

Published in final edited form as:

*Nat Rev Microbiol.* 2009 June ; 7(6): 424–438. doi:10.1038/nrmicro2146.

## Roles of galectins in infection

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### Abstract

Galectins, which were first characterized in the mid-1970s, were assigned a role in the recognition of endogenous ('self') carbohydrate ligands in embryogenesis, development and immune regulation. Recently, however, galectins have been shown to bind glycans on the surface of potentially pathogenic microorganisms, and function as recognition and effector factors in innate immunity. Some parasites subvert the recognition roles of the vector or host galectins to ensure successful attachment or invasion. This Review discusses the role of galectins in microbial infection, with particular emphasis on adaptations of pathogens to evasion or subversion of host galectin-mediated immune responses.

Multicellular organisms and their commensal, symbiotic, or pathogenic microorganisms have co-evolved over millions of years. The evolutionary success of individual consortium members has depended either on the maintenance of a homeostatic balance for mutual benefit (symbiosis or commensalism) or on a colonization of the host that is beneficial to the microorganism but reduces fitness in the host (pathogenic)<sup>1</sup>. These cross-kingdom interactions take place through the sensing of soluble signals, such as hormone-like factors that are produced by both the host and the microorganism<sup>2</sup>, and direct cell–cell contacts<sup>3</sup>. Because virtually all bacterial and eukaryotic cells, as well as many viruses, display surface carbohydrates, it is thought that these molecules have a crucial role in the establishment of cell-to-cell contacts<sup>3</sup>. Indeed, many host–microorganism interactions have recently been shown to involve protein–carbohydrate recognition. Rapid modifications of exposed carbohydrate moieties by either microbial or host glycosyltransferases and glycosidases, and

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### DATABASES

**Entrez Genome Project:** <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeptj>

*Ancylostoma caninum* | *Biomphalaria glabrata* | *Brugia malayi* | *Candida albicans* | *Caenorhabditis elegans* | *Coprinopsis cinerea* | *Crassostrea virginica* | *Entamoeba histolytica* | *Fasciola hepatica* | *Haemophilus influenzae* | *Helicobacter pylori* | *Klebsiella pneumoniae* | *Leishmania infantum* | *Leishmania major* | *Lutzomyia longipalpis* | *Neisseria gonorrhoeae* | *Neisseria meningitidis* | *Perkinsus marinus* | *Phlebotomus papatasi* | *Plasmodium falciparum* | *Porphyromonas gingivalis* | *Pseudomonas aeruginosa* | *Rhodnius prolixus* | *Saccharomyces cerevisiae* | *Staphylococcus aureus* | *Streptococcus pneumoniae* | *Toxoplasma gondii* | *Trichomonas vaginalis* | *Trypanosoma cruzi* | *Trypanosoma rangeli* | *Vibrio parahaemolyticus* | *Vibrio vulnificus*

**UniProtKB:** <http://www.uniprot.org>

GRIFIN | GRP | IL-4 | MIC1 | MYD88 | TICAM1 | TLR2 | TLR9

### FURTHER INFORMATION

**Gerardo R. Vasta's homepage:** <http://www.umbi.umd.edu/comb/faculty-directory/vasta/index.php>

**A Genomics Resource for Animal Lectins:** <http://www.imperial.ac.uk/research/animallectins/>

**Functional Glycomics Gateway:** <http://www.functionalglycomics.org/static/index.shtml>

### Roles of galectins in infection

Gerardo R. Vasta

Galectins are important for recognition of carbohydrate ligands during embryogenesis, development and immune regulation. In addition, recent work has shown that galectins also function as receptors for glycans expressed on the surface of potentially pathogenic microorganisms. In this Review, Gerardo Vasta discusses the roles of galectins in host immunity and how pathogens have evolved to evade or subvert galectin-mediated immune responses.

the equally dynamic patterns of expression of their protein receptors, further add to the complexity of host–microorganism interactions<sup>4,5</sup>. Similarly, within multicellular organisms, protein–carbohydrate recognition is crucial to intracellular processes, such as protein folding and transport, as well as interactions between cells, or cells and the extracellular matrix in functions ranging from cell differentiation, adhesion and migration, to embryogenesis, development and immune responses<sup>3–5</sup>. Therefore, it is recognized that cell surface glycans, including glycoproteins, glycolipids and polysaccharides, encode complex information that is decoded by specific binding to cell surface-associated or soluble carbohydrate–protein receptors, which are mainly known as lectins<sup>6</sup>.

Lectin–glycan interactions are ubiquitous and essential to biological systems, not simply as the ‘glue’ between cells, but as the initiators of a functional crosstalk that modulates their physiology and homeostatic balance<sup>7</sup>. Several microbial lectins are involved in host colonization, including viral haemagglutinins, bacterial adhesins and parasite lectins<sup>8,9</sup>. Likewise, some animal lectins can function as pattern-recognition receptors (PRRs)<sup>10</sup>, a group of diverse, soluble and membrane-associated molecules that include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NODs) and NK cell receptors (FIG. 1).

Galectins (formerly known as S-type lectins) were initially thought to bind only endogenous ‘self’ glycans and mediate developmental processes, including cell differentiation, tissue organization<sup>11</sup> and, more recently, regulation of immune homeostasis<sup>12,13</sup>. In the past few years, however, it has become clear that galectins also bind glycans on the surface of potentially pathogenic microorganisms and parasitic worms, and mediate recognition and effector functions in innate immunity<sup>14</sup>.

In this Review, the roles of galectins in microbial infection are discussed, with a particular emphasis on adaptations to evade or subvert the host galectin-mediated immune responses.

## Lectin–sugar interactions in consortia

The term lectin commonly refers to a wide range of carbohydrate-binding proteins and glycoproteins from viruses, bacteria (including adhesins and soluble lectins), fungi, protista, plants and animals<sup>6–9,15</sup> (see Further information for a link to [A Genomics Resource for Animal Lectins](#) and the [Functional Glycomics Gateway](#)). Most lectins are organized as homooligomers or heterooligomers of non-covalently bound polypeptide subunits displaying carbohydrate-recognition domains (CRDs) that bind to the sugar ligand, usually a non-reducing terminal monosaccharide or oligosaccharide. Lectins have a diverse array of roles in symbioses, host colonization by microbial pathogens and immune responses of the host<sup>7</sup> (FIG. 1).

Lectin–carbohydrate recognition is not only crucial to the establishment of specific mutualistic associations of symbiotic microorganisms with plants, corals and squid, but can also modulate the physiology of either the host or the symbiont<sup>16–18</sup>. This carbohydrate-mediated crosstalk is particularly intriguing in host gut–microbial symbioses<sup>19</sup> in which reciprocal, tightly regulated interactions modulate the composition and function of the microbiota<sup>20</sup>. One mechanism that contributes to the tolerance of the resident microbiota is molecular mimicry, whereby bacteria display surface molecules that resemble those of the host’s surface to render them immunologically inert, while enzymatically modifying the host mucosal glycome for a mutually beneficial microbial colonization of the gut<sup>21</sup>.

During the host colonization process, pathogens ranging from viruses to protists bind to host cell surface glycans through surface lectins<sup>8,9</sup>. Influenza A haemagglutinins bind to sialic acids on the surface glycans of bird and mammalian cells<sup>22</sup>. Similarly, specific adhesins in

pili and fimbriae mediate interactions between bacteria and cell surface carbohydrate ligands on specific host tissues<sup>23</sup>. Host colonization by protozoan parasites, such as *Entamoeba histolytica*<sup>24</sup>, *Plasmodium falciparum*<sup>25</sup>, *Cryptosporidium* species<sup>26</sup> and *Toxoplasma gondii*<sup>27</sup>, is also mediated by lectins. Reciprocally, soluble and membrane-associated lectins of the host cell are crucial recognition molecules that can facilitate the establishment of favourable mutualistic interactions with the colonizing microorganisms, or initiate innate and adaptive immune responses against pathogens. In addition to pathogen recognition, lectins also mediate downstream effector functions, such as agglutination, immobilization, opsonization and phagocytosis of potential pathogens, as well as leukocyte migration and complement activation<sup>7</sup>. The increasing availability of amino acid sequences and crystal structures of C- and S-type characteristic sequence motifs in animal lectins<sup>28</sup> has allowed us to classify them into several families (TABLE 1). In the C- and F-type lectin families, CRDs can be found in combination with a range of unrelated domains to form multifunctional chimerical molecules<sup>29–31</sup>. Although interactions between animal lectins and their legends on the microbial surface are weak compared with other immune recognition molecules, such as antibodies, high avidity for the target is achieved when multiple CRDs interact with the ligand simultaneously, as observed in collectins and other oligomeric lectins<sup>32</sup>. A less frequent organizational plan is the presence of tandemly arrayed CRDs within a polypeptide, such as that observed in the macrophage mannose receptor<sup>33</sup>, immulectins<sup>34</sup>, F-type lectins<sup>30,31</sup> and tandem repeat-type galectins<sup>11</sup>. C-type lectins, ficolins, pentraxins, F-type lectins and galectins are implicated in immune functions<sup>7</sup>.

### Galectins: structure–function relationships

Galectins constitute a lectin family with a characteristic domain organization, affinity for  $\beta$ -galactosides (BOX 1), a characteristic CRD sequence motif and wide taxonomic distribution<sup>35</sup>. Based on structural features, mammalian galectins have been classified as proto, chimera or tandem repeat types<sup>36</sup> (FIG. 2a). Proto-type galectins contain one CRD per subunit, and are usually homodimers of non-covalently linked subunits. Chimera-type galectins have a carboxy-terminal CRD that is similar to the proto type, joined to an amino-terminal peptide that is rich in glycine, tyrosine and proline. In the tandem repeat galectins, two CRDs are joined by a functional linker peptide. The proto and tandem repeat types comprise several distinct subtypes, which have been numbered according to the order of their discovery, and, so far, 15 have been described in mammals<sup>37</sup>. Galectins 1, 2, 5, 7, 10, 11, 13, 14 and 15 are of the proto type, galectin 3 is the only galectin of the chimera type and galectins 4, 6, 8, 9 and 12 are of the tandem repeat type. By contrast, nonmammalian galectins are named based on the organism in which they were discovered<sup>35,37</sup>.

