Galectin-mediated immune recognition: Opsonic roles with contrasting outcomes in selected shrimp and bivalve mollusk species

Gerardo R. Vasta[1]* and Jin-Xing Wang[2]

[1] Department of Microbiology and Immunology, School of Medicine, University of Maryland Baltimore, and Institute of Marine and Environmental Technology, Baltimore, Maryland, USA

[2] Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Sciences, Shandong University, Qingdao, Shandong, China

* Corresponding author: gvasta@som.umaryland.edu

Abstract

Galectins are a structurally conserved family of ß-galactoside-binding lectins characterized by a unique sequence motif in the carbohydrate recognition domain, and of wide taxonomic distribution, from fungi to mammals. Their biological functions, initially described as key to embryogenesis and early development *via* recognition of endogenous ("self") carbohydrate moieties, are currently understood as also encompassing tissue repair, cancer metastasis, angiogenesis, adipogenesis, and regulation of immune homeostasis. More recently, however, numerous studies have contributed to establish a new paradigm by revealing that galectins can also bind to exogenous ("non-self") glycans on the surface of potentially pathogenic virus, bacteria, and eukaryotic parasites, and function both as pathogen recognition receptors (PRRs) and effector factors in innate immunity. Our studies on a galectin from the kuruma shrimp *Marsupenaeus japonicus* (*Mj*Gal), revealed that it functions as a typical PRR. Expression of MjGal is upregulated by infectious challenge, and can recognize both Gram(+) and Gram(-) bacteria. MjGal also recognizes carbohydrates on the shrimp hemocyte surface, and can cross-link microbial pathogens to the hemocytes, promoting their phagocytosis and clearance from circulation. Therefore, MjGal contributes to the shrimp's immune defense against infectious challenge both as a PRR and effector factor. Our studies on galectins from the bivalve mollusks, however, have shown that although they can function in immune defense as MjGal, protistan parasites take advantage of the recognition roles of the host galectins, for successful attachment and host infection. We identified in the eastern oyster *Crassostrea virginica* two galectins (CvGal1 and CvGal2) that not only recognize a large variety of bacterial species, but also the protozoan parasite *Perkinsus marinus*. Like the shrimp MjGal, both oyster galectins function as opsonins, and promote parasite adhesion and phagocytosis. However, *P. marinus* survives intrahemocytic oxidative killing and proliferates, eventually causing systemic infection and death of the oyster host. In the softshell clam *Mya arenaria* we identified a galectin (MaGal1) that displays carbohydrate specificity and recognition properties for sympatric *Perkinsus* species (*P. marinus* and *P. chesapeaki*), that are different from CvGal1 and CvGal2. Our results suggest that although

galectins from bivalves can function as PRRs, *Perkinsus* parasites have co-evolved with their hosts to subvert the galectins' immune functions for host infection by acquisition of carbohydrate-based mimicry.

1. Introduction

A critical step for the host's successful immune response against infectious challenge is the immediate detection of foreign surface structures or soluble products from the potential pathogens and parasites by pattern recognition receptors (PRRs) that can induce multiple downstream effector functions aimed at immobilizing, killing, and clearing the pathogen or parasite from the host's internal milieu (Janeway and Medzhitov. 2002). For example, carbohydrate moieties on bacterial lipopolysaccharides and exopolysacharides, viral envelope glycoproteins, and various surface or soluble glycans from eukaryotic parasites can be recognized by one or more carbohydrate-binding proteins (lectins) from the host (Laine, 1997; Vasta and Ahmed, 2008). Lectins can form soluble or membrane-associated oligomeric assemblies of two or more polypeptide subunits, resulting in multivalent carbohydrate-binding proteins that enable cross-linking of target ligands (Dam and Brewer, 2008, 2010). Thus, lectin-mediated recognition can lead to agglutination and immobilization, opsonization, phagocytosis, and intracellular oxidative killing of the pathogens, as well as activation of complement or prophenoloxidase cascades that can promote their extracellular killing, followed by clearance from the host. Therefore, in both invertebrates and vertebrates, lectins are critical components of innate immune responses to infection (Vasta, *et al* 2007; Wang and Wang, 2013a)

While lacking the typical adaptive immune response of vertebrates characterized by immunoglobulins, and B and T cells, invertebrates share with vertebrates very rapid and effective innate immune mechanisms against infection (Locker *et al,* 2004). These mechanisms include both cell-associated receptors and soluble recognition molecules (Janeway and Medzhitov, 2002; Lemaitre and Hoffmann, 2007; Wang and Wang, 2013a) among which lectins play a key role (Vasta and Ahmed, 2008; Wang and Wang, 2013b). The presence of unique amino acid sequence motifs and conserved structural fold of the carbohydrate recognition domains (CRDs) have enabled the classification of lectins into several major families, such as galectins (formerly S-type lectins), C-, F-, L-, P-, X-, and I-types, heparin-binding, and others. Although lectins from several families are exclusively involved in intracellular functions, C-, F- and X-type lectins have been extensively studied as PRRs (Vasta and Ahmed, 2008; Zhang *et al,* 2014; Xu *et al,* 2014)

2. Galectins: A Structurally Conserved and Functionally Diversified Lectin Family

a. Structural Aspects Among the lectin families described so far, galectins are characterized by their binding affinity for β-galactosides, a unique conserved sequence motif in their CRD, wide taxonomic distribution from fungi to invertebrates and vertebrates (Leffler *et al*, 2004; Vasta and Ahmed, 2008; Cummings *et al,* 2015-2017), and striking functional diversification (Leffler *et al* 2004; Vasta, 2009; Rabinovich and Croci, 2012). Based on structural features, galectins from vertebrates have been classified into the "proto", "chimera", and "tandem-repeat" types (Hirabayashi and Kasai, 1993). The first (proto-type) is characterized by single CRD subunits that usually form non-covalently-linked homodimers. The proto-type galectin-13 ("pregnancy protein 13") is an exception, since in solution the dimer is established by a disulfide bridge between Cys^{136} and Cys^{138} (Than *et al*, 2004). The chimera-type galectin subunits display a C-terminal CRD, and an N-terminal domain rich in proline and glycine that can promote oligomerization into trimers or pentamers. In the tandem-repeat type, two CRDs are joined by a linker peptide, are mostly monomeric. Galectin-related proteins that do not follow the canonical structure of the three major galectin types include the eye lens protein GRIFIN (Galectin related inter-fiber protein), the galectin-related protein GRP (previously HSPC159; hematopoietic stem cell precursor), and the Charcot-Leyden crystals (Galectin 10) (Su *et al,* 2018; Zhou *et al*, 2018; Ogden *et al*, 1998). Galectins from invertebrates can display structural features and domain organizations that do not fit within the three mammalian galectin types describe above, with some molecular species from bivalve mollusks housing four CRDs (Vasta *et al*, 2015), or constituting hybrid structures, such as the chimeric galectin-related protein (GREP) in the snail *B. glabrata* (Dheilly *et al*, 2015).

The structure of mammalian galectin-1 (Gal1) in complex with N-acetyl-lactosamine (LacNAc) enabled the identification of the specific amino acid residues of the CRD that interact with the carbohydrate ligand (Liao *et al*, 1994). The 135 amino acids-long polypeptide subunit of Gal1 folds into a β-sandwich that comprises two antiparallel β-sheets of five and six strands each (S1–S6 and F1–F5). The primary carbohydrate binding site is formed by three strands (S4-S6) in which H^{44}, B^{46}, R^{48}, H^{52}, B^{61}, W^{68}, E^{71}, and R^{73} establish direct interactions with LacNac (Liao *et al*, 1994). Further, W^{68} establishes a hydrophobic interaction with the non-reducing terminal galactose ring, while additional water-mediated interactions between H^{52}, D^{54}, and R^{73} and the nitrogen of the N-acetyl group determine the higher affinity for LacNAc over lactose (Lac). In this regard, the dissociation constants of Gal1 for Lac, LacNAc, and thiodigalactoside (TDG) are in the range of 10^{-5} M, with two binding sites per Gal1 dimer (Schwarz *et al*, 1998). In addition to the primary binding site in the galectin CRD, additional carbohydrate-binding features ("extended binding site") can enhance interactions with more complex glycans. For example, the galectin-3 (Gal3) carbohydrate-binding site is shaped as a cleft open at both ends, resulting in increased affinity for polylactosamines and ABH blood group oligosaccharides (Seetharaman *et al*, 1998). For galectins from invertebrates, such

as the *Caenorhabditis elegans* 16-kDa galectin and the oyster (*Crassostrea virginica*) galectins CvGal1 and CvGal2, their binding specificity for blood group oligosaccharides is determined by a shorter loop 4 in the primary binding cleft in the galectin CRD (Ahmed *et al*, 2002; Feng *et al*, 2013; Vasta *et al*, 2015).

Oligomerization of galectin subunits (proto and chimera types) or the presence of multiple distinct CRDs in TR galectins results in binding multivalency and increased avidity (Dam and Brewer, 2008, 2010), that is critical to galectin function. Interactions of multivalent galectins with multivalent glycans on the cell surface can lead to their cross-linking and formation of clusters, microdomains, and lattices that are critical for signaling or receptor endocytosis, as well as cross-linking of two or more cells, and adhesion of cells to glycosylated surfaces (Rabinovich *et al*, 2007; Garner and Baum, 2008; Kutzner *et al*, 2019) **(Fig 2A)**. Further, while in proto and chimera galectins, the multivalent oligomers display CRDs with identical specificity and affinity, for the vertebrate TR galectins and the multi-CRD galectins from invertebrates the carbohydrate specificity of the CRDs present in the polypeptide may be similar but not identical, enabling the protein to crosslink different glycans or cells (Houzelstein *et al*, 2004; Krejcirikova *et al*, 2011; Vasta *et al*, 2015). In addition, galectins that are bound to carbohydrate ligand are less susceptible to proteolytic cleavage or oxidative inactivation (Liao *et al*, 1994; Stowell *et al*, 2009).

b. Functional Aspects: Galectins can mediate key biological functions in both the intracellular (cytosolic and nuclear) and extracellular compartments. By binding to endogenous ("self") glycans, galectins can mediate developmental processes, and tissue repair, as well as regulation of immune homeostasis, cancer metastasis, angiogenesis, and adipogenesis, among others (Leffler et al, 2004; Vasta and Ahmed, 2008; Cummings *et al*, 2015-2017). Multivalent interactions of extracellular galectins with cell surface glycans can modulate cell function by inducing their reorganization or association in lattices, regulating turnover of endocytic receptors, and modulating signaling pathways (Garner and Baum, 2008; Rabinovich *et al*, 2007; Dam and Brewer, 2010). Furthermore, differences in carbohydrate specificity, affinity, and avidity of the various galectin types, subtypes, and isoforms, endow the galectin repertoire in any given species with substantial diversity in recognition properties, that together with their unique tissue-specific expression, distribution and local concentrations, results in extensive functional diversification (Cooper, 2002; Vasta and Ahmed, 2008; Vasta, 2009; Takeuchi, 2018).

Soon after the discovery of galectins, the observation that those present in chicken muscle were developmentally regulated and preferentially recognized polylactosamines present on the myoblast surface and the extracellular matrix (ECM), suggested a function in embryogenesis and early development (Reviewed in Cummings *et al*, 2017). Later research on Gal1 and galectin-3 (Gal3) revealed their roles in development of notochord, skeletal muscle and central nervous system

(Colnot *et al*, 1997; Colnot *et al*, 2001; Georgiadis *et al*, 2007). The increasing availability of null mice for selected galectins enabled the analyses of developmental phenotypes and the elucidation of their unique functions. More recently, genetically-tractable models such as *C. elegans, Drosophila,* and zebrafish (*Danio rerio*) have become alternative useful systems for addressing the diverse functions of galectins (Pace *et al*, 2002; Ahmed *et al*, 2002; Nemoto-Sasaki *et al*, 2002; Ahmed *et al*, 2009; Craig *et al*, 2010; Shi *et al*, 2014; Feng *et al*, 2015; Eastlake *et al*, 2017; Takeuchi, 2018).

