family members play a wide variety of roles in innate immunity cells and pathogens may actually utilize galectins as mechanisms of host invasion (Fig. 1).

4.2. Gal-1 and infection

Galectins can recognize glycans on different microorganisms including viruses, bacteria, fungi, protozoan parasites and helminths. These glycan structures can be pathogen-specific or shared by host cells, thereby delineating a degree of specificity in glycan-mediated pathogen recognition [5]. In contrast to compelling evidence on the role of Gal-3 during microbial infection [5], less information is available on the role of Gal-1 in host–pathogen interactions.

Nipah (NiV) and Hendra (HeV) viruses are members of the *Henipavirus* genus of the *Paramyxoviridae*. NiV is an emergent paramyxovirus that causes fatal encephalitis in infected patients, and there is increasing evidence of human-to-human transmission [57]. NiV infection of endothelial cells results in cell–cell fusion and

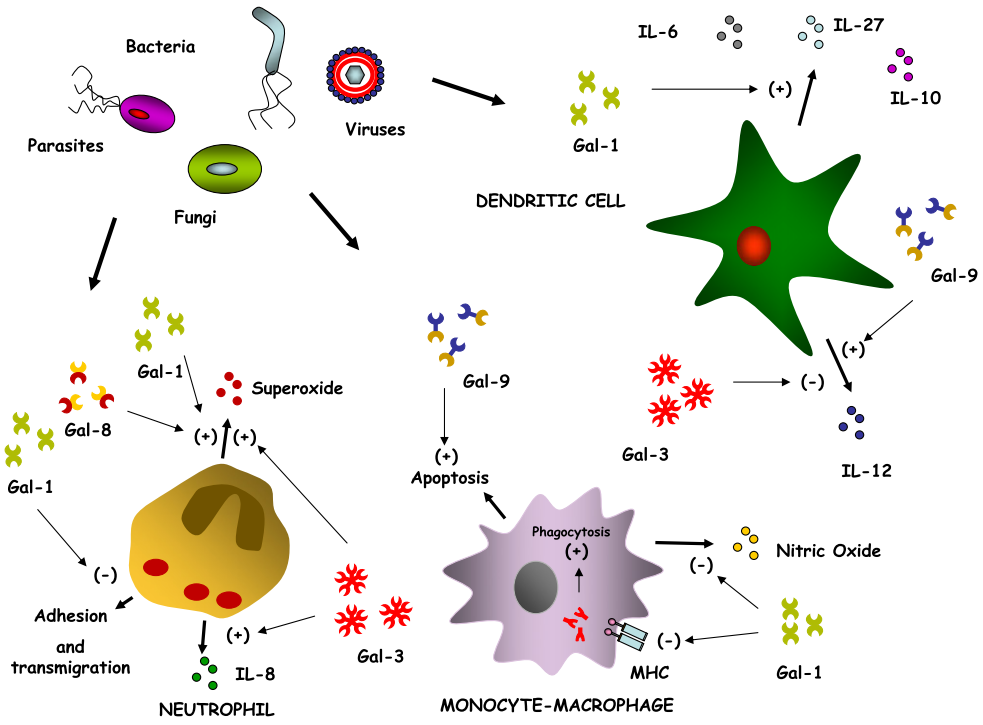

Fig. 1. Galectins participate in host–pathogen interactions influencing the functions of innate immune cells. During infection, galectins recognize glyco-epitopes either restricted to pathogens or shared by host cell and affect different functions of innate immunity cells. While Gal-1 inhibits neutrophil transmigration through the extracellular matrix, Gal-3 favors neutrophil adhesion to endothelia through its oligomerization processes. Galectins also increase superoxide and IL-8 production. In contrast, Gal-1 inhibits nitric oxide production and MHC II-dependent antigen presentation by monocytes/macrophage. Moreover, Gal-9 contributes to monocyte/macrophage apoptosis. In addition, Gal-1 promotes tolerogenic dendritic cells (DCs) inducing secretion of high amounts of IL-27 and IL-10. Furthermore, while Gal-9 stimulates IL-12 production by DCs, Gal-3 inhibits secretion of this cytokine. Thus, galectins can differentially influence innate immune reactions elicited in response to pathogens.