Most galectins are non-glycosylated soluble proteins, although galectins with transmembrane domains have been reported<sup>38,39</sup>. The presence of a galectin fold in the protistan parasite *T. gondii*<sup>40</sup>, and lactose-binding galectin-like proteins in the fungus *Coprinopsis cinerea*<sup>41</sup> and in the sponge *Geodia cydonium*<sup>42</sup> reveal the early emergence and structural conservation of galectins in eukaryotic evolution. By contrast, galectin-like proteins, such as the lens crystallin protein galectin-related inter-fibre protein (GRIFIN)<sup>43</sup> and galectin-related protein (GRP; previously known as HSPC159)<sup>44</sup>, lack carbohydrate-binding activity and are considered to be products of evolutionary co-option<sup>43</sup>.

Glycans that contain poly-lactosamine chains ((Gal $\beta$ 1,4GlcNAc) $n$ ), such as laminin, fibronectin, lysosome-associated membrane proteins and mucins, are the preferred endogenous galectin ligands<sup>45–52</sup> (BOX 1). Most metazoans are endowed with a complex galectin repertoire, with members exhibiting multiple isoforms<sup>53</sup> and subtle variations in carbohydrate specificity. This, together with the plasticity observed in sugar binding<sup>47</sup>, suggests that there is substantial diversity in their glycan-recognition properties.

Although galectins lack a typical secretion signal peptide, they are present not only in the cytosol and the nucleus, but also in the extracellular space<sup>37,54,55</sup>. From the cytosol, galectins can be targeted for secretion by non-classical mechanisms, possibly by direct translocation across the plasma membrane<sup>55</sup> (FIG. 2a). The binding properties and biological functions of galectins in the oxidative extracellular environment, however, may depend on their immediate binding to ligand, which prevents the oxidation of free cysteine residues, as well as galectin susceptibility to proteolysis<sup>45,46,56</sup>. In solution, galectins can form multivalent species in a concentration-dependent equilibrium<sup>57</sup>, and their binding to  $\beta$ -galactoside-containing glycolipids and glycoproteins exposed on the cell surface (FIG.2b) can lead to the formation of lattices that cluster these ligands into lipid raft microdomains required for optimal transmission of signals relevant to cell function<sup>58-60</sup> (BOX 2).

## Roles of galectins in immune homeostasis

Galectins are ubiquitously expressed and distributed in mammalian tissues, including most cells of the innate (dendritic cells, macrophages, mast cells, natural killer cells, gamma and delta T cells, and B1 cells) and adaptive (activated B and T cells) immune systems<sup>61,62</sup>. The roles of galectins in the development and regulation of innate and adaptive immunity homeostasis, as well as responses to infectious and allergic challenge and cancer, have been recently reviewed<sup>12,13</sup>.

During the infection process, galectins have diverse effects on cells involved in innate immune responses, including macrophages, dendritic cells, neutrophils, eosinophils and mast cells (Supplementary information S1 (table)). Galectin 1 displays anti-inflammatory activities by blocking or attenuating signalling events that lead to leukocyte infiltration, migration and recruitment<sup>61,62</sup>. By contrast, galectin 3 shows pro-inflammatory activity, enhances macrophage survival, and positively modulates macrophage recruitment and antimicrobial activity<sup>63</sup>. Galectin 9 induces chemotaxis, activation, oxidative activity and degranulation of eosinophils. Similarly to galectin 1, it also induces monocyte-derived dendritic cell maturation<sup>64</sup>, although through different mechanisms and with different outcomes<sup>65,66</sup>.

With regards to adaptive immune responses, galectins are regulators of immune cell development and homeostasis<sup>4,12,61-65</sup>. Interactions between stromal cells from the bone marrow and thymic compartments and lymphocyte precursors are crucial to their development, selection and further progression to the periphery. Galectins can modulate B cell maturation and differentiation both at the central and peripheral immune compartments<sup>67</sup>. Galectin 1 can have pro- or anti-apoptotic effects on T cells depending on the developmental stage and activation status of the cell, and the microenvironment in which the exposure takes place<sup>68</sup>. The effects of galectin 3 on T cell survival, however, are dependent on whether the protein acts endogenously (anti-apoptotic) or by exogenous exposure (pro-apoptotic)<sup>63</sup>. Galectins also exert regulatory functions in T cell homeostasis, and signalling cascades triggered by their binding and lattice formation at the T cell surface have implications in a range of downstream events that modulate their differentiation, functional activation, and production of pro- and anti-inflammatory cytokines<sup>12,58</sup> (BOX 2). Finally, given the regulatory roles of galectins on cells that mediate both innate and adaptive immune responses, their effects can be beneficial or detrimental for pathological conditions that are based on exacerbated or depressed immune function, such as inflammatory, allergic and autoimmune disorders, and cancer<sup>12,63,69</sup>.

## Galectins as PRRs

Recently, galectins have been discovered to bind glycans on the surface of viruses, bacteria, protista and fungi<sup>14,70–72</sup>. Thus, the potential role of galectins as PRRs has become an area of increased attention.

### Viruses

Galectin 1 can inhibit virus attachment and host cell fusion by binding *N*-linked oligosaccharides from the virion envelope or capsid glycoproteins, and promoting their crosslinking and oligomerization. Infection by the Nipah virus, a paramyxovirus that causes severe encephalitis, takes place by fusion and attachment of its envelope glycoproteins to the host endothelial cells through their ephrinB2, and alternatively ephrinB3 receptors, leading to cell–cell fusion and formation of syncytia<sup>72</sup>. In both Nipah virus and Hendra virus (another paramyxovirus that affects humans and animals), the dimeric galectin 1 specifically crosslinks the *N* glycans displayed in the envelope glycoproteins, causing aberrant oligomerization and blocking cell–cell fusion<sup>73</sup> (FIG. 3a). experimental removal of the Nipah virus *N* glycans resulted in higher fusogenicity, but also increased sensitivity to neutralization by specific antisera, suggesting that although the viral glycans can hinder viral infectivity to some extent, they serve a protective function against the neutralizing host antibodies<sup>74</sup>. Modulatory effects of viral infection (for example, by herpesvirus 1, Newcastle disease and epstein–Barr virus)<sup>75–77</sup> on galectin expression and function suggest that galectins participate at various levels of antiviral defence, from the initial recognition and blocking of envelope and fusion glycoproteins, to the activation and amplification of the innate and adaptive immune responses.

### Bacteria

Galectins can interact directly with bacterial surface glycans. Furthermore, bacterial infection can modulate galectin expression, which in turn regulates leukocyte function and inflammatory responses. Both Gram-positive bacteria, such as *Streptococcus pneumoniae*, and Gram-negative bacteria, such as *Klebsiella pneumoniae*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*, display surface carbohydrate galectin ligands<sup>70,78–81</sup>. Galectin 3 binds to bacterial lipopolysaccharides (LPSs) in a dual manner: the C terminus CRD binds to lactosyl moieties of *K. pneumoniae* LPS, whereas the non-carbohydrate-binding N-terminal domain of galectin 3 binds to the lipid A moiety of *Salmonella enterica* subsp. *enterica* serovar Minnesota LPS<sup>70</sup>. Galectin 3 also binds mycobacterial phosphatidylinositol mannosides, glycolipids that accumulate on the membrane of *Mycobacterium*-containing phagosomes during infection<sup>82</sup>. Recent studies have shown that galectin 3 recognizes the O-antigen side chains on *Helicobacter pylori*, a species that can cause gastritis, in some cases leading to peptic ulcer, gastric adenocarcinoma or gastric lymphoma. Adhesion of *H. pylori* to the gastric lining upregulates expression and secretion of galectin 3 by gastric epithelial cells<sup>83</sup>, suggesting that galectin 3 might play a part in the host response to infection by promoting recruitment of phagocytic cells to the site of infection and induction of an inflammatory response, and by disrupting the interaction of the pathogen with the host cells. Furthermore, transcript profiling of *H. pylori*-infected gastric tissue in rhesus macaques revealed increased expression of galectin 8, and marginally, of galectins 3 and 4, whereas in *in vitro* infected gastric epithelial AGS cells, galectins 1 and 3 were upregulated<sup>84,85</sup>. Galectin 3 also has a role in neutrophil extravasation and recruitment during *S. pneumoniae* infection<sup>86,87</sup>. expression of galectin 9 in periodontal ligament cells is elevated after both *in vitro* and *in vivo* exposure to LPS from *Porphyromonas gingivalis*<sup>88</sup>. The galectin BbtGal-L from the cephalochordate amphioxus (*Branchiostoma belcheri*) specifically recognizes *Vibrio vulnificus* but not *Vibrio parahaemolyticus* or *Staphylococcus aureus*, and its expression is



upregulated by bacterial challenge<sup>89</sup>. Taken together, these observations indicate that host galectins can bind directly to glycoconjugates on the surface of bacteria, either facilitating or inhibiting pathogen entry, followed by positive and negative regulation of host innate and adaptive immunity. Interestingly, a recent report suggests that galectin 3 protects the host from *Salmonella* LPS-induced endotoxin shock, but, paradoxically, favours bacterial survival<sup>90</sup>.

## Fungi

Galectins can recognize surface glycans on the surface of saprophytic or pathogenic fungi. The binding of a galectin 3 homologue from the J774 mouse cell line to polysaccharides and glycolipids that display  $\beta$ 1,2-linked oligomannosides on the cell wall of *Candida albicans* suggested that galectin 3 could function as a macrophage receptor for this opportunistic fungal pathogen<sup>91</sup>, either leading to a successful immune response or enabling intracellular parasitism. The differential recognition of *C. albicans* and *Saccharomyces cerevisiae* by macrophages takes place through a cooperative mechanism that involves TLR2 and galectin 3, which probably binds to carbohydrate ligands that are specific to the *C. albicans* cell wall<sup>92</sup>. In a mouse model of experimental colitis, pre-existing inflammatory conditions strongly promoted *C. albicans* colonization, which in turn augmented the inflammatory process with upregulation of TLR2 expression<sup>93</sup>. This supports the roles of galectin 3 in both inflammation and the regulation of host immune responses to *C. albicans*<sup>93</sup>. Interestingly, the selective binding of galectin 3 to the *C. albicans* cell wall glycans is fungicidal<sup>94</sup>, an observation that underscores how galectins have direct effector functions in innate immunity.