Galectins are expressed and distributed in most mammalian tissues, including cells of the innate and adaptive immune system (Stowell *et al*, 2008; Rabinovich *et al*, 2012). Galectins are regulators of innate immune homeostasis (DiLella *et al*, 2011), and can function as pro- or anti-inflammatory factors (Hirashima *et al*, 2002; Stowell *et al*, 2008; Liu *et al*, 2012; Toledo *et al*, 2014). Galectins are also modulators of lymphocyte development and adaptive immune responses (Rabinovich *et al*, 2012; Liu *et al*, 2012). Gal1-mediated interactions of lymphocyte precursors with stromal cells in bone marrow and thymus are a key step for their further development, selection, and migration to the periphery (Rossi *et al*, 2006). Further, galectins can also either promote or attenuate apoptosis or proliferation, modulate cytokine synthesis and secretion by T cells, and promote feto-maternal tolerance (Rabinovich *et al*, 2012; Liu *et al*, 2012; Blois *et al*, 2019). Thus, it has become firmly established that galectins can modulate immune homeostasis with either beneficial or detrimental effects on pathological conditions that result from depressed or exacerbated immune function, such as cancer, inflammation, allergy, and autoimmune disorders (Rabinovich *et al*, 2012; Liu *et al*, 2012; Hsu and Liu, 2008; Martínez Allo *et al*, 2020).

The multiple roles of galectins in cancer has been addressed with increasing interest over the past two decades (Reviewed in Méndez-Huergo *et al*, 2017). Expression profiles of selected galectins in melanoma, prostate and ovarian cancer can be associated with malignancy stage or metastatic potential (Blidner *et al*, 2015; Hill *et al*, 2010), and increased angiogenesis (Croci *et al*, 2014). In early stages of prostate adenocarcinoma, Gal3 expression is silenced by promoter methylation (Ahmed *et al*, 2009; Ahmed and AlSadek, 2015), but is expressed in later stages and enhances tumor angiogenesis, tumor-endothelial cell adhesion, metastasis, and evasion of immune surveillance (Guha *et al*, 2013). In recent years, the adipogenic roles of Gal12 and Gal3 and their associations with type 2 diabetes, obesity, and inflammation have been characterized in detail (Yang *et al*, 2011; Hsu *et al*, 2018; Pejnovic *et al*, 2013; Pang *et al*, 2013).

During the past decade, evidence has mounted in support of new paradigm by revealing that galectins can also bind to exogenous (“non-self”) glycans on the surface of potentially pathogenic virus, bacteria, and eukaryotic parasites and function as PRRs. Furthermore, downstream of pathogen recognition, galectins also function as effector factors in innate immunity, as they can

inhibit pathogen adhesion and/or entry into the host cell, or either directly kill certain pathogens by disrupting its surface structures, or promote their agglutination, phagocytosis or encapsulation, and clearance from circulation (Vasta 2009, Vasta *et al*, 2012, Shi *et al*, 2014).

3. Galectins as PRRs in Defense Against Infection: Recognition of Exogenous ("Non-self") Glycans and Effector Functions

The recent finding that galectins can recognize glycans on the surface of viruses, bacteria, protistan parasites, and fungi, has supported their proposed roles in innate immunity as PRRs (Reviewed in Vasta, 2009) **(Fig 2B)**. As discussed above, the considerable diversity in fine carbohydrate specificity of the galectin repertoire(s) in each species, suggest a broad capacity for recognition of potential pathogens and parasites. Furthermore, the capacity for multivalent binding of all three galectin types enables the direct recognition of pathogens and parasites, their crosslinking to the host's phagocytic cells, and the activation of signaling pathways and downstream processes that lead to modulation of immune responses (Di Lella *et al*, 2011). Additionally, as multiple innate immune receptors such as TLRs and C-type lectins are present in any single species, cooperative defense functions of galectins with other receptors have been described (Esteban *et al*, 2011; Jouault *et al*, 2006). As will be discussed in the following sections, the binding of host galectins to the pathogen or parasite surface glycans, and their crosslinking to host cell receptors can lead to various outcomes beneficial to the host, including direct killing of the pathogen, blocking of pathogen attachment to the host cell surface, or opsonization, phagocytosis, encapsulation, and clearance of the pathogen. However, this is not always the case, and recognition of infectious agent by galectins can also lead to detrimental outcomes for the host, by facilitating attachment, entry and infection by pathogens and parasites. This subversion of the defense role(s) of the host galectins by the pathogens and parasites is likely the product of the host-pathogen co-evolutionary process (Vasta, 2009).

Galectins of all three major types have been experimentally shown to recognize a variety of glycans on the surface of virus, bacteria, protozoan parasites and yeasts (Reviewed in Vasta, 2009). Upon recognition of the pathogen or parasite by a galectin, several different outcomes have been reported. Some galectins can not only bind to, but also directly kill the pathogens. The tandem repeat galectins Gal4 and Gal8 are expressed in the human intestinal tract, and can specifically recognize and kill *Escherichia coli* strains that display B-blood group oligosaccharides; other *E. coli* strains or bacterial species are not affected (Stowell *et al*, 2010). Recognition is carried out by the C-CRD, whereas the N-CRD would be responsible for the bactericidal activity by disrupting the target cell surface integrity (Stowell *et al*, 2010). Similarly, Gal3 displays recognition, agglutination and bactericidal effect against *Helicobacter pylori* and displays a potent, associated with drastic changes

in the bacterial morphology (Park *et al*, 2016). Galectin-3 has also been reported to bind to and directly kill fungal pathogens such as *C. albicans* and *C. neoformans,* although the detailed mechanism(s) involved in the fungicidal activity has not been fully elucidated (Kohatsu *et al*, 2006; Almeida *et al*, 2017).

Galectins can bind to the pathogen or parasite surface and while not killing or inactivating it, they can hinder their attachment to the host cell surface and prevent infection. Gal1 and Gal9 can bind to the envelope glycoprotein of influenza A virus and reduce infection severity (Yang *et al*, 2011; Machala *et al*, 2019; Hattori et al, 2013; Katoh *et al*, 2014). Gal1 can also recognize Dengue virus inhibiting viral adhesion and internalization into host cells *in vitro* (Toledo *et al*, 2014). Direct interaction of Gal1 with the envelope glycoproteins of the Nipah virus Gal1 can reduce cell–cell fusion and attenuate the pathophysiologic effects of infection Levroney *et al*, 2005; Garner *et al*, 2015). Similarly, the zebrafish Gal1 and Gal3 can bind to the infectious hematopoietic necrosis virus and hinder adhesion to the fish epithelial cell surface (Nita-Lazar *et al*, 2016; Gosh *et al*, 2019). A similar mechanism has been proposed for Gal4, which hinders attachment of *Bordetella pertussis* and *Helicobacter pylori* by binding to their gut epithelial cell surface receptors (Danielsen *et al*, 2006; Ideo *et al*, 2005).

Galectins can also function as opsonins by binding to and promoting phagocytosis of pathogens and parasites, which are killed by intracellular oxidative burst, and cleared from the internal milieu. If the infectious particle is too large to be phagocytosed by a single cell, it can be encapsulated by deposition multiple layers of cells, and killed either by oxidative stress or melanization (Vazquez *et al*, 2009; Nakamura *et al*, 2012; Xia *et al*, 2017).

In the following sections we discuss two examples of the role(s) of galectins from invertebrates in recognition, opsonization, and phagocytosis of pathogens and parasites, with contrasting outcomes. In the first example, we illustrate the successful opsonic role of a in innate immune defense in an invertebrate with the galectin MjGal from the kuruma shrimp, *Marsupenaeus japonicus*. In the second example, the subversion of the opsonic role of galectins from bivalve mollusks by the parasite *Perkinsus* sp. is discussed.

4. A Shrimp Galectin is a Successful Opsonin for Bacterial Pathogens

Our studies on a galectin (MjGal) from the kuruma shrimp *Marsupenaeus japonicus*, revealed that it functions as an inducible typical PRR and effector factor that is effective in the recognition and clearance of bacterial pathogens (Shi *et al*, 2014). MjGal was identified in our *M. japonicus* EST database as a 33.5 kDa galectin that displays a single 127-residue long CRD on the N-terminal region, followed by a C-terminal polypeptide extension (**Fig 2A**). In this regard it resembles the

chimera-type galectins described in mammals (**Fig 1B**), but with the CRD located on the opposite end of the polypeptide.

In the naïve shrimp MjGal was expressed in all tissues examined (**Fig 2B),** and can bind to both Gram-positive and Gram-negative bacteria (**Fig 2C**). Interestingly, the binding to bacteria could not be inhibited by lactose or related sugars that are typical inhibitors for most vertebrate and invertebrate galectins (Shi *et al*, 2014). In contrast, recognition of some bacterial species by MjGal could be partially inhibited by preincubation of the galectin with lipopolysaccharide (LPS), while lipoteichoic acid (LTA) behaved as a more effective inhibitor (**Fig 2C**). These results suggest that MjGal binds to bacteria through the recognition of either LTA or LPS, and that the binding is strong enough that simple sugars cannot outcompete these interactions. The alternative explanation that bacterial recognition could take place via a non-CRD region of MjGal was ruled out, as the mutant r*Mj*Gal$^{\Delta 102\text{-}106}$ that lacks the two cysteine residues and three additional residues in the CRD showed weaker recognition for the tested bacteria, or the isolated LPS and LTA (Shi *et al*, 2014).

Upon experimental infectious challenge with *V. anguillarum*, one of the bacterial species strongly recognized by MjGal (**Fig 2C**), galectin expression was upregulated in circulating phagocytic cells (hemocytes) and hepatopancreas (**Fig 2D**). These results suggested that MjGal functions in innate immune defense as a PRR that is inducible by microbial infection. In addition to binding to the surface of a variety of G^+ and G^- bacteria, MjGal also binds to the shrimp hemocyte surface (**Fig 3A**). It is noteworthy, however, that unlike the recognition of bacteria MjGal binds to the hemocyte in a lactose dependent-manner (Shi *et al* 2014), as typically described for most galectins. The binding to bacteria, presumably by binding to LTA or LPS, and the binding to the hemocyte surface carbohydrate moieties enable MjGal to function as an opsonin, cross-linking the potentially pathogenic bacteria to the hemocyte surface and promoting their phagocytosis (**Fig 3B**).