syncytia formation triggered by the fusion and attachment of NiV envelope glycoproteins that are enriched in Gal-1-specific glycans [58]. Recent studies demonstrated that Gal-1 interacts with specific *N*-glycans on the Nipah envelope proteins [58] and blocks fusion events [58]. Interestingly, this effect appeared to be highly specific as Gal-1 did not inhibit fusion triggered by envelope glycoproteins of other viruses, such as retroviruses and poxvirus, but inhibited fusion triggered by envelope glycoproteins of the closely related Hendra virus and other paramyxovirus. In addition, Gal-1 increased DC production of pro-inflammatory cytokines such as IL-6 favoring the antiviral effects and augmenting innate immune responses [58]. Yet, how these effects are reconciled with the tolerogenic effects of Gal-1 on DCs is still not clear. It is possible that different effects are induced by this endogenous lectin depending on its relative concentrations, monomeric *versus* dimeric forms of the protein, activation or differentiation status of the cells or timeline at which Gal-1 was incorporated to cell cultures [59].

Although the information on the role of Gal-1 in infection is limited, it has been reported, using cDNA microarrays, that *Lgals1* is up-regulated by *Helicobacter pylori* in gastric epithelial AGS cells [60]. In this regard, other authors reported binding of galectins to specific glycans present in *H. pylori* [61]. Interestingly, in elegant studies, Stowell and colleagues demonstrated that Gal-4 and Gal-8 but not Gal-1, when expressed along the intestinal tract, selectively recognize and kill *Escherichia coli*-expressing human blood group antigens, while fail to kill other Gram-negative or Gram-positive microorganisms. The killing activity of both Gal-4 and Gal-8 was mediated by their C-terminal domains, occurred independently of complement and was accompanied by disruption of membrane integrity [62]. These findings demonstrated that galectins can provide innate immunity against pathogens that display blood group self-antigens on their surface, thus filling a gap generated by the absence of group-specific antibodies in these groups of individuals.

Interestingly, Gal-1 can bind to several parasites, including low affinity interactions with *Leishmania major* [63] and *Trichomonas vaginalis* [64]. However, the major number of studies reported to date was aimed at clarifying the effects of the Gal-1 during *Trypanosoma cruzi* infection. In this regard, *in vitro* treatment with low concentrations of Gal-1 increased *T. cruzi* replication and attenuated the release of soluble mediators required for parasite killing, such as IL-12 and nitric oxide, whereas high doses of this lectin were able to commit cells to apoptosis and suppress parasite replication [65] (Table 1). In this regard, anti-Gal-1 autoantibodies have been reported in sera from patients with acute Chagas' disease [66]. Interestingly, anti-Gal-1 IgG immunoreactivity was found to correlate with the severity of cardiac damage at chronic stages of Chagas' disease. Interestingly, in these assays no specific immunoreactivity against *T. cruzi* antigens was observed using a specific anti-Gal-1 polyclonal antibody. Remarkably, Gal-1 was found to be dramatically up-regulated in cardiac tissue from patients with severe Chagas' cardiac disease, compared to cardiac tissue from normal individuals [66].

An interesting implication of Gal-1 induction by viruses is reflected by the emerging role of this lectin in viral-induced lymphoproliferative disorders. Post-transplant lymphoproliferative disorders (PTLDs) are potentially fatal Epstein Barr Virus (EBV)-driven B-cell malignancies that develop in immunocompromised solid organ or hematopoietic stem cell recipients. Ouyang et al. recently found that EBV-transformed lymphoblastoid B-cell lines (LCLs) and primary PTLDs overexpress Gal-1 through mechanisms involving the EBV antigens LMP2A and LMP1. Gal-1 expression in LCLs was both AP-1 and PI3K-dependent [67]. Thus, viral induction of Gal-1 may contribute to amplify the immunosuppressive microenvironment characteristic of these malignancies.