## Protistan parasites

It has been recently recognized that host galectins can also directly bind carbohydrate moieties displayed on the parasite surface and function as PRRs. Lipophosphoglycan (LPG) on the surface of *Leishmania* parasites is recognized by both galectin 3 and 9, although with different outcomes<sup>95,96</sup>. *Leishmania* species are intracellular protozoan parasites that are transmitted by phlebotomine sandflies to vertebrate hosts, including humans, where they invade macrophages, and eventually reside within their fusogenic phagolysosomes and block macrophage activation<sup>97</sup>. Depending on the *Leishmania* species, the disease can take cutaneous (for example, *Leishmania major*, *Leishmania pifanoi* and *Leishmania mexicana*) or visceral (for example, *Leishmania donovani* and *Leishmania infantum*) clinical forms. *Leishmania* promastigotes, the parasite's infective stage, have a complex glycocalyx composed of proteophosphoglycans, glycosylinositolphospholipids, the protease gp63 and abundant LPG. LPG consists of a phosphoglycan domain linked through a hexasaccharide glycan core to a 1-*O*-alkyl-2-lyso-phosphatidylinositol lipid anchor<sup>97</sup>. The LPG phosphoglycan moieties share a common backbone of  $\text{PO}_4\text{-6Gal}(\beta 1,4)\text{Man}_1$  repeating units, in which the C3 position of the Gal residue can be either unsubstituted (for example, *L. donovani*), partially substituted with glucose side chains (for example, *L. donovani*, *L. mexicana* and *Leishmania chagasi*) or completely substituted with side chain sugars that terminate primarily in glucose and arabinose (for example, *Leishmania tropica*) or galactose (for example, *L. major*)<sup>71,98</sup>. In *L. major*, the poly- $\beta$ -galactosyl moieties ( $\text{Gal}\beta 1,3)_n$  are recognized with similar affinity by galectin 3 and 9 (REFS 95,96). The significantly lower affinity of galectin 1 for ( $\text{Gal}\beta 1,3)_n$  can be attributed to the lack of an extended CRD that in galectin 3 would accommodate oligosaccharide repeats (BOX 1). Interestingly, *Trichomonas vaginalis* LPG is strongly recognized by galectin 1, which facilitates attachment of the parasite to the cervical epithelial cells<sup>99</sup>, suggesting that there are substantial differences in the structure of LPG from both parasite species. Furthermore, the recently elucidated structure of *T. vaginalis* LPG revealed the presence of poly-lactosamine<sup>100</sup>, which could be a good target for galectin 3 recognition.

The binding of oligomeric galectin 3 to *L. major* during the early stages of infection leads to its cleavage by the gp63 protease in the parasite glycocalyx<sup>96</sup>. Pelletier and Sato have proposed that this cleavage might lead to lattice disruption and initiation of signalling pathways that skew the immune response balance towards a T helper 1 (T<sub>H</sub>1)-type profile (BOX 2), thereby inducing a strong local inflammation that limits the infection to the dermal tissues and results in cutaneous lesions<sup>96</sup>. *Leishmania* species, such as *L. donovani*, that fail to cleave galectin 3 and only promote a limited inflammatory response at the primary site of infection would migrate further, leading to the visceral form of the disease<sup>96</sup>. Therefore, it is tempting to speculate that by its sensitivity to proteolytic attack, galectin 3 could also function as a 'sensor' for microbial infection. By contrast, the binding of galectin 9 would actually promote interactions between *L. major* and the host macrophages, although the underlying mechanisms that control this binding have not been characterized in detail<sup>95</sup>.

In the early stages of infection by the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, recognition of the parasite glycosylphosphatidylinositol anchors and DNA is carried out by TLR2 and TLR9, respectively, on macrophages and dendritic cells. This leads to the activation of both myeloid differentiation primary-response gene 88 (MYD88)- and TIR domain-containing adapter molecule 1 (TICAM1; also known as TRIF)-dependent pathways<sup>101</sup>. However, *T. cruzi* trypomastigotes are also specifically recognized by galectin 3 through its C terminus CRD, which mediates adhesion of the parasite to laminin and possibly facilitates entry into human coronary artery smooth muscle cells<sup>102</sup>. Reciprocally, *T. cruzi* infection upregulates expression of galectin 3 and its ligands on dendritic cells, which affects dendritic adhesion and migration<sup>103</sup>. This is concurrent with intense thymus atrophy followed by depletion of CD4<sup>+</sup>CD8<sup>+</sup> immature cortical thymocytes through galectin 3-mediated migration and premature escape to the peripheral compartment, and increased apoptosis<sup>104</sup>. It also leads to interleukin-4 (IL-4)-induced survival of activated B cells, favouring their differentiation towards a plasma cell pathway, and increasing immunoglobulin (Ig) production and parasite clearance<sup>105</sup>. Unlike galectin 3, galectin 1 does not recognize *T. cruzi* surface glycans, although its upregulation during *T. cruzi* infection can induce macrophage apoptosis and inhibit parasite replication<sup>106</sup>.

Infection by the apicomplexan parasite *T. gondii* is characterized by a T<sub>H</sub>1-polarized immune response in which galectin 3 has important roles both as a proinflammatory factor and in the regulation of dendritic cell function<sup>107</sup>.

## Helminths and nematodes

Glycans are also present on the surface of parasitic worms, such as trematode helminths, commonly known as blood (*Schistosoma* spp.) or liver (*Fasciola* spp.) flukes, and nematodes, and are recognized by soluble and cell surface-associated C-type lectin receptors that modulate macrophage cytokine production and dendritic cell maturation, and initiate innate immune responses<sup>108</sup>. During *Schistosoma mansoni* infection, galectin 3 functions as a PRR that mediates LacdiNAc (LDN; composed of GalNAc $\beta$ 1,4GlcNAc) recognition and phagocytosis by macrophages<sup>109</sup>. Galectin 1 does not recognize LDN, possibly because galectin 3 can accommodate the O-2 *N*-acetyl moiety of the GalNAc residue, whereas in galectin 1 the histidine residue at position 52 may prevent GalNAc binding<sup>48</sup>. A comparative study on acute and chronic phases of *S. mansoni* infection in wild-type and galectin 3<sup>-/-</sup> mice suggests that galectin 3 has an essential role in the modulation of monocyte-macrophage and B cell-plasma cell differentiation<sup>110</sup>, although this is under debate<sup>111</sup>.

Intestinal parasitic nematodes can upregulate galectin expression. In sheep, larval nematode infection leads to upregulation of a galectin-like protein (galectin 11) that is secreted from epithelial cells into the gut lumen in association with an eosinophil-rich inflammatory

response that is typical for larval infections<sup>112</sup>. As a functional analogue of galectin 1, galectin 11 might crosslink mucus glycans and form an effective physical barrier as part of the inflammatory response to the parasite. The higher rate of expression during a secondary challenge suggests that galectin 11 might be involved in both the innate and adaptive immune response to gastrointestinal parasite infection<sup>112</sup>.

In summary, the experimental evidence for galectin binding to the surface of virus, bacteria, protista and helminth pathogens and parasites suggests that galectins can function as effective PRRs. Furthermore, the considerable diversity of the galectin repertoire in each organism and the substantial or subtle variations in the specificity of each galectin towards the target glycans, which are determined by oligosaccharide repeats, branchings or substitutions, suggest that there is extensive diversity and plasticity in the capacity of galectins for non-self recognition. The presence of canonical and extended CRDs<sup>45–48</sup>, and the carbohydrate-independent binding properties of the N terminus region of galectin 3 (REF. 63), further suggests that galectins have a high recognition capacity. Moreover, because galectins from all three types (proto, chimera or tandem repeat) can form oligomers, their multivalent binding properties, including increased avidity<sup>32</sup>, clearly enable galectins to participate effectively both in direct recognition of pathogens and parasites, and downstream processes that lead to activation of innate and adaptive immune responses. Whether galectin-mediated recognition is an effective defence mechanism with a clear benefit for the host is not entirely clear, except for a few examples. It is noteworthy that a particular glycan on the surface of a microorganism or parasite can be recognized by multiple galectins, and that the outcome of the interaction differs substantially depending on the galectin type involved and the concentration of the galectin in a particular cell surface or extracellular microenvironment. This, in turn, determines the level of oligomerization and cooperative binding to ligand, and the potentially antagonistic or synergistic activation of pathogen signalling pathways (for example, modulation of immune cell activation, or cytokine production and secretion).

## Subversion of galectin PRR function

Several pathogens and parasites can subvert the roles of host or vector galectins as PRRs to attach to or gain entry into their cells.

### HIV-1 infection

In contrast to the inhibitory role of galectin 1 in paramyxovirus infection, elegant work by Sato and colleagues has shown that galectin 1, 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 facilitating virus attachment to the host cell surface glycans<sup>113</sup> (FIG. 3b). Furthermore, galectin 1 enhances HIV adsorption kinetics on monocyte-derived host macrophages, facilitating HIV-1 infectivity by shortening the time required to establish an infection<sup>114</sup>. Galectin 1 could also function as a soluble scavenger receptor and enhance the uptake of the virus by macrophages, thereby facilitating sexual transmission of HIV-1 (REF. 114). Galectin 3 has no effect on HIV-1 adsorption, entry or infection<sup>113,114</sup>, although its expression is upregulated by the HIV Tat protein in several human cell lines<sup>115</sup>, and in cells infected with other retroviruses<sup>116,117</sup>, suggesting that it may participate in regulation of antiviral immunity. This underscores the relevance of the subtle differences in galectin specificity and affinity that may determine different recognition and effector outcomes.