In vivo experimental infections in mature shrimp with *V. anguillarum* that had been pre-exposed to either r*Mj*Gal, Trx-His, or PBS alone showed that MjGal significantly contributes to the clearance of the injected bacteria from circulation over the baseline levels (**Fig 4A**). This was confirmed by an *in vivo* loss-of-function approach, through silencing expression of MjGal by RNAi. The injection of *Mj*Gal dsRNA into mature shrimp could significantly inhibit levels of the *Mj*Gal transcript in hemocytes (**Fig 4B**). As a result, the *Mj*Gal dsRNA-injected animals were less effective in the clearance of *V. anguillarum* than the GFP dsRNA-injected controls (Shi *et al,* 2014) (**Fig 4C**). Therefore, *Mj*Gal plays a key role in the ability of the shrimp to recognize and clear a bacterial infectious challenge (**Fig 5**). The detailed mechanisms involved in the opsonization activity remain to be unraveled, but in order to explain the cross-linking of bacterial surface carbohydrates (LPS or LTA) to the hemocyte surface glycans both interactions taking place via the *Mj*Gal CRD, it is

possible that *Mj*Gal can form oligomers, dimers or trimers, as illustrated in the cartoon (**Fig 5A**). Furthermore, although the bacterial numbers in circulation are reduced by the opsonic activity of *Mj*Gal, it remains to be elucidated whether the phagocytosed bacteria are killed by the typical hemocyte oxidative burst and digested by lysosomal activity (**Fig 5B,C**), as observed in other invertebrate models (Vazquez *et al*, 2009; Nakamura *et al*, 2012; Xia *et al*, 2017).

5. The Opsonic Activity of Galectins from Bivalve Mollusks can Promote Parasite Infection

During our initial study on the mechanism(s) for uptake of the protozoan parasite *Perkinsus marinus* by the eastern oyster (*Crassostrea virginica*) hemocytes we identified in EST databases two galectins (CvGal1 and CvGal2) of unique CRD organization (Tasumi and Vasta, 2007; Feng *et al*, 2013, 2015). A similar search on our RNAseq database from the softshell clam *Mya arenaria* we identified one galectin transcript (MaGal1) that was about half the length of the oyster galectins, this observation supported by the electrophoretic mobilities of the recombinant proteins **(Fig 6A) (**Vasta *et al*, 2020). Based on sequence analysis, CvGal1 and CvGal2 have 4 tandemly arrayed CRDs, whereas the clam galectin (MaGal1) has 2 CRDs in each polypeptide **(Fig 6B)** Vasta *et al*, 2020. Within each polypeptide, the 4 CRDs are structurally similar but not identical, suggesting that they differ in their fine specificity. As follows below, our biochemical, structural and functional studies on these galectins are illustrated with CvGal1. Our *in vitro* binding-inhibition studies showed that the recombinant CvGal1 prefers GalNAc to Gal, and strongly binds to glycoproteins that are rich in blood group A oligosaccharides such as the porcine stomach mucin (PSM) (Tasumi and Vasta, 2007; Feng *et al*, 2013, 2015).

Analysis of CvGal1 specificity on a glycan microarray confirmed these results **(Fig 7A)** and showed that oligosaccharides that display nonreducing terminal GalNAc linked in α1–3 to a subterminal Gal, such as the complex bi-antennary GalNAcα1–3(Fucα1–2)-Galβ1– 4GlcNAcβ1–2Manα1– 6[GalNAcα1–3(Fucα1–2)-Galβ1–4GlcNAcβ1–2Manα1–3]Manβ1–4GlcNAcβ1–4GlcNAcβ and GalNAcα1–3(Fucα1–2)-Galβ1–3GlcNAcβ1–2Manα1– 6[GalNAcα1–3(Fucα1–2)-Galβ1–3GlcNAcβ1–2Manα1–3]Manβ1– 4GlcNAcβ1– 4-(Fucα1–6)GlcNAcβ were the strongest recognized ligands. These structures further suggested that the anomeric linkage linked in β1–4 or β1–3 of the subterminal Gal to the GlcNAc affects the binding and that CvGal1 prefers type 2 backbone structures Galβ1–4GlcNAc in N-acetyllactosamine, typical of most galectins described to date, and shows weaker binding to type 1 backbones Galβ1–3GlcNAc. In addition, a Fucα1–2 bound to the subterminal Gal, as typical of blood group A oligosaccharides, further strengthens the binding. CvGal2 also preferentially recognizes ABH oligosaccharides, but here are significant differences in specificity with CvGal1 and MaGal1. The assessment of the binding affinity of CvGal1 by surface plasmon resonance (SPR) and solid phase binding studies revealed that this oyster galectin prefers

the blood group A tetrasaccharide over the trisaccharide **(Fig 7B)**. A model of the CvGal1 binding site and docking of the blood group A tetrasaccharide enabled the rationalization of the structural basis for the CvGal1 preference for the blood group A tetrasaccharide **(Fig 7C)** (Feng *et al*, 2013)**.**

CvGal1 is expressed in most tissues tested but the transcripts are most abundant in hemocytes suggesting that the signals observed in other tissues may originate in infiltrating hemocytes **(Fig 8A)**. In circulating hemocytes most of the CvGal1 protein remains as an intracellular protein, but when hemocytes attach and spread on a foreign surface, the CvGal1 is secreted to the extracellular space **(Fig 8B)**. The secreted CvGal1 binds to the external surface of the attached and spread hemocytes, as shown by immunofluorescence on non-permeabilized hemocytes (-TritonX_{100}) **(Fig 8C)**. Based on the results from the specificity analysis of CvGal1, we tested the hemocytes for the potential presence of blood group A surface moieties with an anti-A monoclonal antibody and obtained strong staining as assessed by FACS **(Fig 8D)**. Pretreatment of the hemocytes with GalNAc'ase, a glycolytic enzyme that cleaves non-reducing terminal GalNAc, reduced the anti-A monoclonal antibody to the baseline **(Fig 8E)**. As GalNAc is the key sugar that characterizes blood group A oligosaccharides and determines the specificity of anti-A antibodies and lectins, these results led to the conclusion that CvGal1 recognizes blood group A moieties on the hemocyte surface (Tasumi and Vasta, 2007; Feng *et al*, 2013, 2015). This was rigorously confirmed by a glycoproteomic study by MS on the oyster hemocyte isolated glycoproteins (αβintegrins and dominin) that are recognized by CvGal1 (Feng *et al*, 2013; Kurz *et al*, 2013).

In addition to binding to the endogenous ("self") hemocyte surface glycans, the oyster CvGal1 can also recognize and bind to a wide variety of bacteria, both G+ and G-, with variable degrees of preference for specific species and strains **(Fig 9A)**, and also several species of microalgae that are components of the phytoplankton on which the oyster feeds (Tasumi and Vasta, 2007; Vasta, 2009; Vasta *et al*, 2015; Vasta *et al*, 2020). Most importantly, it can also recognize the parasite *Perkinsus marinus* trophozoites with the highest affinity **(Fig 9B)**, and this binding is specific for galactosyl moieties, as it can be completely inhibited by thiodigalactoside (TDG) but not by glucose (Glc) **(Fig 9C) (**Tasumi and Vasta, 2007).

The strong recognition of the parasite *P. marinus* trophozoites by oyster hemocytes leads to their adhesion to the hemocyte cell surface as the first step to their phagocytosis **(Fig 10A)**. Pre-exposure of the parasite *P. marinus* trophozoites to CvGal1 or CvGal2 significantly enhances their adhesion of the *P. marinus* trophozoites surface **(Fig 10B)**, whereas pre-incubation of the oyster hemocytes with anti-CvGal1 or anti-CvGal2 antibodies significantly hinders trophozoite adhesion to the hemocyte surface **(Fig 10C)**. These results strongly suggest that function as active opsonins for *P. marinus* trophozoites, promoting their adhesion to the phagocytic cell surface. Taken together, these results strongly suggested that both extracellular oyster galectins CvGal1 or CvGal2 can function as

opsonins that recognize *P. marinus* trophozoites and promote their adhesion to the phagocytic cell surface. This opsonic activity contributes to phagocytosis of the *P. marinus* trophozoites **(Fig 11A)**. The galectin MaGal1 also effectively recognizes *P. marinus* trophozoites, but the details of its potential role as opsonin for the parasite remain to be elucidated **(Fig 11A)**. The *P. marinus* trophozoites are endowed of a very powerful anti-oxidative enzyme repertoire that includes two SODs and two APXs, and the phagocytosed parasites survive intracellular killing by inhibiting or degrading the ROS produced by the oyster hemocytes **(Fig 11B)** (Schott *et al*, 2003a,b, 2020; Fernández-Robledo *et al*, 2008). Therefore, the trophozoites take advantage of the intra-hemocytic environment for nutrition, they multiply intracellularly, and eventually cause a systemic infection and kill the oyster host **(Fig 11C) (**Vasta, 2009).

6. Summary and Conclusions

As discussed throughout this review, while galectins can participate in multiple regulatory functions by recognizing endogenous (self) cell surface, ECM, and soluble glycans, evidence has accumulated in the past few years to support that galectins can also recognize exogenous (non-self) glycans present on the surface of viruses, bacteria, and eukaryotic parasites. Therefore, they can be considered *bona fide* pattern recognition receptors (PRRs). An illustrative example of the recognition of structural topologies ("patterns") on distinct glycans by a galectin is the binding of the proto-type galectin from the toad *Bufo arenarum* to different carbohydrates with similar affinity (Bianchet *et al*, 2000). The crystal structures of the toad galectin in complex with LacNAc and TDG revealed that the protein establishes identical interactions with the non-reducing terminal Gal that is shared by both disaccharides, but different contacts with the sub-terminal sugar unit (GlcNAc in LacNAc, and Gal in TDG). The number and quality of H-bonds, however, is similar in both complexes, revealing the structural plasticity of the galectin CRD to establish compensatory protein/sugar interactions for the two chemically distinct disaccharides that result in similar binding affinity (Bianchet *et al*, 2000). Galectins, however, do not rigorously fit the definition of PRRs as described by Medzhitov and Janeway (2002). PRRs recognize pathogens *via* widely distributed, highly conserved microbial molecules (pathogen- or microbe-associated molecular patterns, PAMPs or MAMPs) such as LPS or peptidoglycan, which are absent in the host. In contrast, galectins can recognize similar carbohydrate ligands that are displayed on both the host and the pathogen cell surface. This apparent paradox reveals our limited knowledge about the actual diversity in recognition of the host galectin repertoire, and as well as the structural and biophysical aspects of multivalent ligand binding (Vasta, 2009; Dam and Brewer, 2008,2010)

As discussed above, the recognition of glycans on the surface of bacteria and parasites, and on the surface of the invertebrate phagocytic cells (hemocytes) enables soluble galectins to function as

effective opsonins. Thus, the host galectins promote adhesion of the pathogen to the hemocyte and contribute to the phagocytosis of the infectious agent. In the shrimp, the opsonic activity of MjGal leads to enhanced clearance of the circulating bacteria, supporting their effective role in innate immune response to infection. In the oyster, however, although galectins are equally capable of recognizing multiple environmental bacteria, the parasite *Perkinsus marinus* appears to subvert galectin function by taking advantage of this recognition system to enter the host hemocytes, block intracellular oxidative killing, and proliferate to produce a systemic infection. Thus, the opsonic activity of invertebrate galectins, can lead to various and sometimes opposite outcomes, that benefit either the host or the pathogen. The key roles played by galectins in development, tissue repair, and immune homeostasis via recognition of endogenous glycans, has most likely imposed severe functional constraints against evolutionary structural changes, resulting in the striking conservation of this lectin family in both structure and specificity. Therefore, it should be not surprising that pathogens would have co-evolved with their hosts by adapting their surface glycocomes to "hijack" the recognition and opsonic properties of host galectins for attachment and entry into the host cells in a "Trojan horse" model (Vasta, 2009).

7. Acknowledgments

Studies reviewed in this article were supported by grants IOB-0618409, IOS-0822257 and IOS-1063729 from the National Science Foundation, grant NA05NMF4571243 from the National Oceanic and Atmospheric Administration, and grant R01 GM070589 from the National Institutes of Health to GRV, and grants from the National Natural Science Foundation of China (No.31630084, 31930112), the National Key Research and Development Program of China (Grant No. 2018YFD0900502) to JXW. We are grateful to Prof. Richard D. Cummings, Dr. David Smith, and Dr. Jamie Molinaro from the Core H, Consortium for Functional Glycomics, Emory University, Atlanta, GA, and National Center for Functional Glycomics, Harvard University, Boston, MA for the glycan array analysis of galectins discussed in this review.