Table 1
Role of Gal-1 in host–pathogen interactions.

Microorganism	Gal-1 effects	Reference
Virus		
Nipah	Gal-1 interacts with specific *N*-glycans on envelope proteins and blocks cell–cell fusion.	[57], [58]
	Gal-1 increases production of IL-6 by DCs favoring the antiviral effects and augmenting innate immune responses.	[58]
Hendra	Gal-1 inhibits fusion triggered by envelope glycoproteins of the virus.	[58]
Epstein–Barr virus (EBV)	Gal-1 is overexpressed in EBV-driven B-cell malignancies	[67]
Bacteria		
Helicobacter pylori	Gal-1 is up-regulated in infected gastric epithelial cells.	[60]
Parasites		
Leishmania major	Gal-1 interacts with low affinity with the parasite.	[63]
Trichomonas vaginalis	Gal-1 interacts with high affinity with the parasite and mediates infection.	[64]
Trypanosoma cruzi	At low concentrations, Gal-1, increases *in vitro T. cruzi* replication and inhibits cytokines (IL-12) involved in parasite killing. At high doses, Gal-1 inhibits parasite replication and commits infected cells to apoptosis.	[65]

5. Modulation of microbial infection by galectins

It has been proposed that several pathogens could infect host target cells by subverting the role of host galectins as PRRs, and/or modulating cell–cell attachment or entry into target cells. In this regard, Gal-1 promotes HIV-1 infection either by enhancing binding of the virus to host cells, increasing the absorption of HIV-1 by monocyte-derived host macrophages or acting as a soluble scavenger receptor to facilitate viral uptake by macrophages [68,69]. In addition, Gal-1 has been proposed to serve as receptor for *Trichomonas vaginalis,* an extracellular pathogen capable of adhering to lipophosphoglycan (LPG) structures rich in galactose and *N*-acetylglucosamine. These saccharide structures are specifically recognized by Gal-1 that is expressed in inflamed tissues. As Gal-7 does not attach to this parasite, it has been speculated that Gal-1 has some binding preferences for *T. vaginalis* [70]. Although much remains to be learned, these results encourage a more in-depth study of the role of galectins as potential PRRs that modulate microbial–host cell interactions and innate immune signaling.

6. Influence of glycosylation on TLR structure and function

Regulated glycosylation can modulate immune response and host–pathogen interactions through exposure of glyco-epitopes that are specific for endogenous lectins [5]. However, it has been proposed that glycosylation also controls other processes including trafficking and cellular distribution of TLRs as well as specific interactions with PAMPs. TLRs are type I transmembrane proteins with ectodomains containing leucine-rich repeats (LRR) that mediate the recognition of PAMPs, transmembrane domains, and intracellular Toll–IL-1 receptor (TIR) domains required for downstream signal transduction. So far, 10 and 12 functional TLRs have been identified in humans and mouse respectively, with TLR1–TLR9 being conserved in both species. Mouse TLR10 is not functional because of a retrovirus insertion, and TLR11, TLR12 and TLR13 have been lost from the human genome [1]. An important area in TLR research is related to the understanding of the biochemical and structural bases of PAMP recognition [71,72]. The trafficking of TLRs could be affected by glycosylation. It has been well-established that LRR domains expressed in all human TLRs are *N*-glycosylated and these glycan structures appear to be essential for proper function. Homology models and quantitative analyses demonstrated that human TLR3, TLR7, TLR8, and TLR9 contain the highest number of putative *N*-glycosylation sites. However, glycosylation of some TLRs can also regulate their cell surface distribution; yet in most cases the ligand binding pocket is located in non-glycosylated regions. On the other hand, biochemical and structural analysis of TLR2 and TLR4, demonstrated that *N*-linked glycosylation plays an essential role in TLR secretion and localization [73,74]. Sun et al. have characterized the contribution of glycosylation to TLR3 structure and function. The authors found that inhibition of glycosylation prevented TLR3-induced NF-κB activation, but did not affect expression or cell surface localization of this receptor confirming that *N*-linked glycosylation is required for the bioactivity of this receptor [75]. Based on these data, some TLRs belong to a group of glycoproteins that require glycosylation sites for their secretion and localization. Meanwhile in other TLRs, receptor glycosylation is required for modulating their biological activity and function [75].