### Attachment of *Leishmania* to the insect vector

After a sandfly feeds on blood from a host infected with a *Leishmania* species, the ingested *Leishmania* amastigotes mature into promastigotes, which attach to the insect midgut



epithelium to prevent their excretion along with the digested blood meal (FIG. 4A). These promastigotes then undergo numerous divisions before differentiating into free-swimming infective metacyclics<sup>71</sup>. The *Phlebotomus papatasi* sandfly midgut receptor for the procyclic *L. major* LPG was identified as a 35.4 kDa tandem repeat galectin (PpGalec) that is only expressed by epithelial midgut cells and is upregulated in blood-feeding females<sup>98</sup>. Binding of PpGalec is restricted to *Leishmania* promastigotes that express poly-Gal( $\beta$ 1–3) side chains on their LPG, such as those of *L. major*, and is responsible for specific attachment of the parasite to the vector's midgut linings. These polygalactose epitopes are masked with arabinose during *L. major* metacyclogenesis, and therefore the free-swimming, infective, metacyclic promastigotes are unable to bind to PpGalec and are released from the midgut for transmission from the sandfly to the mammalian host<sup>98</sup>. Thus, the sandfly midgut galectin is not only crucial for parasite survival during the promastigote stage, but also determines the species specificity of the vector– parasite interaction. expression of PpGalec is restricted to *P. papatasi* and *Phlebotomus duboscqi*, closely related sandfly species that naturally transmit *L. major*. unlike *P. papatasi*, the sandfly *Lutzomyia longipalpis* is broadly permissive to the development of different *Leishmania* species<sup>118,119</sup> and expresses four putative galectin-like proteins, of which LulongGale A is a tandem-repeat galectin with high similarity to PpGalec<sup>120</sup>. However, *L. infantum* (also known as *L. chagasi*), a species transmitted by *L. longipalpis*, does not display galactose residues on its surface LPG<sup>120</sup>, and lectin-like molecules on the *Leishmania* surface bind to the sandfly midgut glycoproteins<sup>118,119</sup>, a reverse interaction that might contribute to the adaptation of *Leishmania* species to new vectors and expand their geographical distribution<sup>119</sup>. Furthermore, the *Leishmania*–midgut interactions might not be limited simply to parasite anchoring to avoid expulsion, as a comparative analysis of the *Lutzomyia longipalpis* midgut transcriptome revealed that, during blood meal digestion, the parasite alters the expression profile of midgut factors that could be crucial to its survival in the sandfly<sup>121</sup>.

Galectins have also been identified in other insect species<sup>122–125</sup>, some of which are parasite vectors or intermediate hosts. A galectin identified in *Anopheles* spp. is upregulated in response to *Plasmodium* infection<sup>123</sup>, and galactose-specific lectins mediate attachment of *Trypanosoma rangeli* to the salivary glands of its reduvid vector, *Rhodnius prolixus*<sup>124</sup>. A galectin has been recently characterized from the argasid tick *Ornithodoros moubata*, a vector of various viral and borrelian diseases<sup>125</sup>. whether these parasites similarly misuse recognition by the insect vector's galectins remains to be determined.

### Perkinsus marinus entry into phagocytic cells

The protozoan parasite *P. marinus* is a facultative intracellular parasite that causes Dermo disease in the eastern oyster *Crassostrea virginica*, and is responsible for catastrophic damage to shellfisheries and the estuarine environment in North America<sup>126</sup>. Oyster haemocytes (which are phagocytic cells) recognize *P. marinus* through a galectin (CvGal) that has four canonical CRDs, a domain organization that is unlike any of the known galectin types<sup>127</sup> (FIG. 4B). CvGal is present in the cytoplasm of circulating haemocytes, and following recognition of a foreign surface, CvGal is translocated to the cell periphery and secreted. Some CvGal binds to the cell surface, whereas the remaining galectin is released to the extracellular environment. Interestingly, the soluble CvGal binds in a carbohydrate-specific manner to bacteria, phytoplankton components and, preferentially, to *Perkinsus* spp. trophozoites, suggesting that CvGal has a direct role in recognition and opsonization of potential microbial pathogens and algal food. Inhibition of phagocytosis of *P. marinus* trophozoites by pre-treatment of haemocytes with anti-CvGal antibodies confirmed that the haemocyte surface-associated CvGal is a phagocytosis receptor for *P. marinus*. *P. marinus* might therefore have evolved in such a way that its glycocalyx is strongly recognized by CvGal, thereby subverting the innate immune and feeding

recognition mechanism of the oyster to gain entry into host cells<sup>127</sup>. A two-CRD galectin (BgGal) was subsequently identified in the embryonic cell line and circulating haemocytes of the snail *Biomphalaria glabrata*, the intermediate host of *S. mansoni*, and was proposed to mediate snail–parasite interactions<sup>128</sup>.

### T. vaginalis attachment to host epithelia

A recent study identified galectin 1 as the receptor for the protozoan parasite *T. vaginalis*<sup>99</sup>, the causative agent of the most prevalent non-viral sexually transmitted human infection in both women and men. As an obligate extracellular parasite, establishment and persistence of *T. vaginalis* infection requires adherence to the host epithelial cell surface<sup>129</sup>. *T. vaginalis* displays a surface LPG rich in galactose and *N*-acetyl glucosamine. This LPG is recognized in a carbohydrate-dependent manner by galectin 1, which is expressed by the epithelial cells in the cervical linings, as well as in other tissue colonized by the parasite, such as placenta, prostate, endometrial and decidual tissue<sup>99,129</sup>. Galectin 7 neither binds to nor mediates attachment of *T. vaginalis* to host cells, suggesting that the interaction of galectin 1 and *T. vaginalis* has some specificity, although it does not exclude the possibility that other galectins may also contribute to this interaction<sup>99</sup>.

The examples discussed above reveal that some pathogens and parasites subvert the roles of galectins as PRRs to either attach to suitable epithelia in their insect vector or final host, or to enter the host cells to proliferate and disseminate systemically. These interactions not only confirm the role of galectins as non-self recognition receptors, but also reveal the co-evolutionary adaptations of the glycocalyx in these highly specialized microorganisms.

### Parasite galectins that promote infection

Galectin-like proteins have been also identified in parasitic worms and protozoa. Initial studies on parasitic nematodes and helminths identified galectins in *Onchocerca volvulus*<sup>130</sup>, *Teladorsagia circumcincta*<sup>131</sup>, *Haemonchus contortus*<sup>132</sup> and *Trichostrongylus colubriformis*<sup>133</sup>. The flatworms *Taenia serialis* and *Fasciola hepatica* also express galectin-like cross-reactive molecules<sup>133</sup>.

The galectins from the free-living nematode *Caenorhabditis elegans*<sup>134</sup> have been characterized in most detail and are thought to mediate developmental processes. By contrast, galectins from parasitic nematodes and helminths might contribute to parasitic invasion or downregulation of the host immune response<sup>135</sup>, suggesting that galectins from free-living worms and protista might have been co-opted during evolution to parasitism to ensure their entry and survival in the host environment. Products secreted or excreted by parasitic nematodes and helminths significantly modulate the host immune response<sup>135,136</sup>. Proteomic analysis of these products in *T. circumcincta*, *Brugia malayi* and *Ancylostoma caninum* has identified galectin-like proteins, in addition to a range of proteolytic, antioxidative and glycolytic enzymes<sup>137–139</sup>. The eosinophil-specific chemokinetic activity of the *H. contortus*-infective larvae (L3 stage) is mediated by the nematode galectins in a carbohydrate-dependent manner, and may mimic the activity of the host's galectin 9 (a known eosinophil chemokine) to the parasite's advantage<sup>140</sup>. Perhaps the strongest evidence for a crucial role of parasite galectins in infection is the observation that in sheep resistant to the gastrointestinal nematode *T. colubriformis*, antibodies specific for the galectins expressed by the infective larvae are dominant<sup>141</sup>. A recent vaccination trial in goats that used the recombinant galectins rHco-gal-m and rHco-gal-f from *H. contortus* as immunogens significantly reduced faecal egg output and worm burdens, and increased IgG levels in the vaccinated groups relative to the controls<sup>142</sup>. Taken together, these findings suggest that worm galectins actively participate in the infection process.

The protistan parasite *T. gondii* has lactose-binding lectin activity that is associated with micronemal protein 1 (MIC1)<sup>143</sup>. Although further studies identified a galectin-like domain in the MIC4–MIC1–MIC6 protein complex of the microneme, this domain was devoid of carbohydrate-binding activity<sup>40</sup>. The MIC1 tandem MAR domains resemble thrombospondin type 1 repeats<sup>27</sup>. These repeats recognize negatively charged sulphated glycosaminoglycans, which, together with sialic acid, have been implicated as cell surface glycan receptors for *T. gondii* attachment<sup>144</sup>. The current evidence therefore suggests that the *T. gondii* galectin domain only participates indirectly in host invasion, by facilitating micronemal protein assembly.

## Conclusions and perspectives

A growing body of evidence from recent studies indicates that host galectins can function as PRRs that target glycans on the surfaces of viruses, bacteria and helminths. The specific carbohydrate moieties galectins recognize in these surface glycans can be identified tentatively in a limited number of species, based on the available information about galectin binding specificity and affinity<sup>45–52,91,94–96,99,109,113,114,145</sup> (FIG. 5). In most cases, however, these carbohydrate ligands remain largely unknown and rigorous studies for each galectin–microorganism (or galectin–parasite) interaction will be required to experimentally isolate, identify and characterize them.

Nevertheless, a perplexing paradox arises, as the host galectins also recognize endogenous lactosamine-containing glycans on the host cell surface, which are important for certain developmental processes and regulation of immune homeostasis. Based on the Janeway and Medzhitov model<sup>10</sup> for non-self recognition, PRRs recognize pathogens through highly conserved and widely distributed microbial surface molecules, such as LPS or peptidoglycan, which are absent from the host. Thus, this premise does not seem to apply to galectins, which bind similar self or non-self molecular patterns. This paradox underscores, first, the oversimplification in the use of PRR terminology, and second, the gaps in our knowledge of recognition properties of the host galectin repertoire and the dynamic and mechanistic aspects of the subcellular compartmentalization and secretion of its members, as well as the detailed structural and biophysical aspects of their interactions with microbial carbohydrate moieties. All these aspects warrant further investigation.