8. List of abbreviations

*Mj*Gal, *Marsupenaeus japonicus* galectin; *Mj*Gal$^{\Delta 102\text{-}106}$, deletion mutant of MjGal; CvGal1, *Crassostrea virginica* galectin 1; CvGal2, *Crassostrea virginica* galectin 2; MaGal1, *Mya arenaria* galectin 1; BaGal1, *Bufo arenarum* galectin-1; hGal-1, human galectin-1; CGL2, *Coprinus cinereus* galectin-2; CRD, carbohydrate recognition domain; GalNAc, N-acetyl-D-galactosamine; GlcNAc, N-acetyl-D-glucosamine; LacNAc, N-acetyllactosamine; LTA, lipoteichoic acid from *Staphylococcus aureus*; LPS, lipopolysaccharide from *Escherichia coli*; PGN, peptidoglycan from *Micrococcus*

luteus; TDG, thiodigalactose; BSM, bovine submaxillary mucin; OSM, ovine submaxillary mucin; PSM, porcine stomach mucin; Trx-His, recombinant thioredoxin carrying a His tag; RNAi, RNA interference; dsRNA, double stranded RNA; SPR, surface plasmon resonance, PRR, pattern recognition receptors.

9. Figure Legends

Figure 1. Galectin structure, types, and functions in "self"/"non-self" recognition. A. Crystal structure of galectin-1 dimer in complex with LacNAc. **B.** Galectins are classified into three main types: proto, chimera and tandem-repeat types. Proto-type galectins contain one carbohydrate-recognition domain (CRD) per subunit and are usually homodimers of non-covalently linked subunits (Galectins 1, 2, 5, 7, 10, 11, 13, 14 and 15). In contrast, chimera-type galectins are monomeric with a carboxy-terminal CRD, joined to an amino-terminal peptide that contains a collagen-like sequence rich in proline and glycine, and can oligomerize as trimers (Galectin 3). In the tandem-repeat galectins, two CRDs are covalently joined by a linker peptide (Galectins 4, 6, 8, 9 and 12). **C,** Recognition of "self" glycans by galectins: In the extracellular space, galectins form multivalent oligomers that cross-link cell surface glycoproteins and glycolipids, form microdomains and lattices, and activate signaling pathways. **D.** Recognition of "non-self" glycans by galectins: Proto, chimera, and tandem-repeat galectins can function as pattern recognition receptors (PRRs) and establish trans-interactions with the host cell surface and microbial glycans.

Figure 2. The shrimp galectin MjGal: Domain organization, tissue-specific expression, recognition of bacteria, and upregulation of expression upon infectious challenge: A. Domain organization of MjGal: the cartoon illustrates the N-terminal CRD, followed by the peptide extension at the C-terminal end; **B.** Expression of *MjGal* in different tissues of naïve animals as assessed by RT-PCR, with β-actin serving as the reference gene. **C.** Binding of recombinant *Mj*Gal (r*Mj*Gal) to bacteria by Western blot, and inhibition by LPS and LTA**:** Purified, His-tagged r*Mj*Gal (500 μg/ml) was pre-incubated with TBS alone or excess LPS or LTA for 1 h, after which bacterial suspensions were added and incubated at room temperature for 1 h. The bacteria were centrifuged, washed four times with TBS, and treated with 7% SDS for 1 min. The eluate was collected by centrifugation, subjected to SDS-PAGE, and the eluted rMjGal detected by Western blot using anti-His antibody. **D.** The time-course expression of MjGal was upon *V. anguillarum* challenge was analyzed by qRT-PCR in hemocytes (Left panel) and hepatopancreas (Right panel), with β-actin serving as the reference gene. The histograms show the statistical analysis of the quantitative real-time PCR results. The asterisks indicate significant differences (** $P < 0.01$, *** $P < 0.001$) between the *V. anguillarum*-

challenged and PBS-injected group. Error bars represent ± SD of three independent PCR amplifications and quantifications. (Adapted from Shi *et al*, 2014)

Figure 3. *In vivo* binding of rMjGal to shrimp hemocytes, and opsonization of bacteria. A. rMjGal, Trx-His, or PBS were injected into shrimp and the hemocytes were collected and analyzed by immunohistochemistry. The left panel indicated the binding signal detected by the anti-Histidine antibody for the recombinant protein (Green), the middle panel showed the hemocyte nucleus location (Blue), and the right panel showed the merge of previous two panels. Bar = 20 mm. **B.** Bacteria phagocytosis assay: fluorescently labeled *V. anguillarum* ($5x10^7$ cells) were coated with either Trx-His (Top panel, control) or rMjGal (Middle panel) and injected into shrimp. The hemocytes were collected after 30 min and placed onto the glass slides. Subsequently, trypan blue solution (2 mg/ml PBS) (Amresco) was added to quench the fluorescence of non-phagocytosed bacteria. The phagocytosis by hemocytes was observed at 400 x magnification, and phagocytosed bacteria counted at 200 x magnification under fluorescence microscope. The phagocytic percentages of two groups (*$P < 0.05$) are shown in the lower panel. Bar = 50 mm. (Adapted from Shi *et al*, 2014)

Figure 4. The opsonic activity of rMjGal in the clearance of *Vibrio anguillarum.* A. *Vibrio anguillarum* (2×10^7 cells) pre-incubated with rMjGal (15 μg) were injected into the shrimp. Hemolymph was collected, diluted 1:1000, and plated on 2216E agar plates. Bacterial colonies were counted after 37 °C cultivation for 12 h. Results represent as the number of bacteria from three independent repeats and expressed as mean values ±S.D. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). **B.** RNA interference assay: shrimp were injected with dsRNAs twice to silence *Mj*Gal expression, and or with GFP dsRNAs as control. *Vibrio anguillarum* (2×10^7 cells) were injected into shrimp after the second injection. Hemolymph was collected from the two groups at 5, 15, 60 min post-infection, and serially diluted with PBS. Fifty μl of each sample was plated onto 2216E agar plates and incubated at 37 °C overnight. RNA was extracted from hemocytes and gills of two groups, and tested by RT-PCR to confirm silencing of *Mj*Gal expression**. C.** The bacterial counts in hemolymph samples collected at 5, 15 and 60 min from shrimp from the ds*Mj*Gal and dsGFP groups (Adapted from Shi *et al*, 2014).

Figure 5. Schematic model of the proposed role of the shrimp galectin MjGal in the uptake and clearance of bacteria by hemocytes. A. The galectin MjGal from the kuruma shrimp *M. japonicus* can opsonize and cross-link the circulating bacteria to carbohydrate moieties on the hemocyte surface. It remains unknown if MjGal forms multivalent oligomers (dimers or trimers) that may contribute to the cross-linking of bacterial and hemocyte glycans. **B.** MjGal-mediated recognition and opsonization of bacteria promotes their phagocytosis. The phagocytosed bacteria

are most likely killed by the typical oxidative burst. **C**. The phagocytosed bacteria are cleared from the shrimp internal milieu.

Figure 6. Galectins CvGal1 and CvGal2 from the eastern oyster, and MaGal1 from the softshell clam. A. Coomassie (CBB) stain of recombinant CvGal1, CvGal2, and MaGal1 containing either four or two CRDs. Arrows indicate the expected molecular weight of the proteins. B. Schematic illustration of 4 CRDs of CvGal1 and CvGal2 and 2 CRDs of MaGal1. [Adapted from Tasumi and Vasta, 2007, with permission from the American Association of Immunologists]

Figure 7. Analysis of the carbohydrate specificity of rCvGal1 by glycan array and surface plasmon resonance analyses, and homology modeling of the interactions with the ligand. A. Glycan array analysis: The six best glycans ranked by their affinity for rCvGal1 in glycan array analysis. The negative percentages are the evaluation of the % change in the fluorescent signal ((F_j – F_i)/F_i × 100%). **B.** Surface plasmon resonance: Blood group A tetrasaccharide-BSA (A Tetra-BSA; left panel) or blood group A trisaccharide-BSA (A Tri-BSA; middle panel) were immobilized up to 1000 response units on CM5 chips, and the binding of rCvGal1 was assessed by SPR with rCvGal1 being injected as analyte. The SPR sensorgrams were recorded with 2-fold serial dilutions of the analyte starting from 100µg/ml. Solid phase analysis: A Tetra-BSA, A Tri-BSA, or *N*-acetylgalactosamine-BSA (GalNAc-BSA) were added at the concentrations indicated (serial dilution starting from 10 µg/ml, 100 µl/well) into 96-well plates and the binding of 0.2 µg/ml of rCvGal1 was assessed by ELISA. Data show optical density at 450 nm (OD_{450nm}) in triplicates with standard error (SEM). **C**. Homology modeling of the interactions of CvGal1 with blood group A tetrasaccharide: A2 blood tetrasaccharide docked at the binding pocket of the CvGal1 model of the first CRD. CvGal1-binding site is shown as semi-transparent solvent-accessible surface colored by its vacuum electrostatic potential (positive in *blue* to negative in *red*). The schematic model of the protein is visible across the surface showing the interacting residues in a *stick* representation. H-bonds recognizing hydroxyl groups of the A2 oligosaccharide are displayed as *dashed lines* with their distances (in Å) between heavy atoms indicated. [Adapted from Feng et al 2013, with permission from the American Society for Biochemistry & Molecular Biology].

Figure 8. The oyster galectin CvGal1: Tissue-specific expression, secretion, and binding to the hemocyte surface blood group A moieties: A. Expression of CvGal1 different tissues of eastern oysters as assessed by RT-PCR, with β-actin serving as the reference gene. **B.** Upon hemocyte attachment and spreading, CvGal1 is secreted. Western blotting of unattached hemocytes, plasma, attached-spread hemocytes, and supernatant. **C.** Immunofluorescence staining with anti-CvGal1 and DAPI staining of attached-spread hemocytes with (+) or without (-) Triton X treatment, showing the presence of CvGal1 in the cytoplasm, and on the external surface of the hemocyte plasma membrane, respectively. Scale bar, 10 µm. **D.** Fixed hemocytes were stained with

dilutions of anti-blood group A antibody (*red,* 1:2000; *blue,* 1:500; *yellow,* 1:100) or buffer only (*black*) in flow cytometry analysis. **E.** Binding of rCvGal1 to unattached hemocytes with α-*N*-acetylgalactosaminidase treatment (GalNAc'ase) or no treatment (Untreated) were measured by flow cytometry, whereby a sample without rCvGal1 was the control (*Ctrl*). Data show mean fluorescence intensity (*MFI*) ± S.E. of each sample. * indicates significant difference ($p < 0.05$) between samples from One-Way ANOVA analysis. [Adapted from Tasumi and Vasta, 2007with permission from the American Association of Immunologists and the American Society for Biochemistry & Molecular Biology]

Figure 9. The oyster galectin CvGal1 binds to a variety of environmental bacteria and to the parasite *Perkinsus marinus*. A. CvGal1 binds to a variety of environmental bacteria: Absorption test of rCvGal1 with selected microorganisms. Results are shown as relative absorption. Bars show SDs of quadruplicate experiments. **B** Absorption test of rCvGal1 *P. marinus* trophozoites at various cell pellet-to-CvGal1 solution ratios. Bars show SDs of quadruplicate experiments. **C**, Binding of rCvGal to *P. marinus* trophozoites: Carbohydrate-specific binding of rCvGal1 to P. marinus trophozoites in the presence and absence of thiodigalactose (inhibits rCvGal1 binding) or glucose (does not inhibit rCvGal1 binding) was analyzed by immunofluorescence staining. Scale bar, 10 μm. [Adapted from Tasumi and Vasta, 2007, with permission from the American Association of Immunologists and the American Society for Biochemistry & Molecular Biology.