7. Identification of microbial 'galectin-like' structures

Although the expression and function of host galectins have been extensively appreciated, there is still scarce information on the repertoire of 'galectin-like molecules' expressed by pathogens and associated to their virulence. Although these structures are theoretically present in diverse species and represented in multiple databases [20], their biological function remains a mystery. In this regard, 'galectin-like' molecules which show high similarity in their amino acid sequences have been isolated from helminth parasites, including *Fasciola hepatica,* which causes atrophy in the liver parenchyma and periportal cirrhosis in humans [76]. Also, immediately prior to invasion, *Toxoplasma gondii* tachyzoites release a large number of micronemal proteins (TgMICs) that participate in host cell attachment and penetration. The TgMIC4–MIC1–MIC6 complex was the first to be identified in *T. gondii* and has been recently shown to be critical in invasion. Instead, the TgMIC1 'galectin-like' domain stabilizes TgMIC6, which provides the basis for a highly specific quality control mechanism for successful exit from the early secretory compartments and for subsequent trafficking of the complex to the micronemes [14,77]. Interestingly, MIC1 has been originally identified as a galactose-binding protein present in tachyzoites of a virulent *T. gondii* strain [78]. Of interest, it has been demonstrated that feeding of *Caenorhabditis elegans* with a mushroom 'galectin-like' protein (CGL2) isolated from *Coprinopsis cinerea* inhibited development and reproduction and ultimately killed the nematode. Importantly, the effects of CGL2 were dependent of the interaction between the galectin and a fucose-containing glycoconjugate [79]. On the other hand, Kim et al. isolated a Gal-9 homologue gene (Tl-gal) from an adult worm of the canine gastrointestinal nematode parasite *Toxascaris leonina.* The deduced amino acid sequence of the Tl-gal gene shared a 35% identity with the human Gal-9 gene (*Lgals9*). The authors confirmed that recombinant rTl-GAL like mammalian galectins have a distinctive CRD. The clinical symptoms of inflammatory bowel disease in mice treated with dextran sulfate sodium (DSS) were attenuated when mice were pre-treated with rTl-GAL, as shown by increased levels of the anti-inflammatory cytokines TGF-β and IL-10. These observations demonstrated that, similar to host Gal-1, rTl-GAL inhibits inflammatory reactions by skewing the balance towards Th2 and T regulatory cytokine profiles. This is the first study demonstrating that a nematode galectin may function similarly to a host galectin serving

as a regulatory backup mechanism to dampen potentially harmful immune responses [80].

8. Conclusions and future perspectives

In recent years there has been an increasing appreciation of the role of galectins and glycans in the homeostatic control of immune responses as well as their role in host–microbe interactions [58–66]. This evidence also clarified some of the mechanisms underlying tumor-immune escape and recovery of autoimmune inflammation [42–45]. Moreover, emerging data hint to a novel role of galectins as PRRs that control pathogen attachment, entry and invasion [4]. Host-derived galectins may contribute to amplify, fine-tune or attenuate antimicrobial immune responses by coupling glycan-dependent structures on pathogenic microorganisms to innate immune cells. On the other hand, the role of glycosylation in TLR trafficking, secretion, localization and function suggests another possible target of modulation. Finally, the presence of candidate 'galectin-like' molecules in a diversity of pathogens [76–80] suggests their potential function as virulence factors involved in microbial invasion, which add to the complexity of the host 'galectinomics'. Collectively, these results highlight the versatility of the mammalian and microbial 'glycome' during infection. Understanding the role of cell surface glycosylation during host–pathogen interactions may help to delineate novel therapeutic strategies based on galectin–glycan interactions as immunomodulators, inhibitors of pathogen invasion and potential adjuvant of novel or conventional vaccines.

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