From an evolutionary standpoint, it has been proposed that the microbial and host glycomes and their receptors continuously evolve to escape mutual recognition, in a process known as the Red Queen effect<sup>146</sup>, by which the microorganisms avoid recognition by the host PRRs, while the host avoids colonization through microbial factors (agglutinins, adhesins and lectins). Given the key parts played by galectins in host development and immunoregulation through the recognition of self lactosamine moieties, strong functional constraints should prevent galectins from undergoing dramatic evolutionary changes in carbohydrate specificity, which is to some extent supported by the apparent structural conservation within this lectin family. Furthermore, as pathogens and parasites seem to subvert host galectins to attach or gain entrance into host cells, it seems plausible that instead of avoiding recognition by the host, they have evolved their glycomes to mimic their hosts' in a 'Trojan horse' model<sup>127</sup> and rely on the host's self-recognition molecules, such as galectins, for attachment to the vector or host invasion. It is noteworthy that most (if not all) of these pathogens and parasites are endowed with diverse and powerful mechanisms to evade intracellular killing by the host and/or downregulate downstream immune responses. The complex strategies developed by microbial pathogens to successfully colonize, enter, proliferate and disseminate within and among their vectors or hosts are the products of strong selective pressures. Such pressures have led to adaptations that ensure the survival of microorganisms in the most hostile environment of all, and therefore represent a substantial challenge for the

development of novel strategies for intervention in human disease. The novel insights provided by the realization that galectins are directly involved in pathogen recognition has opened new avenues of research aimed at disrupting their roles in parasite–vector interactions or host invasion.

### Online-only summary

- Virtually all bacterial and eukaryotic cells, as well as many viruses, display surface carbohydrates, which have a crucial role in the establishment of host-microorganism complex interactions through their recognition by protein receptors, mainly known as lectins.
- Lectin-glycan interactions are ubiquitous and essential to biological systems, not simply as the ‘glue’ between cells, but as the initiators of a functional crosstalk that modulates their physiology and homeostatic balance. Microbial lectins, including viral haemagglutinins, bacterial adhesins and parasite lectins, are involved in host colonization, whereas some animal lectins can function as pattern recognition receptors in immune responses against microbial pathogens and parasites.
- Among the various lectin families, the galectins are proteins that are characterized by a unique binding-site sequence motif, affinity for  $\beta$ -galactosides and wide taxonomic distribution. Most metazoans are endowed with a complex galectin repertoire, with members exhibiting multiple isoforms and subtle variations in carbohydrate specificity, which together with a certain level of plasticity in sugar binding suggests they have substantial diversity in recognition properties.
- Galectins were initially thought to only bind endogenous ‘self’ glycans and mediate developmental processes, including cell differentiation and tissue organization, and more recently, regulation of immune homeostasis. In the past few years, however, it has become clear that galectins also bind non-self glycans on the surface of potentially pathogenic microorganisms (viruses, bacteria, protista and fungi) and parasitic worms, and mediate recognition and effector functions in innate immunity.
- Some pathogens and parasites subvert the roles of galectins as PRRs to either attach to suitable epithelia in their insect vector or final host, or to enter the host cells to proliferate and disseminate systemically. Furthermore, galectins from parasites might contribute directly or indirectly to host invasion, or downregulation of the host immune response.
- In summary, the recent evidence discussed in this Review indicates that host galectins can function as recognition receptors that target non-self glycans on the surfaces of viruses, bacteria, protista and helminth pathogens and parasites, and either prevent or facilitate infection. Because galectins also bind self glycans on the host cell surface as the first step in immunoregulation and developmental processes, galectins do not fit current models of innate immune self or non-self recognition or defence.
- Gaps in our knowledge about the diversity of the host galectins, their subcellular compartmentalization and secretion, and structural and biophysical aspects of their interactions with the microbial carbohydrate moieties warrant further investigation. The novel insights provided by the realization that galectins are directly involved in pathogen recognition has opened new avenues of research aimed at disrupting their roles in parasite-vector interactions or host invasion.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The author thanks M. Bianchet, Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, for providing the galectin structural models in BOX 1. He also thanks R.D. Cummings, Department of Biochemistry, Emory University School of Medicine, S. Sato, Research Center for Infectious Diseases, Laval University, and L.G. Baum, Department of Pathology and Laboratory Medicine, UCLA School of Medicine, for useful comments on FIG. 5. Research in the authors laboratory was supported by grant R01 GM070589-01 from the National Institutes of Health, grants IOB-0618409 and IOS-0822257 from the National Science Foundation and grant NA05NMF4571243 from the National Oceanic and Atmospheric Administration. The author apologizes to the numerous investigators whose articles could not be cited owing to space constraints.

## Biography

### Author biography

Gerardo R. Vasta earned his Ph.D. degree in biochemistry from the university of La Plata, Argentina, in 1978, and his Ph.D. degree in zoology in 1980. He conducted postdoctoral studies at the Roswell Park Cancer Institute, New York, uSA, and at the Medical university of South Carolina, uSA. He is currently Professor at the university of Maryland Biotechnology Institute, Maryland, uSA. His primary scientific interests include structural, functional and evolutionary aspects of protein-carbohydrate interactions in innate immunity, host-parasite associations and development.

## Glossary

<b>Pattern-recognition receptor</b>	Soluble or membrane-associated receptor displayed by the metazoan host that can recognize complex molecular patterns on the surface of microorganisms.
<b>Innate immunity</b>	Constitutive immunity that in contrast to acquired or adaptive immunity, is not inducible to specific antigens and does not require prior sensitization through an antigen from, for example, an infection or vaccination.
<b>Glycome</b>	The entire carbohydrate repertoire, whether free or present in more complex molecules, of an organism.
<b>Adaptive immune response</b>	A response to a stimulating agent, such as protein, carbohydrate, or a pathogen, that improves recognition of the agent and, after neutralization of the agent, allows the system to retain immunological memory.
<b>Collectin</b>	A C-type lectin characterized by a collagen-like domain. A coiled-coil neck domain connects the carbohydrate recognition domains with the collagen-like domain, and a cysteine-rich domain is present at the amino terminus. Collectin subunits are usually organized as trimers, and trimeric units are assembled in numbers that differ among members of the collectin family.
<b>Apoptosis</b>	Programmed cell death in multicellular organisms that is characterized by cell shrinkage, cytoskeleton break-up, chromatin condensation and chromosomal fragmentation. In contrast to



	necrosis, elimination of apoptotic cells or their fragments has no detrimental effects on the organism.
<b>Metacyclogenesis</b>	The process by which non-infective promastigotes mature into the infective parasite life stage. In <i>Leishmania</i> spp., this process takes place in the midgut of the insect vector, and the changes in metabolism (such as protein kinase A activity) and expression of surface molecules that take place during the process are major determinants of parasite virulence.
<b>Microneme</b>	Small, osmiophilic, elongated secretory organelle that forms part of the apical complex located within the extreme apical region of the apicomplexan parasite, under the inner membrane complex. Micronemes discharge their contents (micronemal proteins) during initial contact of the parasite's apical pole with the host cell surface.

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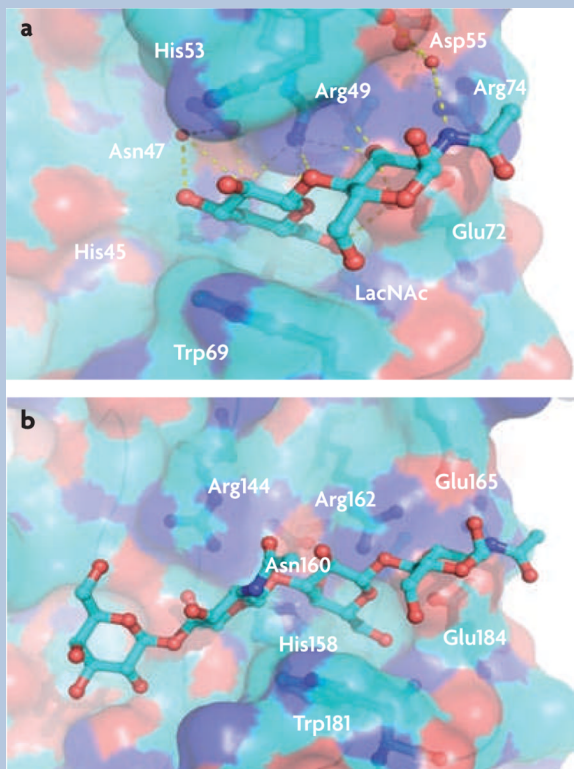
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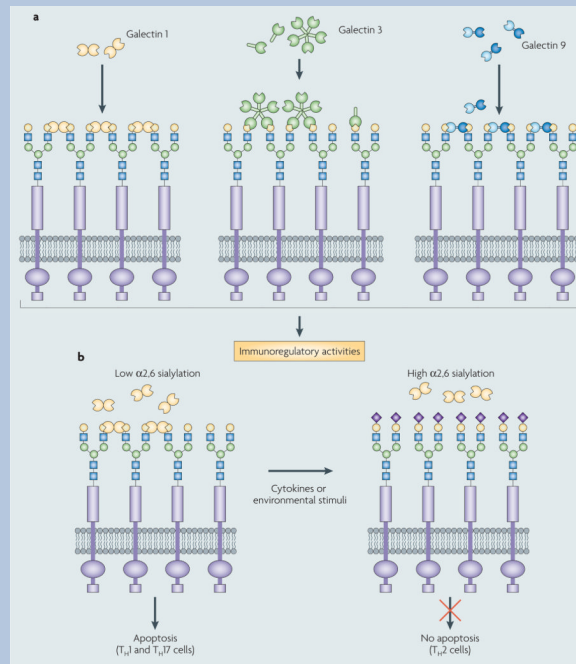
### Galectin–oligosaccharide complexes



Galectins are  $\beta$ -galactoside-binding lectins. Their preferred ligands are *N*-acetylglucosamine (LacNAc; Gal $\beta$  1,4GlcNAc) and related disaccharides, and they have dissociation constants in the order of  $10^{-5}$  M<sup>31</sup>. Glycans that contain polylactosamine chains ((Gal $\beta$  1,4GlcNAc)*n*), such as laminin, fibronectin, lysosome-associated membrane proteins and mucins, are the preferred endogenous ligands for galectins from vertebrates<sup>48–51</sup>. Determination of the crystal structure of bovine galectin 1 complexed with LacNAc (see the figure, part **a**) revealed the galectin structural fold, and enabled the identification of the amino acids in the galectin polypeptide and the hydroxyl groups of the sugar ligands that participate in protein-carbohydrate interactions<sup>44–47</sup>. Unlike galectin 1, galectin 3 (see the figure, part **b**) has an extended carbohydrate-binding site formed by a cleft open at both ends, which leads to increased affinity for glycans with multiple lactosamine units and substitution of the non-reducing terminal galactose moiety with ABH blood group oligosaccharides (Fuca 1,2, GalNAc $\alpha$  1,3 (Fuca 1,2) and Gala 1,3(Fuca 1,2))<sup>47,48</sup>.