Figure 10. The oyster galectin CvGal1 promotes adhesion of *Perkinsus marinus* trophozoites to the hemocyte surface A. SEM of attached oyster hemocytes with *Perkinsus marinus* trophozoites attached to the cell surface and in the process of being phagocytosed. **B.** Pre-incubation of *P. marinus* trophozoites with rCvGal1 or rCvGal2 for 1 h significantly enhances their adhesion to the hemocyte cell surface. **C**. Pre-incubation of oyster hemocytes with anti-CvGal1 or anti-CvGal2 purified Igs for 1 h significantly reduces the adhesion of *P. marinus* trophozoites to the hemocyte cell surface. * indicates significant difference ($p < 0.05$) from control sample. [Adapted from Tasumi and Vasta, 2007, with permission from the American Association of Immunologists and the American Society for Biochemistry & Molecular Biology]

Figure 11. Schematic model of the proposed roles of an oyster galectin (CvGal1 or CvGal2) in the uptake of *Perkinsus marinus* trophozoites by hemocytes. A. The 4-CRD galectin (CvGal1 or CvGal2) from the eastern oyster (*C. virginica*) is secreted by the hemocytes and can cross-link the blood group oligosaccharide moieties on the hemocyte surface to glycans of similar topology on the surface of the parasite *P. marinus*. **B.** CvGal1-mediated recognition and opsonization promotes adhesion of the trophozoites and leads to enhanced phagocytosis. The *P. marinus* trophozoites escape oxidative killing by expression of anti-oxidative enzymes (Superoxide dismutases, ascorbate-

dependent peroxidases, etc). **C.** Surviving phagocytosed *P. marinus* trophozoites proliferate within the hemocyte, eventually causing systemic infection and death of the oyster host.

9. References

Ahmed, H., Bianchet, M. A., Amzel, L. M., Hirabayashi, J., Kasai, K., Giga-Hama, Y., Tohda, H., and Vasta, G. R. (2002) Novel carbohydrate specificity of the 16- kDa galectin from *Caenorhabditis elegans*: binding to blood group precursor oligosaccharides (type 1, type 2, Talpha, and Tbeta) and gangliosides. Glycobiology 12, 451-461

Ahmed, H., Cappello, F., Rodolico, V., and Vasta, G. R. (2009) Evidence of heavy methylation in the galectin 3 promoter in early stages of prostate adenocarcinoma: development and validation of a methylated marker for early diagnosis of prostate cancer. Transl Oncol 2, 146-156

Ahmed H, Al Sadek DM. (2015) Galectin-3 as a Potential Target to Prevent Cancer Metastasis. Clin Med Insights Oncol.25;9:113-21

Almeida F, Wolf JM, da Silva TA, DeLeon-Rodriguez CM, Rezende CP, Pessoni AM, Fernandes FF, Silva-Rocha R, Martinez R, Rodrigues ML, Roque-Barreira MC, Casadevall A. Galectin-3 impacts *Cryptococcus neoformans* infection through direct antifungal effects. Nat Commun. 2017 Dec 6;8(1):1968. doi: 10.1038/s41467-017-02126-7. PMID:29213074

Bianchet MA, Ahmed H, Vasta GR, Amzel LM. Soluble beta-galactosyl-binding lectin (galectin) from toad ovary: crystallographic studies of two protein-sugar complexes. Proteins. 2000 Aug 15;40(3):378-88. PMID: 10861929

Blidner, A. G., Mendez-Huergo, S. P., Cagnoni, A. J., and Rabinovich, G. A. (2015) Re-wiring regulatory cell networks in immunity by galectin-glycan interactions. FEBS letters 589, 3407-3418

Blois, S. M., Ilarregui, J. M., Tometten, M., Garcia, M., Orsal, A. S., Cordo-Russo, R., Toscano, M. A., Bianco, G. A., Kobelt, P., Handjiski, B., Tirado, I., Markert, U. R., Klapp, B. F., Poirier, F., Szekeres-Bartho, J., Rabinovich, G. A., and Arck, P. C. (2007) A pivotal role for galectin-1 in fetomaternal tolerance. Nature medicine 13, 1450-1457

Blois SM, Dveksler G, Vasta GR, Freitag N, Blanchard V, Barrientos G. Pregnancy Galectinology: Insights Into a complex network of glycan binding proteins. Front Immunol. 2019 May 29;10:1166.

Cooper, D. N. 2002. Galectinomics: finding themes in complexity. Biochim Biophys Acta. 1572: 209-231.

Colnot, C., Ripoche, M., Fowlis, D., Cannon, V., Scaerou, F., Cooper, D. N. W., and Poirier, F. (1997) The role of galectins in mouse development. Trends Glycosci Glycotechnol 9, 31-40

Colnot, C., Sidhu, S. S., Balmain, N., and Poirier, F. (2001) Uncoupling of chondrocyte death and vascular invasion in mouse galectin 3 null mutant bones. Developmental biology 229, 203-214

Croci, D. O., Cerliani, J. P., Dalotto-Moreno, T., Mendez-Huergo, S. P., Mascanfroni, I. D., Dergan-Dylon, S., Toscano, M. A., Caramelo, J. J., Garcia- Vallejo, J. J., Ouyang, J., Mesri, E. A., Junttila, M. R., Bais, C., Shipp, M. A., Salatino, M., and Rabinovich, G. A. (2014) Glycosylation-dependent lectin- receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. Cell 156, 744-758

Cummings RD, Liu FT, Vasta GR. Galectins. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Darvill AG, Kinoshita T, Packer NH, Prestegard JH, Schnaar RL, Seeberger PH, editors. Essentials of Glycobiology. 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017. Chapter 36.

Di Lella S, Sundblad V, Cerliani JP, Guardia CM, Estrin DA, Vasta GR, Rabinovich GA., When galectins recognize glycans: from biochemistry to physiology and back again. Biochemistry, 2011. 50 (21848324): p. 7842-7857.

Dam, T. K. & C. F. Brewer. 2008. Effects of clustered epitopes in multivalent ligand-receptor interactions. Biochemistry. 47: 8470-8476.

Dam, T. K., and Brewer, C. F. 2010. Maintenance of cell surface glycan density by lectin-glycan interactions: a homeostatic and innate immune regulatory mechanism. Glycobiology 20, 1061-106

Danielsen E M, Hansen G H.Lipid raft organization and function in brush borders of epithelial cells. Mol MembrBiol (2006) 23:71–9.doi:10.1080/09687860500445604

Dheilly NM, Duval D, Mouahid G, Emans R, Allienne JF, Galinier R, et al. A family of variable immunoglobulin and lectin domain containing molecules in the snail *Biomphalaria glabrata*. Dev Comp Immunol. 2015 48:234-43.

Esteban A, Popp MW, Vyas VK, Strijbis K, Ploegh HL, Fink GR. Fungal recognition is mediated by the association of dectin-1 and galectin-3 in macrophages. Proc Natl Acad Sci USA (2011) 108:14270–5.doi:10.1073/pnas.1111415108

Feng C, Nita-Lazar M, González-Montalbán N, Wang J, Mancini J, Ravindran C, Ahmed H, Vasta GR. Manipulating galectin expression in zebrafish (*Danio rerio*). (2015) Methods Mol Biol.1207:327-41

Feng, C., Ghosh, A., Amin, M. N., Giomarelli, B., Shridhar, S., Banerjee, A., Fernandez-Robledo, J. A., Bianchet, M. A., Wang, L. X., Wilson, I. B., and Vasta, G. R. (2013) The galectin CvGal1 from the eastern oyster (*Crassostrea virginica*) binds to blood group A oligosaccharides on the hemocyte surface. The Journal of biological chemistry 288, 24394-24409

Feng, C., Ghosh, A., Amin, M. N., Bachvaroff, T. R., Tasumi, S., Pasek, M., Banerjee, A., Shridhar, S., Wang, L. X., Bianchet, M. A., and Vasta, G. R. (2015) Galectin CvGal2 from the Eastern Oyster (*Crassostrea virginica*) Displays Unique Specificity for ABH Blood Group Oligosaccharides and Differentially Recognizes Sympatric Perkinsus Species. Biochemistry 54, 4711-4730

Fernández-Robledo, J. A., Schott, E. J., and Vasta, G. R. (2008) *Perkinsus marinus* superoxide dismutase 2 (PmSOD2) localizes to single-membrane subcellular compartments. Biochem. Biophys. Res. Commun. 375, 215-219

Garner, O. B., Yun, T., Pernet, O., Aguilar, H. C., Park, A., Bowden, T. A., Freiberg, A. N., Lee, B., and Baum, L. G. (2015) Timing of galectin-1 exposure differentially modulates Nipah virus entry and syncytium formation in endothelial cells. Journal of virology 89, 2520-2529

Garner OB, Baum LG. Galectin-glycan lattices regulate cell-surface glycoprotein organization and signalling. Biochem Soc Trans. 2008 Dec;36(Pt 6):1472-7. doi: 10.1042/BST0361472.

Georgiadis, V., Stewart, H.J., Pollard, H.J., Tavsanoglu, Y., Prasad, R., Horwood, J., Deltour, L., Goldring, K., Poirier, F. & Lawrence-Watt, D.J. (2007) Lack of galectin-1 results in defects in myoblast fusion and muscle regeneration. Dev Dyn. 236, 1014-1024 (2007).

Ghosh A, Banerjee A, Amzel LM, Vasta GR, Bianchet MA. Structure of the zebrafish galectin-1-L2 and model of its interaction with the infectious hematopoietic necrosis virus (IHNV) envelope glycoprotein. Glycobiology. 2019 May 1;29(5):419-430. doi: 10.1093/glycob/cwz015.

Guha, P., Kaptan, E., Bandyopadhyaya, G., Kaczanowska, S., Davila, E., Thompson, K., Martin, S. S., Kalvakolanu, D. V., Vasta, G. R., and Ahmed, H. (2013) Cod glycopeptide with picomolar affinity to galectin-3 suppresses T- cell apoptosis and prostate cancer metastasis. Proceedings of the National Academy of Sciences of the United States of America 110, 5052-5057

Hattori, T; Arikawa, T; Fujioka, Y; Maruyama, J; Nakayama, Y; Ohba, Y; Niki, T; Miyazaki, T; Hirashima, M; Kida, H. Inhibition of influenza A virus infection by Galectin-9. Japanese Journal of Veterinary Research 61(1&2): 5-18, 2013

Hill, M., Mazal, D., Biron, V. A., Pereira, L., Ubillos, L., Berriel, E., Ahmed, H., Freire, T., Rondan, M., Vasta, G. R., Liu, F. T., Iglesias, M. M., and Osinaga, E. (2010) A novel clinically relevant animal model for studying galectin-3 and its ligands during colon carcinogenesis. Journal of Histochemistry and Cytochemistry. 58, 553-565

Hirabayashi, J. and K. Kasai, The family of metazoan metal-independent beta-galactoside binding lectins: structure, function and molecular evolution. Glycobiology, 1993. 3(8400545): p. 297-304.

Hirashima M, Kashio Y, Nishi N, Yamauchi A, Imaizumi TA, Kageshita T, Saita N, Nakamura T. (2002) Galectin-9 in physiological and pathological conditions.Glycoconj J. 19(7-9):593-600

Houzelstein,D.,Goncçalves,I.R.,Fadden,A.J.,Sidhu,S.S., Cooper, D. N. W., Drickamer, K., Leffler, H., and Poirier, F. (2004) Phylogenetic analysis of the vertebrate galectin family. Mol. Biol. Evol. 21 (7), 1177–1187.

Hsu, D. K., and Liu, F.-T. (2008) Regulation of Immune Responses by Galectin- 3. in Animal Lectins: A Functional View (Vasta, G. R., and Ahmed, H. eds.), CRC Press, Boca Raton, FL.

Hsu YA, Kuo YH, Chen CS, Chen YC, Huang CC, Chang CY, Lin CJ, Lin CW, Lin HJ, Liu FT, Wan L.(2018) Galectin-12 is Involved in Corn Silk-Induced Anti-Adipogenesis and Anti-Obesity Effects. Am J Chin Med. 5:1-19.

Ideo H, Seko A,Yamashita K. Galectin-4 binds to sulfated glycosphingolipids and carcinoembryonic antigen inpatches on the cell surface of human colon adenocarcinoma cells. J Biol Chem (2005) 280:4730–7.doi:10.1074/jbc.M410362200

Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. Annu. Rev. Immunol. 20:197–216.

Jouault T, ElAbed-ElBehi M, Martínez-Esparza M,Breuilh L,Trinel PA, Chamaillard M, etal. Specific recognition of *Candida albicans* by macrophages requires galectin-3 to discriminate *Saccharomyces cerevisiae* and needs association with TLR2 for signaling. J Immunol (2006) 177:4679–87.doi:10.4049/jimmunol.177.7.4679

Katoh S, Ikeda M, Shimizu H, Mouri K, Obase Y, Kobashi Y, Fukushima K, Hirashima M, Oka M. Increased levels of plasma galectin-9 in patients with influenza virus infection. Tohoku J Exp Med. 2014 Apr;232(4):263-7

Kohatsu L, Hsu DK, Jegalian AG, Liu FT, Baum LG. Galectin-3 induces death of *Candida* species expressing specific beta-1,2-linked mannans. J Immunol. 2006 Oct 1;177(7):4718-26

Krejcirikova, V., Pachl, P., Fabry, M., Maly, P., Rezacova, P., and Brynda, J. (2011) Structure of the mouse galectin-4 N-terminal carbohydrate-recognition domain reveals the mechanism of oligosaccharide recognition. Acta Crystallogr D Biol Crystallogr. 67: 204-211

Kurz, S., Jin, C., Hykollari, A., Gregorich, D., Giomarelli, B., Vasta, G. R., Wilson, I. B., and Paschinger, K. (2013) Hemocytes and plasma of the eastern oyster (*Crassostrea virginica*) display a diverse repertoire of sulfated and blood group A-modified N-glycans. The Journal of biological chemistry 288, 24410-24428

Kutzner TJ, Gabba A, FitzGerald FG, Shilova NV, García Caballero G, Ludwig AK, Manning JC, Knospe C, Kaltner H, Sinowatz F, Murphy PV, Cudic M, Bovin NV, Gabius HJ. How altering the modular architecture affects aspects of lectin activity: case study on human galectin-1. Glycobiology. 2019 May 15. pii: cwz034. doi: 10.1093/glycob/cwz034. [Epub ahead of print]

Laine RA. The information-storing potential of the sugar code, in Glycosciences: status and perspectives (Gabius, H-J & Gabius, S, eds). 1997 pp. 5-14:Chapman & Hill, Weinheim.

Leffler H, Carlsson S, Hedlund M, Qian Y, Poirier F (2004) Introduction to galectins. Glycoconj J 19: 433-440

Lemaitre B, Hoffmann J (2007) The host defense of *Drosophila melanogaster*. Annu Rev Immunol 25: 697-743.

Levroney, E. L., Aguilar, H. C., Fulcher, J. A., Kohatsu, L., Pace, K. E., Pang, M., Gurney, K. B., Baum, L. G., and Lee, B. (2005) Novel innate immune functions for galectin-1: galectin-1 inhibits cell fusion by Nipah virus envelope glycoproteins and augments dendritic cell secretion of proinflammatory cytokines. J Immunol 175, 413-420

Liao, D-I, Kapadia, G., Ahmed, H., Vasta, G.R. and Herzberg, O. Structure of S-lectin, a developmentally regulated vertebrate b-galactoside-binding protein. Proc. Natl. Acad Sci. 91: 1428-1432, 1994

Liu, FT, Yang RY, Hsu DK. Galectins in acute and chronic inflammation 2012 Ann N Y Acad Sci. 1253, 80-91

Loker ES, Adema CM, Zhang SM, Kepler TB (2004) Invertebrate immune systems--not homogeneous, not simple, not well understood. Immunol Rev. 198:10-24.

Machala EA, McSharry BP, Rouse BT, Abendroth A, Slobedman B. Gal power: the diverse roles of galectins in regulating viral infections. J Gen Virol. 2019 Mar;100(3):333-349. doi: 10.1099/jgv.0.001208. Epub 2019 Jan 16.

Martínez Allo VC, Hauk V, Sarbia N, Pinto NA, Croci DO, Dalotto-Moreno T, Morales RM, Gatto SG, Manselle Cocco MN, Stupirski JC, Deladoey Á, Maronna E, Marcaida P, Durigan 6, Secco A, Mamani M, Dos Santos A, Catalán Pellet A, Pérez Leiros C, Rabinovich GA, Toscano MA. Suppression of age-related salivary gland autoimmunity by glycosylation-dependent galectin-1-driven immune inhibitory circuits. Proc Natl Acad Sci U S A. 2020 Mar 24;117(12):6630-6639. doi: 10.1073/pnas.1922778117. Epub 2020 Mar 11.

Méndez-Huergo SP, Blidner AG, Rabinovich GA.(2017) Galectins: emerging regulatory checkpoints linking tumor immunity and angiogenesis. Curr Opin Immunol. 45:8-15.

Nakamura O, Watanabe M, Ogawa T, Muramoto K, Ogawa K, Tsutsui S, Kamiya H. Galectins in the abdominal cavity of the conger eel *Conger myriaster* participate in the cellular encapsulation of parasitic nematodes by host cells. Fish Shellfish Immunol. 2012 Oct;33(4):780-7

Nemoto-Sasaki Y, Hayama K, Ohya H, Arata Y, Kaneko MK, Saitou N, Hirabayashi J, Kasai K. (2008) *Caenorhabditis elegans* galectins LEC-1-LEC-11: structural features and sugar-binding properties.Biochim Biophys Acta. 2008 Oct;1780(10):1131-42.

Nita-Lazar, M., Mancini, J., Feng, C., Gonzalez-Montalban, N., Ravindran, C., Jackson, S., Heras-Sanchez Ade, L., Giomarelli, B., Ahmed, H., Haslam, S. M., Wu, G., Dell, A., Ammayappan, A., Vakharia, V. N., and Vasta, G. R. (2016) The zebrafish galectins Drgal1-L2 and Drgal3-L1 bind in vitro to the infectious hematopoietic necrosis virus (IHNV) glycoprotein and reduce viral adhesion to fish epithelial cells. Dev Comp Immunol 55, 241-252

Ogden AT, Nunes I, Ko K, Wu S, Hines CS, Wang AF, et al. GRIFIN, a novel lens-specific protein related to the galectin family. The Journal of biological chemistry. 1998 273:28889-96.

Pace, K.E., Lebestky, T., Hummel, T., Arnoux, P., Kwan, K. & Baum, L.G. Characterization of a novel Drosophila melanogaster galectin. J Biol Chem. 277, 13091-13098 (2002).

Park AM, Hagiwara S, Hsu DK, Liu FT, Yoshie O. Galectin-3 Plays an Important Role in Innate Immunity to Gastric Infection by *Helicobacter pylori*. Infect Immun. 2016 Mar 24;84(4):1184-1193

Pejnovic, N. N., Pantic, J. M., Jovanovic, I. P., Radosavljevic, G. D., Djukic, A., Arsenijevic, N. N., and Lukic, M. L. (2013) Galectin-3 is a regulator of metaflammation in adipose tissue and pancreatic islets. Adipocyte 2, 266-271

Rabinovich, G. A., and Croci, D. O. (2012) Regulatory circuits mediated by lectin-glycan interactions in autoimmunity and cancer. Immunity 36, 322-335

Rabinovich, G., Toscano, M.A., Jackson, S.S., and Vasta, G.R. (2007) Functions of cell surface galectin-glycoprotein lattices. Curr. Opin. Struct. Biol. 17:513-520

Rabinovich, G. A., van Kooyk, Y., and Cobb, B. A. (2012) Glycobiology of immune responses. Ann N Y Acad Sci 1253, 1-15

Rossi, B., Espeli, M., Schiff, C., and Gauthier, L. (2006) Clustering of pre-B cell integrins induces galectin-1-dependent pre-B cell receptor relocalization and activation. J Immunol 177, 796-803

Schott, E. J., and Vasta, G. R. (2003) The PmSOD1 gene of the protistan parasite *Perkinsus marinus* complements the sod2Delta mutant of Saccharomyces cerevisiae, and directs an iron superoxide dismutase to mitochondria. Mole. Biochem. Parasit. 126, 81-92

Schott, E. J., Robledo, J. A. F., Wright, A. C., Silva, A. M., and Vasta, G. R. (2003) Gene organization and homology modeling of two iron superoxide dismutases of the early branching protist *Perkinsus marinus*. Gene 309, 1-9

Schott EJ, Di Lella S, Bachvaroff TR, Amzel LM, Vasta GR. Lacking catalase, a protistan parasite draws on its photosynthetic ancestry to complete an antioxidant repertoire with ascorbate peroxidase. BMC Evol Biol. 2019 Jul 19;19(1):146. doi: 10.1186/s12862-019-1465-5.

Schwarz FP, Ahmed H, Bianchet MA et al. (1998) Thermodynamics of bovine spleen galectin-1 binding to disaccharides: correlation with structure and its effect on oligomerization at the denaturation temperature. Biochemistry 37:5867–77.

Seetharaman J, Kanigsberg A, Slaaby R et al. (1998) X-ray crystal structure of the human galectin-3 carbohydrate recognition domain at 2.1-A resolution. J Biol Chem 273:13047–52.

Shi, X.-Z., Wang, L., Xu, S., Zhang, X.-W., Zhao, X.-F., Vasta, G. R., and Wang, J.-X. (2014) A Galectin from the Kuruma Shrimp (*Marsupenaeus japonicus*) Functions as an Opsonin and Promotes Bacterial Clearance from Hemolymph. *PloS one* **9**, e91794

Stowell, S. R., Arthur, C. M., Dias-Baruffi, M., Rodrigues, L. C., Gourdine, J.-P., Heimburg-Molinaro, J., Ju, T., Molinaro, R. J., Rivera-Marrero, C., Xia, B., Smith, D. F., and Cummings, R. D. (2010) Innate immune lectins kill bacteria expressing blood group antigen. *Nature medicine* **16**, 295-301

Stowell, S. R., Qian, Y., Karmakar, S., Koyama, N. S., Dias-Baruffi, M., Leffler, H., McEver, R. P., and Cummings, R. D. (2008) Differential roles of galectin-1 and galectin-3 in regulating leukocyte viability and cytokine secretion. J Immunol 180, 3091-3102

Stowell, S.R., Cho, M., Feasley, C. L., Arthur, C. M., Song, X., Colucci, J. K., Karmakar, S., Mehta, P., Dias-Baruffi, M., McEver, R. P., and Cummings, R. D. (2009) Ligand reduces galectin-1 sensitivity to oxidative inactivation by enhancing dimer formation. J. Biol. Chem. 284 (8), 4989–4999

Su, J, Gao J, Si Y, Cui L, Song C, Wang Y, Wu R, Tai G, Zhou Y. Galectin-10: a new structural type of prototype galectin dimer and effects on saccharide ligand binding. Glycobiology. 2018 Mar 1;28(3):159-168. doi: 10.1093/glycob/cwx107.