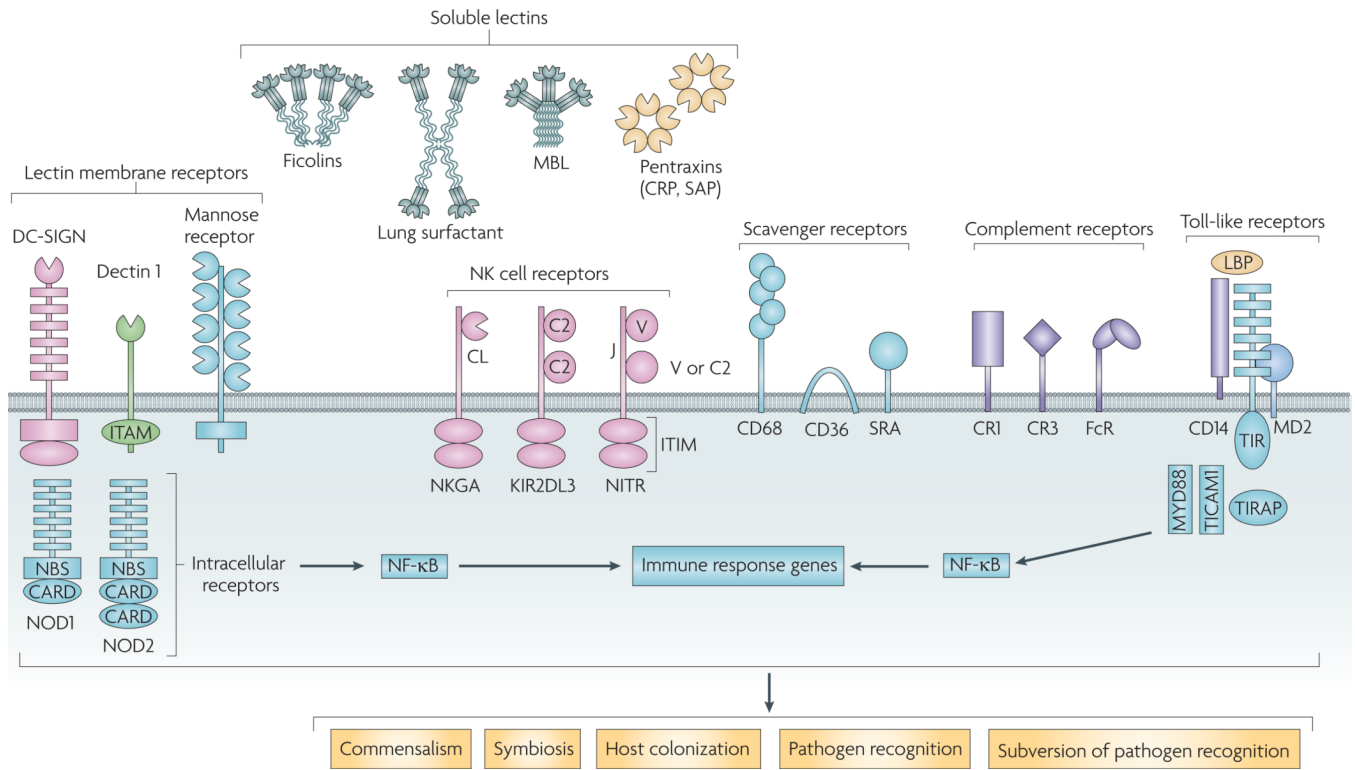


### Galectin–ligand lattices at the cell surface lead to immunoregulatory activity



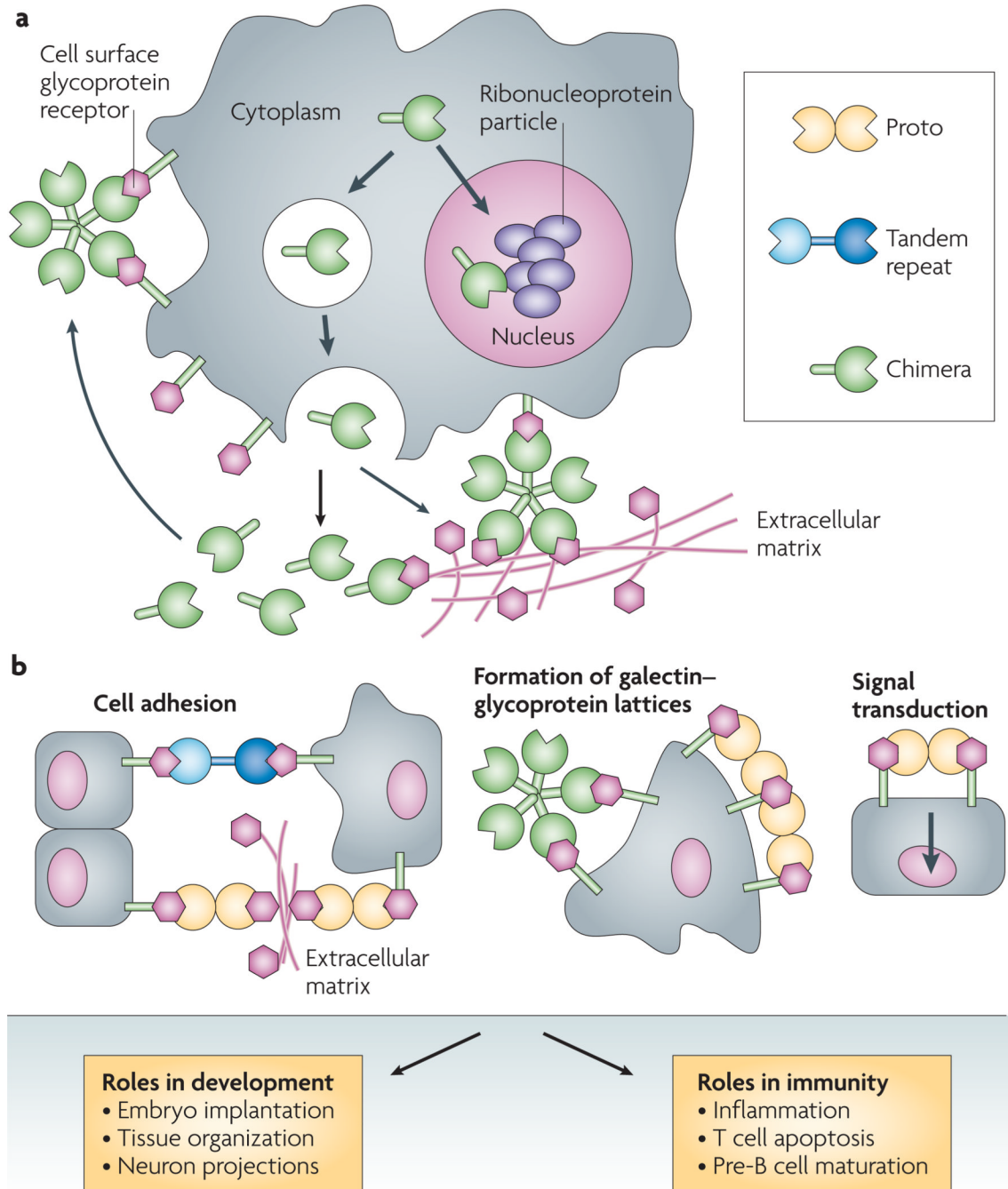
Proto-type galectins (for example, galectin 1) associate as non-covalently bound dimers through a hydrophobic interphase, whereas chimera galectins (galectin 3) associate through their amino-terminal domain to form oligomers that, in the presence of multivalent oligosaccharides, display binding cooperativity<sup>31</sup> (see the figure, part **a**). Bivalent tandem repeat-type galectins (for example, galectin 9) can recognize ligands with a single polypeptide, although they can also form higher-order aggregates that enhance their avidity<sup>31,56</sup>. Galectin-mediated lipid raft assembly can modulate turnover of endocytic receptors, signal transduction pathways that lead to T cell activation and cytokine secretion or apoptosis, B cell maturation, activation and tolerance, and neutrophil activation that leads to phagocytosis, oxidative burst, and protease and cytokine release<sup>57</sup>. Galectin-glycoprotein lattices at the cell surface have therefore been proposed to function as an ‘on-off switch’ that regulates cell proliferation, differentiation and survival, including immune cell responsiveness and tolerance<sup>31,57</sup>.

Galectins form cell surface lattices and polarize the T helper response ( $T_H1$ ,  $T_H2$  or  $T_H17$ ) through galectin 1-induced apoptosis (see the figure, part **b**). Differential sialylation of cell surface glycoproteins selectively influences the formation of galectin-carbohydrate lattices in distinct  $T_H$  cells, thereby regulating their susceptibility to galectin 1-induced cell death.  $T_H1$  and  $T_H17$  effector cells express cell surface glycans that enable the formation of galectin-glycoprotein lattices. By contrast,  $T_H2$  cells are protected from galectin 1 by differential  $\alpha2,6$ -sialylation of surface glycoproteins. Figure adapted, with permission, from REF. 58 © (2007) Elsevier Science.



**Figure 1. Pattern-recognition receptors and their participation in microbial-metazoan interactions**

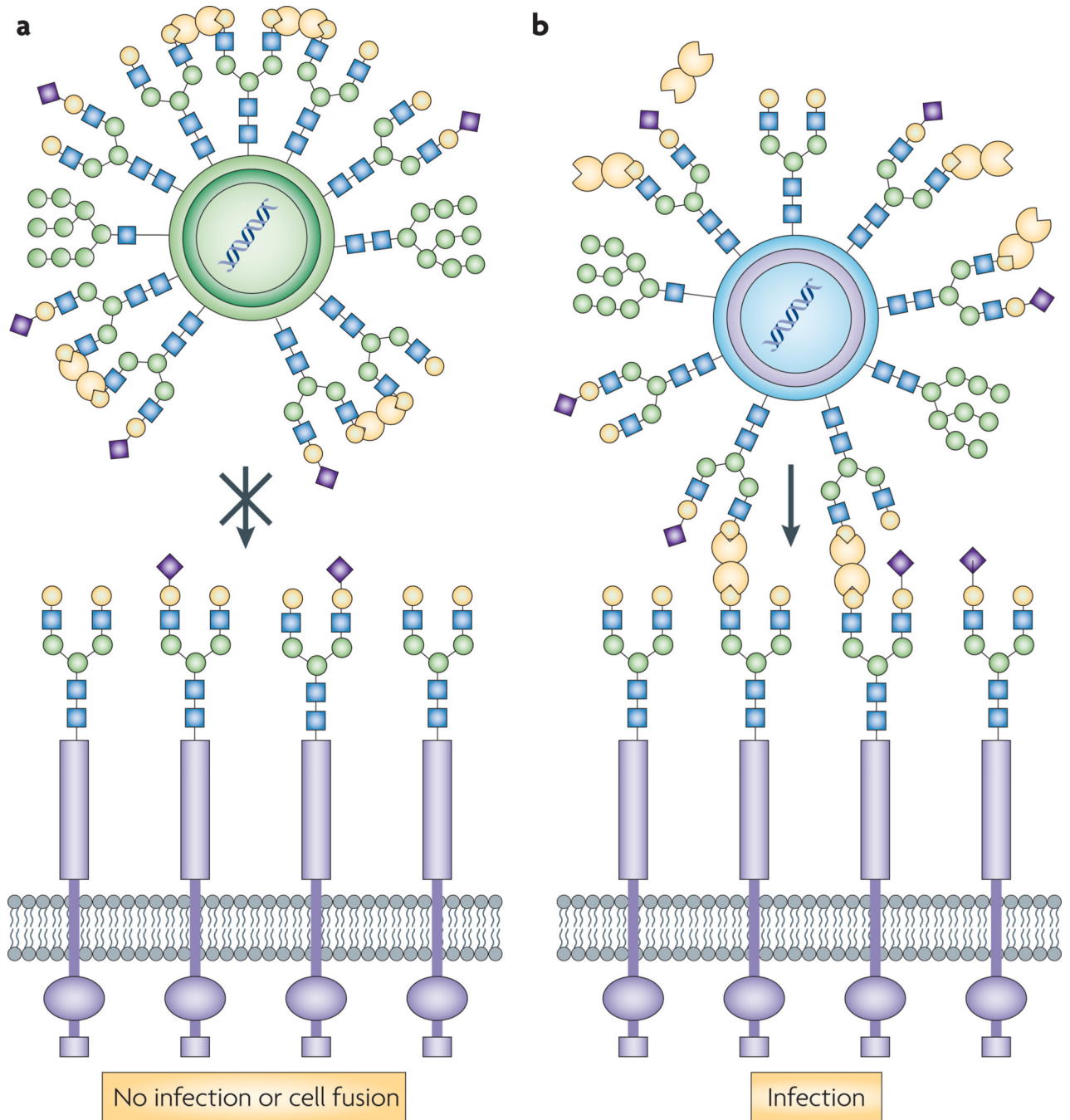
Extracellular pattern-recognition receptors (PRRs) include the soluble lectins, such as collectins (for example, mannan-binding lectins and lung surfactants), ficolins and pentraxins (for example, C-reactive proteins and serum amyloid-P), and integral membrane C-type lectins, including dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), dectin 1 and the macrophage mannose receptor. Other extracellular receptors shown are the natural killer (NK) cell receptors, scavenger receptors and complement receptors. The extracellular Toll-like receptors and the intracellular nucleotide-binding oligomerization domain (NOD) receptors, both of which are rich in leucine rich repeats, activate immune genes through nuclear factor- $\kappa$ B (NF- $\kappa$ B). Soluble and membrane-associated lectins mediate interactions with microorganisms that may lead to mutualistic interactions (commensalism or symbiosis), host colonization, immune recognition by the host or ‘subversion’ of the non-self recognition functions of the receptors by the microorganisms for attachment to the vector or host invasion. CARD, caspase recruitment domain; CRP, C-reactive protein; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; KIR2DL3, killer cell immunoglobulin-like receptor 2DL3; LBP, lipopolysaccharide-binding protein; MBL, mannan-binding lectin; NBS, nucleotide-binding site; NKG2A, natural killer glycoprotein C-lectin receptor; NITR, novel immune-type receptor; SAP serum amyloid protein; TICAM1, TIR domain-containing adapter molecule 1; TIRAP, Toll/interleukin-1 receptor domain-containing adapter protein. Figure adapted, with permission, from REF. 147 ©(2003) Elsevier Science.



**Figure 2. galectin types, binding activity and biological roles at the cell surface**

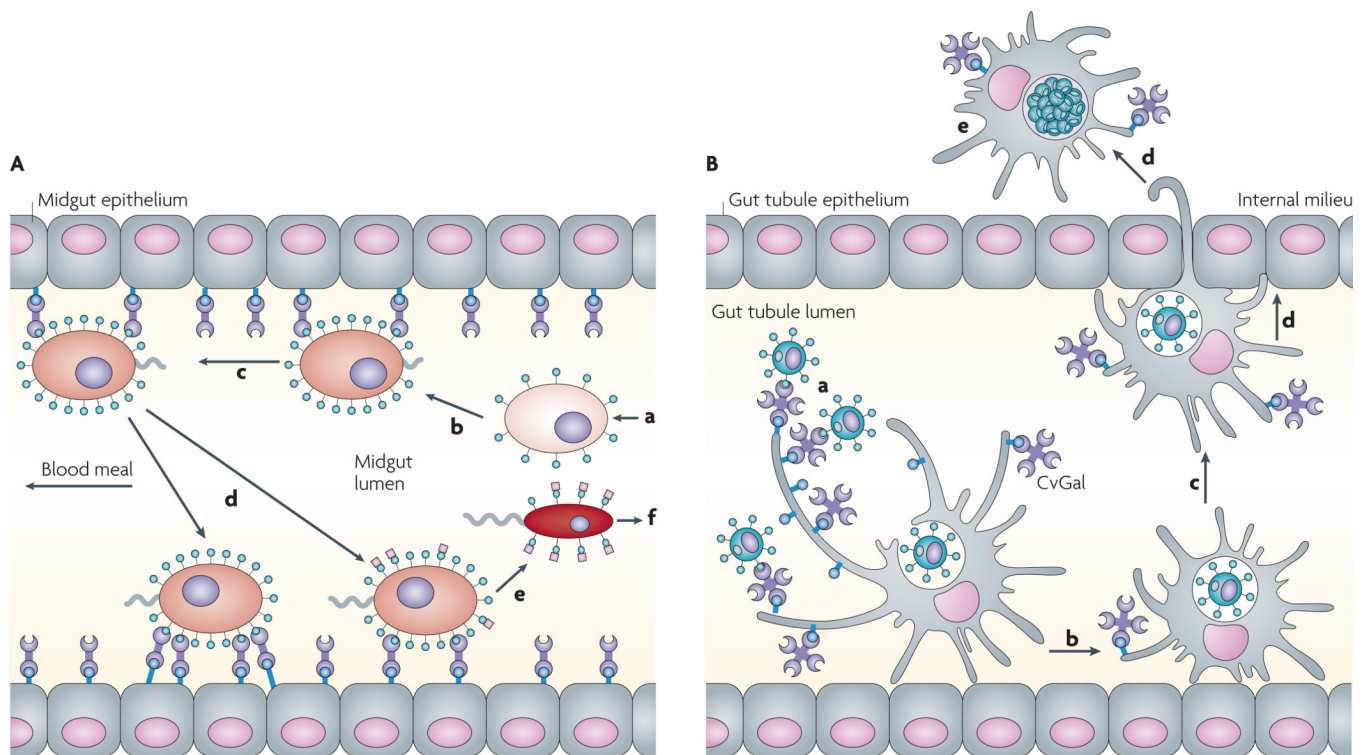
**a** | Subcellular localization and secretion. Based on structural features, galectins have been classified into three types: proto, chimera and tandem repeat. Proto-type galectins contain one carbohydrate-recognition domain (CRD) per subunit and are usually homodimers of non-covalently linked subunits (such as mammalian galectins 1, 2, 5, 7, 10, 11, 13, 14 and 15). By contrast, chimera-type galectins are monomeric with a carboxy-terminal CRD that is similar to the proto type, joined to an amino-terminal peptide that contains a collagen-like sequence rich in proline and glycine (such as mammalian galectin 3). Tandem-repeat galectins, in which two CRDs are joined by a linker peptide, are also monomeric (such as mammalian galectins 4, 6, 8, 9 and 12). From the cytosol, galectins can be translocated into

the nucleus or into vesicles, or can accumulate at sub-cytosolic sites. Although galectins lack a typical secretion signal peptide, they can be targeted for secretion by non-classical mechanisms by direct vesicle translocation across the plasma membrane. Once in the extracellular space, they can bind to cell surface glycans and crosslink them with carbohydrate moieties in the extracellular matrix. **b** | Binding activity at the cell surface and biological roles. Secreted galectins can bind to the cell surface *in cis* or *in trans*, and exert a range of functions. The binding of galectins to cell surface glycans can crosslink glycans on neighbouring cells, leading to cell adhesion. Galectins can also form homogeneous lattices at the cell surface that can activate signalling pathways of functional relevance in the control of receptor endocytosis, host-pathogen interactions, and activation and homeostasis of immune cells. Part **a** adapted, with permission, from REF. 148 ©(2003) Oxford Univ. Press.