Takeuchi, T. Galectins in Invertebrates with a focus on *Caenorhabditis elegans.* Trends in Glycoscience and Glycotechnology. 30 (2018) Issue 172 Pages SE67-SE74

Tasumi, S., and Vasta, G. R. (2007) A galectin of unique domain organization from hemocytes of the Eastern oyster (*Crassostrea virginica*) is a receptor for the protistan parasite *Perkinsus marinus*. J Immunol 179, 3086-3098

Than, NG, Pick E, Bellyei S, Szigeti A, Burger O, Berente Z, et al. Functional analyses of placental protein 13/galectin-13. Eur J Biochem. (2004) 271:1065–78. doi: 10.1111/j.1432-1033.2004.04004.x

Toledo, K. A., Fermino, M. L., Andrade Cdel, C., Riul, T. B., Alves, R. T., Muller, V. D., Russo, R. R., Stowell, S. R., Cummings, R. D., Aquino, V. H., and Dias- Baruffi, M. (2014) Galectin-1 Exerts Inhibitory Effects during DENV-1 Infection. PloS one 9, e112474

Vasta, G. R., and Ahmed, H. (2008) Animals lectins: A Functional View. in Animals lectins: A Functional View (Vasta, G. R., and Ahmed, H. eds.), CRC Press, Boca Raton, FL. Pp

Vasta, G. R., Ahmed, H., Tasumi, S., Odom, E. W., and Saito, K. (2007) Biological roles of lectins in innate immunity: molecular and structural basis for diversity in self/non-self recognition. Adv Exp Med Biol 598, 389-406

Vasta, G. R., and Ahmed, H. (2008) Animal Lectins: A Functional View, CRC Press

Vasta, G. R., et al. 2004. Galectins in teleost fish: Zebrafish (*Danio rerio*) as a model species to address their biological roles in development and innate immunity. Glycoconj J. 21: 503-521.

Vasta, G. R. 2009. Roles of galectins in infection. Nat Rev Microbiol. 7: 424-438

Vasta GR, Ahmed H, Bianchet MA, Fernández-Robledo JA, Amzel LM. (2012) Diversity in recognition of glycans by F-type lectins and galectins: molecular, structural, and biophysical aspects. Ann N Y Acad Sci.1253(1):14-26

Vasta GR, Amzel LM, Bianchet MA, Cammarata M, Feng C, Saito K. (2017) F-Type Lectins: A highly diversified family of fucose-binding proteins with a unique sequence motif and structural fold, involved in self/non-self-recognition. Front Immunol. Nov 29;8:1648.

Vasta GR, Feng C, Bianchet, MA, Bachvaroff TR and Tasumi S (2015) Structural, functional, and evolutionary aspects of galectins in aquatic mollusks: From a sweet tooth to the Trojan horse. Fish and Shellfish Immunol 46(1):94-106

Vasta GR, Feng C, Tasumi S, Abernathy K, Bianchet MA, Wilson IBH, Paschinger K, Wang LX, Iqbal M, Ghosh A, Amin MN, Smith B, Brown S, Vista A. Biochemical characterization of oyster and clam galectins: Selective recognition of carbohydrate ligands on host hemocytes and *Perkinsus* parasites. Front Chem. 2020 Feb 25;8:98. doi: 10.3389/fchem.2020.00098.

Vazquez L, Alpuche J, Maldonado G, Agundis C, Pereyra-Morales A, Zenteno E. Review: Immunity mechanisms in crustaceans. Innate Immun. 2009 Jun;15(3):179-88. doi: 10.1177/1753425909102876.29198776

Wang XW Wang JX(a). Pattern recognition receptors acting in innate immune system of shrimp against pathogen infections. Fish Shellfish Immunol. 2013 Apr;34(4):981-9.

Wang XW Wang JX(b). Diversity and multiple functions of lectins in shrimp immunity. Dev Comp Immunol. 2013 Jan; 39(1-2):27-38.

Xia X, You M, Rao XJ, Yu XQ. Insect C-type lectins in innate immunity. Dev Comp Immunol. 2018 Jun; 83:70-79. doi: 10.1016/j.dci.2017.11.020. Epub 2017 Dec 1. Review.

Xu YH, Bi WJ, Wang XW, Zhao YR, Zhao XF, Wang JX. Two novel C-type lectins with a low-density lipoprotein receptor class A domain have antiviral function in the shrimp *Marsupenaeus japonicus*. Dev Comp Immunol. 2014 Feb; 42(2):323-32

Xu S, Wang L, Wang X-W, Zhao Y-R, Bi W-J, Zhao X-F Wang J-X. L-Type lectin from the kuruma shrimp *Marsupenaeus japonicus* promotes hemocyte phagocytosis. Dev Comp Immunol. 2014 Jun; 44(2):397–405.

Yang, M. L., Chen, Y. H., Wang, S. W., Huang, Y. J., Leu, C. H., Yeh, N. C., Chu, C. Y., Lin, C. C., Shieh, G. S., Chen, Y. L., Wang, J. R., Wang, C. H., Wu, C. L., and Shiau, A. L. (2011) Galectin-1 binds to influenza virus and ameliorates influenza virus pathogenesis. *Journal of virology* **85**, 10010-10020

Yang, R. Y., Yu, L., Graham, J. L., Hsu, D. K., Lloyd, K. C., Havel, P. J., and Liu, F. T. (2011) Ablation of a galectin preferentially expressed in adipocytes increases lipolysis, reduces adiposity, and improves insulin sensitivity in mice. Proceedings of the National Academy of Sciences of the United States of America 108, 18696-18701

Zhang KY, Yuan WJ, Xu JD, Wang JX. Cation-dependent mannose-6-phosphate receptor functions as a pattern recognition receptor in anti-bacterial immunity of *Marsupenaeus japonicus*. Dev Comp Immunol. 2018; 89:122–130.

Zhang Q, Wang XQ, Jiang HS, Jia WM, Zhao XF, Wang JX. Calnexin functions in antibacterial immunity of *Marsupenaeus japonicus*. Dev Comp Immunol. 2014;46(2):356–363.

Zhou D, Ge H, Sun J, Gao Y, Teng M, Niu L. (2008) Crystal structure of the C-terminal conserved domain of human GRP, a galectin-related protein, reveals a function mode different from those of galectins. Proteins 15;71(3):1582-8.

Figure 1

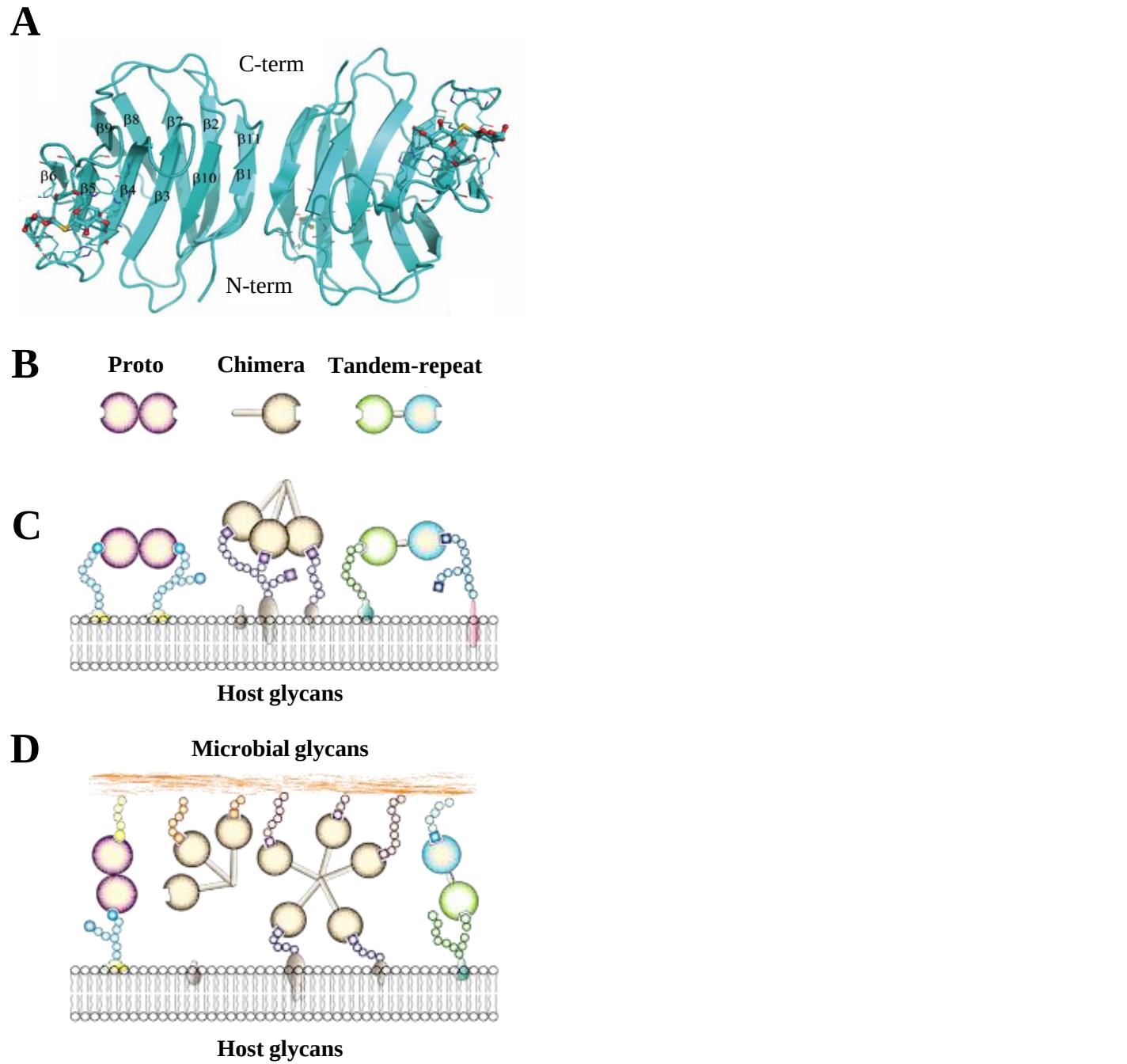

A
C-term
β9
β8
β7
β2
β11
β6
β5
β4
β3
β10
β1
N-term
B
Proto
Chimera
Tandem-repeat
C
Host glycans
D
Microbial glycans
Host glycans