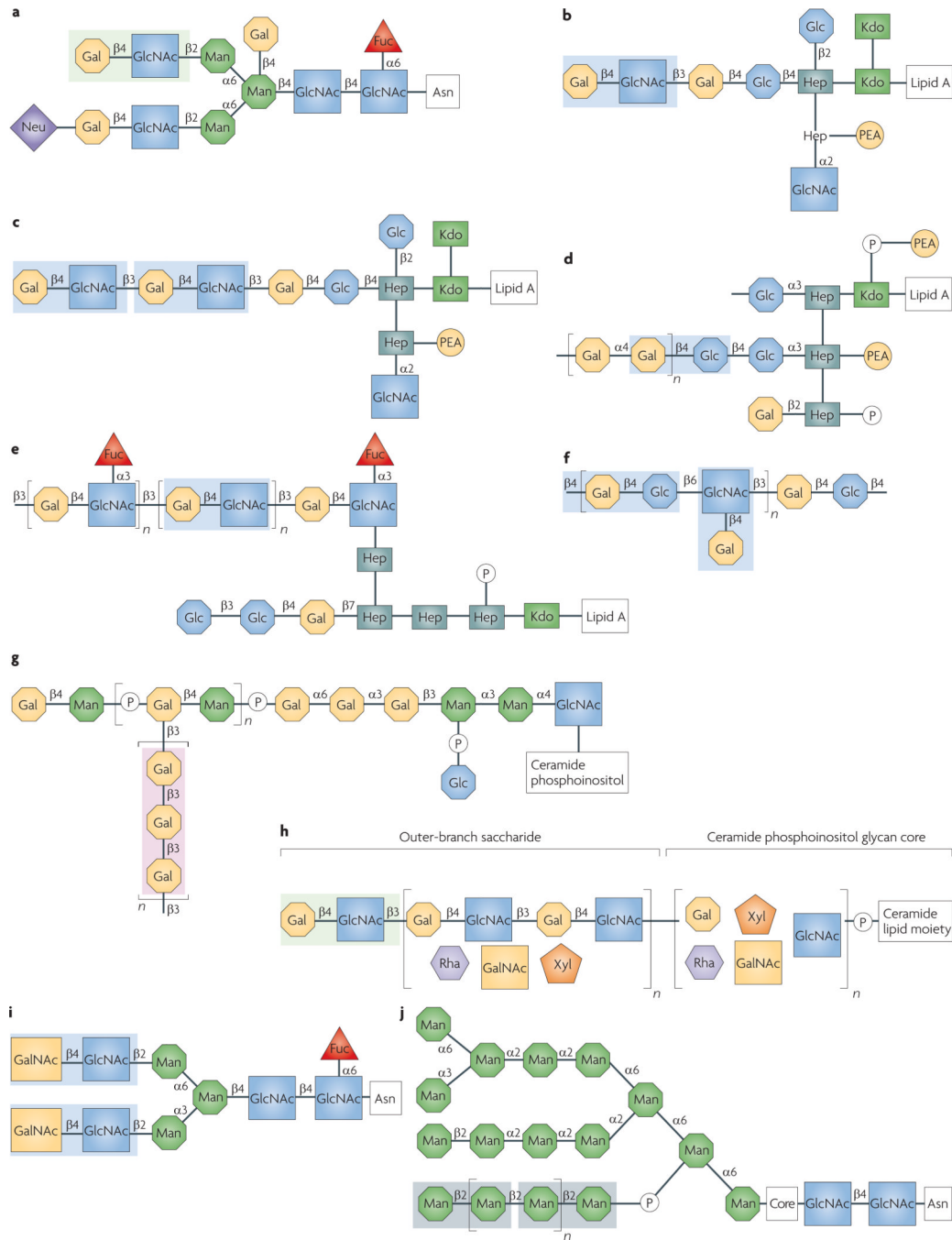
**Figure 3. galectin 1 functions as a pattern-recognition receptor for paramyxoviruses and HIV-1**  
**a** | In both Nipah virus and Hendra virus (*Paramyxoviridae* family), galectin 1 specifically crosslinks the N glycans displayed in the envelope glycoproteins, causing aberrant oligomerization and blocking cell–cell fusion<sup>73</sup>. **b** | Galectin 1 promotes infection by HIV-1 by mediating viral attachment to host cell surface glycans<sup>113</sup>. Galectin 1 enhances HIV-1 adsorption kinetics on monocyte-derived host macrophages, which facilitates HIV-1 infectivity by shortening the time required to establish an infection<sup>114</sup>.





**Figure 4. galectins function as protein-recognition receptors for parasites**

**A** | Recognition of *Leishmania major* lipophosphoglycan (LPG) by the sandfly galectin PpGalec facilitates attachment of the parasite to the midgut. The *L. major* amastigotes (**a**) ingested by the *Phlebotomus papatasi* sandfly from an infected host attach to the vector midgut epithelium (**b**) to prevent their excretion along with the digested blood meal, mature into promastigotes (**c**) and undergo numerous divisions (**d**) before differentiating into infective metacyclics (**e,f**)<sup>71</sup>. The parasite binds through PpGalec, a tandem repeat galectin expressed by the sandfly midgut that recognizes poly-Gal( $\beta$ 1–3) side chains (green circles) on the parasite LPG. During metacyclogenesis, PpGalec can be downregulated or capped with arabinose (pink squares), and is no longer recognized by PpGalec. This allows the free-swimming infective metacyclic promastigotes to detach from the midgut and migrate forward for transmission from the sandfly to the mammalian host<sup>98</sup>. **B** | Recognition of *Perkinsus marinus* trophozoites by the oyster (*Crassostrea virginica*) galectin CvGal facilitates infection<sup>127</sup>: CvGal displays four canonical galectin carbohydrate-recognition domains (CRDs), a domain organization that is unlike any of the known galectin types. CvGal is translocated to the periphery and secreted by attached haemocytes, and binds to the cell surface. *P. marinus* trophozoites (**a**) ingested by filter-feeding are recognized by CvGal on the surface of haemocytes that coat the gut tubules, phagocytosed (**b**) and transported through the gut epithelium (**c,d**) into the internal milieu. The parasite inhibits intracellular killing by the host haemocytes and proliferates (**e**), thereby causing systemic infection and eventually death of the host.



**Figure 5. glycans on the surface of selected microorganisms and protistan or metazoan parasites potentially recognized by galectins 1, 3 and 9**

Complex type N-linked oligosaccharide from the HIV-1 gp120 envelope glycoprotein<sup>149</sup> (a); meningococcal (*Neisseria meningitidis*) lipopolysaccharide (LPS)<sup>150,151</sup> (b); gonococcal (*Neisseria gonorrhoeae*) lipooligosaccharide<sup>151</sup> (c); *Haemophilus influenzae* LPS<sup>152</sup> (d); *Helicobacter pylori* LPS O-antigen side chain<sup>153</sup> (e); *Streptococcus pneumoniae* polysaccharide type XIV<sup>154</sup> (f); *Leishmania major* lipophosphoglycan (LPG)<sup>71,96,155</sup> (g); *Trichomonas vaginalis* LPG<sup>100</sup> (h); *Schistosoma mansoni* LdN<sup>156</sup> (i); *Candida albicans* oligomannan<sup>94,148,157</sup> (j). Carbohydrate moieties that could be recognized by galectins are highlighted: galectin 1, light green; galectin 3, light blue; galectins 3 and 9,

pink. Because there is glycan microheterogeneity and variability within and among microbial and parasite strains and species, either only the most abundant or representative glycan is shown. In the *T. vaginalis* LPG sequence (only partially elucidated), the presence of polylectosamine has been confirmed<sup>100</sup> (**h**). Although these carbohydrate moieties are probably strongly recognized by galectin 3, because only recognition of the *T. vaginalis* LPG by galectin 1 has been reported to date<sup>99</sup>, only the terminal LacNAc disaccharide is highlighted. For the *C. albicans* oligomannan (**j**), the boxed oligosaccharide indicates its requirement for recognition by galectin 3, without implying direct binding<sup>91–94</sup>. Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Hep, heptose; KDO, 2-keto-3-deoxyoctonate; Man, mannose; Rha, rhamnose; Neu, *N*-acetylneuraminic acid; PEA, phosphoethanolamine; Xyl, xylose.

**Table 1**

## Classification of animal lectins

lectin family	Ca <sup>2+</sup> use	Specificity	Defining features	localization
C type	Yes	Variable	C-type sequence motif	Cell membrane; extracellular
Calnexin and calreticulin	Yes	Glc <sub>1</sub> Man <sub>3-9</sub>	Calnexin sequence motif	ER
Chilectins	No	Chitooligosaccharides	TIMβ or TIMα barrel fold	Extracellular
F box	No	GlcNAc <sub>2</sub>	F-box sequence motif	Cytoplasm
F type	Variable	L-fucose	F-type sequence motif and F-type fold	Extracellular
Ficolin	Yes	GlcNAc and GalNAc	Ficolin sequence motif	Cell membrane; extracellular
Galectins	No	β-galactosides	S-type sequence motif	Cytoplasm and nucleus; extracellular
Heparin-binding type	No	Heparin or Heparan-SO <sub>4</sub> <sup>2-</sup>	Basic amino acid clusters	Cytoplasm and nucleus; extracellular
Siglecs (I type)	No	Variable	Immunoglobulin-like domains	Cell membrane
L type	Yes	Variable	L-type sequence motif	ER, ERGICs and Golgi
M type	Yes	Man <sub>8</sub>	M-type sequence motif	ER
P type (M-6-P R)	Variable	Man-6-P	P-type sequence motif	Cell membrane
Pentraxins	Most	Phosphocholine and galactosides	Multimeric binding motif	Extracellular
R type	No	Variable	R-type sequence motif	Golgi and cell membrane
Intelectins (X type)	Yes	Gal, galactofuranose and pentoses	Intelectin sequence motif	Cell membrane; extracellular

ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; Man, mannose.