Figure 2

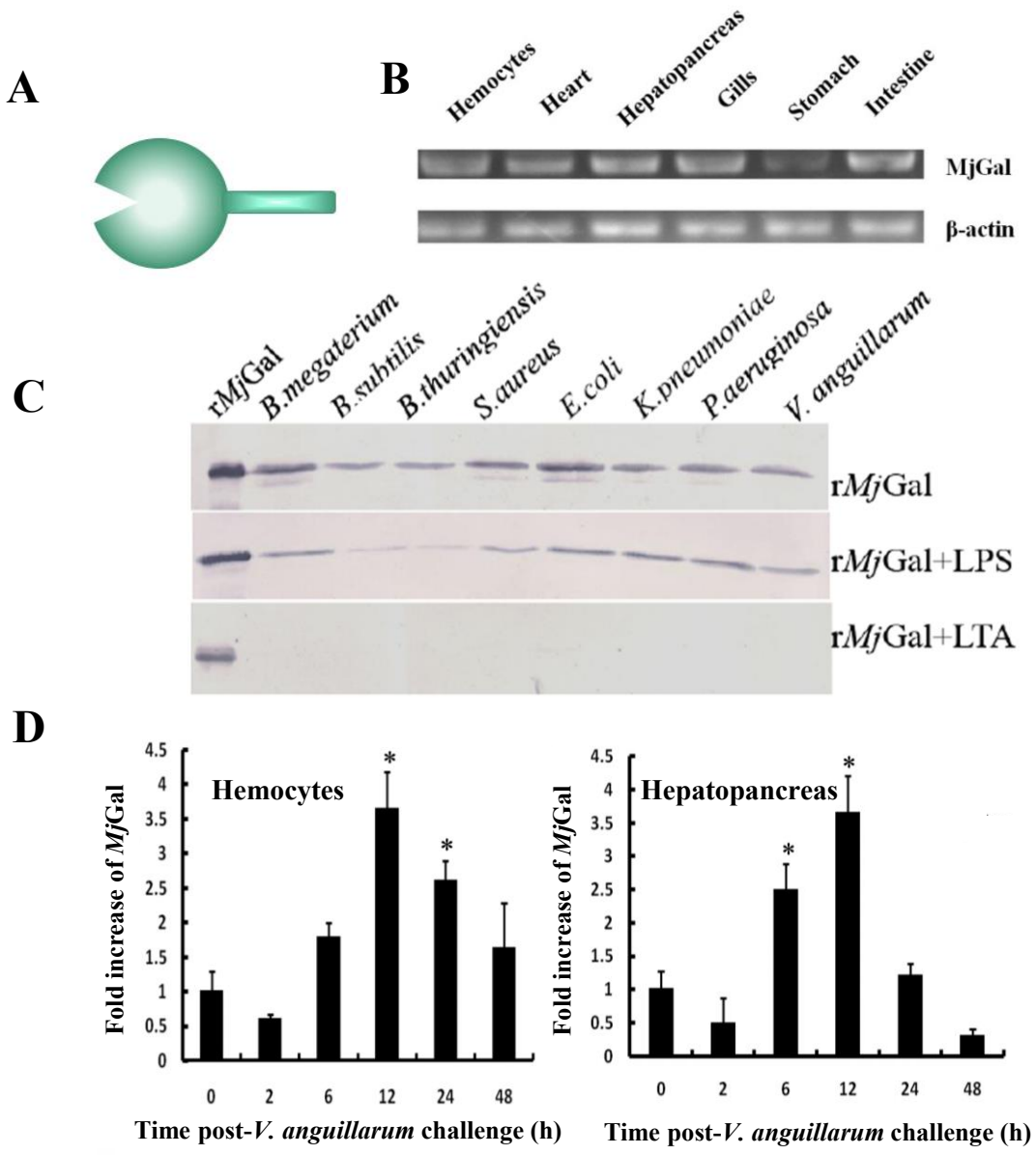

A
B
Hemocytes
Heart
Hepatopancreas
Gills
Stomach
Intestine
MjGal
β-actin
C
rMjGal
B.megaterium
B.subtilis
B.thuringiensis
S.aureus
E.coli
K.pneumoniae
P.aeruginosa
V.anguillarum
rMjGal
rMjGal+LPS
rMjGal+LTA
D
Hemocytes
Hepatopancreas
Fold increase of MjGal
Time post-V. anguillarum challenge (h)
0
2
6
12
24
48
0.5
1
1.5
2
2.5
3
3.5
4
4.5
*

Figure 3

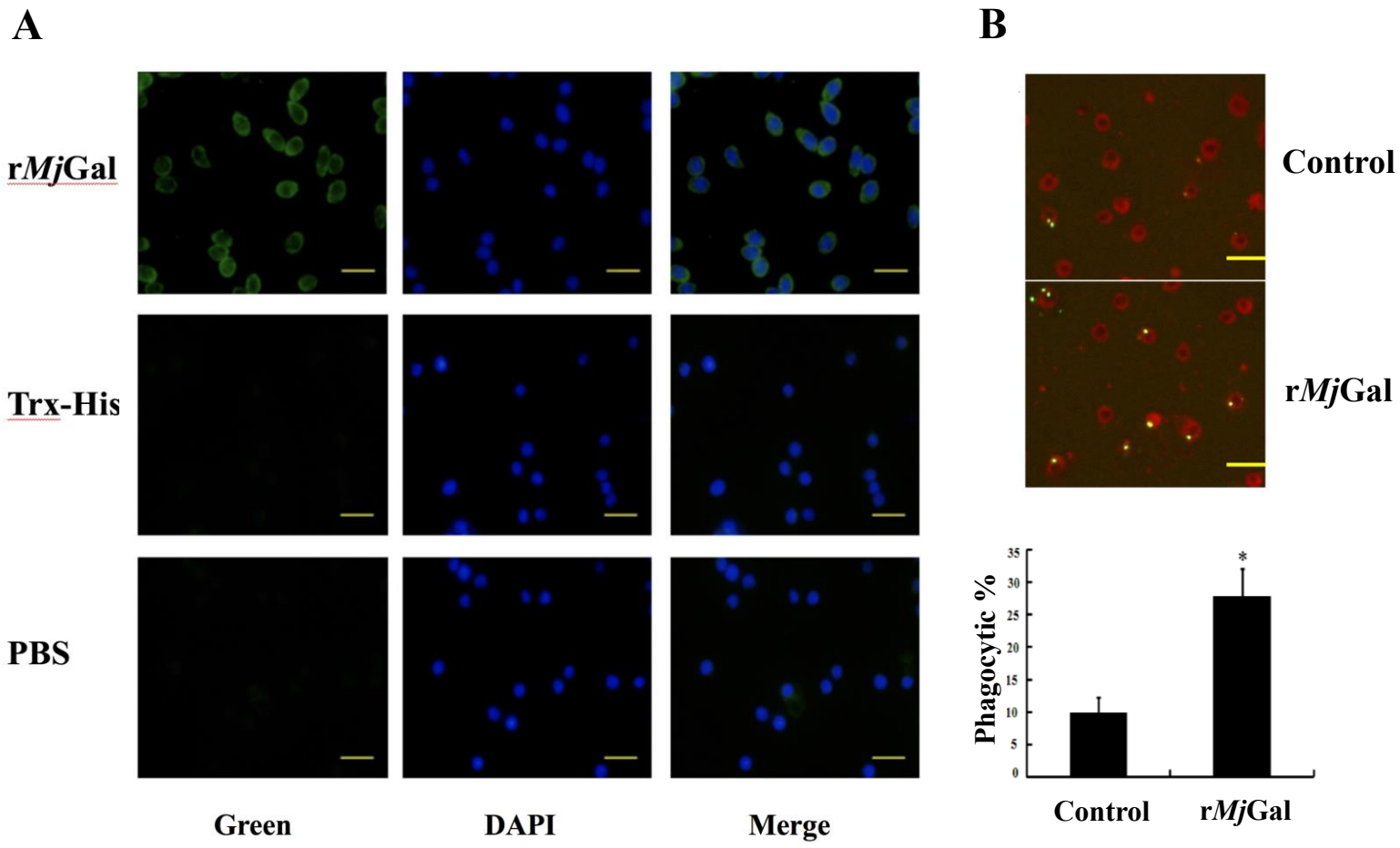

A
B
rMjGal
Trx-His
PBS
Green
DAPI
Merge
Control
rMjGal
Phagocytic %
35
30
25
20
15
10
5
0
*
Control
rMjGal

Figure 4

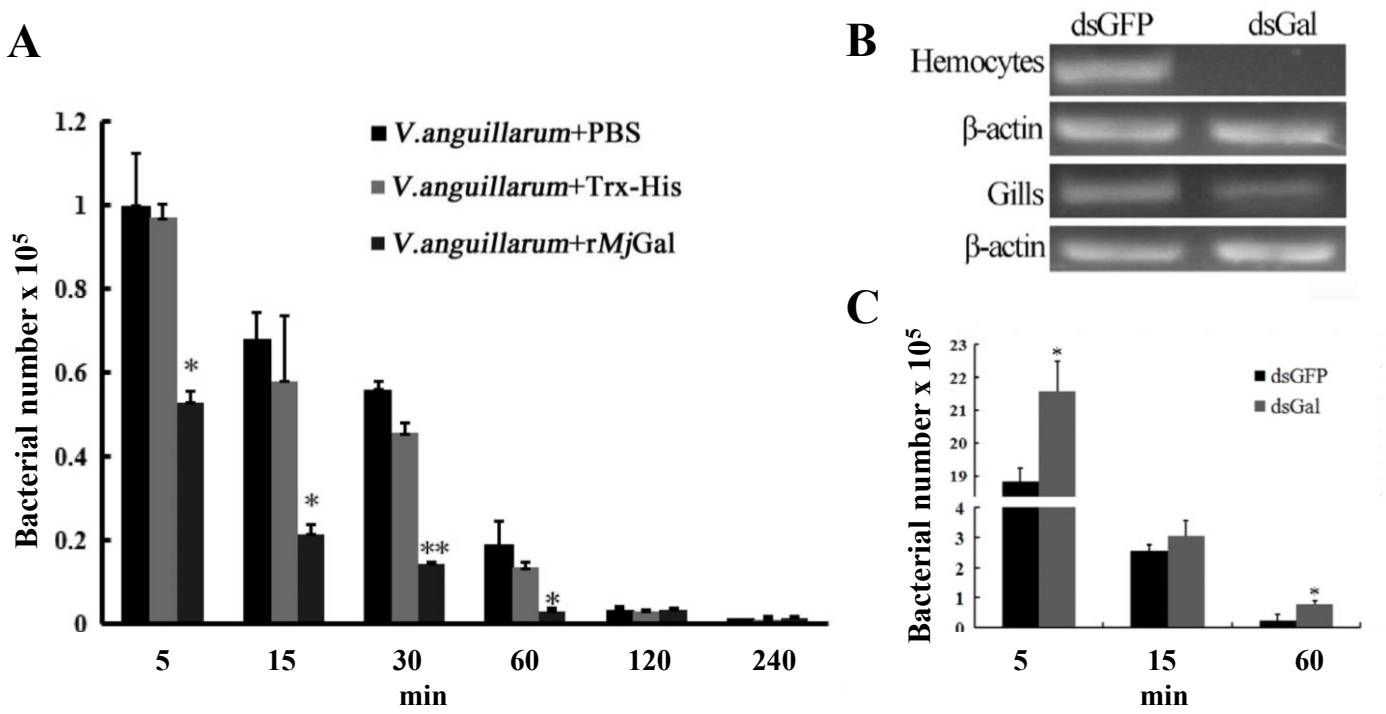

A
V.anguillarum+PBS
V.anguillarum+Trx-His
V.anguillarum+rMjGal
Bacterial number x 10^5
1.2
1
0.8
0.6
0.4
0.2
0
*
*
**
*
5
15
30
60
120
240
min
B
dsGFP
dsGal
Hemocytes
β-actin
Gills
β-actin
C
Bacterial number x 10^5
23
22
21
20
19
4
3
2
1
0
dsGFP
dsGal
*
*
5
15
60
min

Figure 5

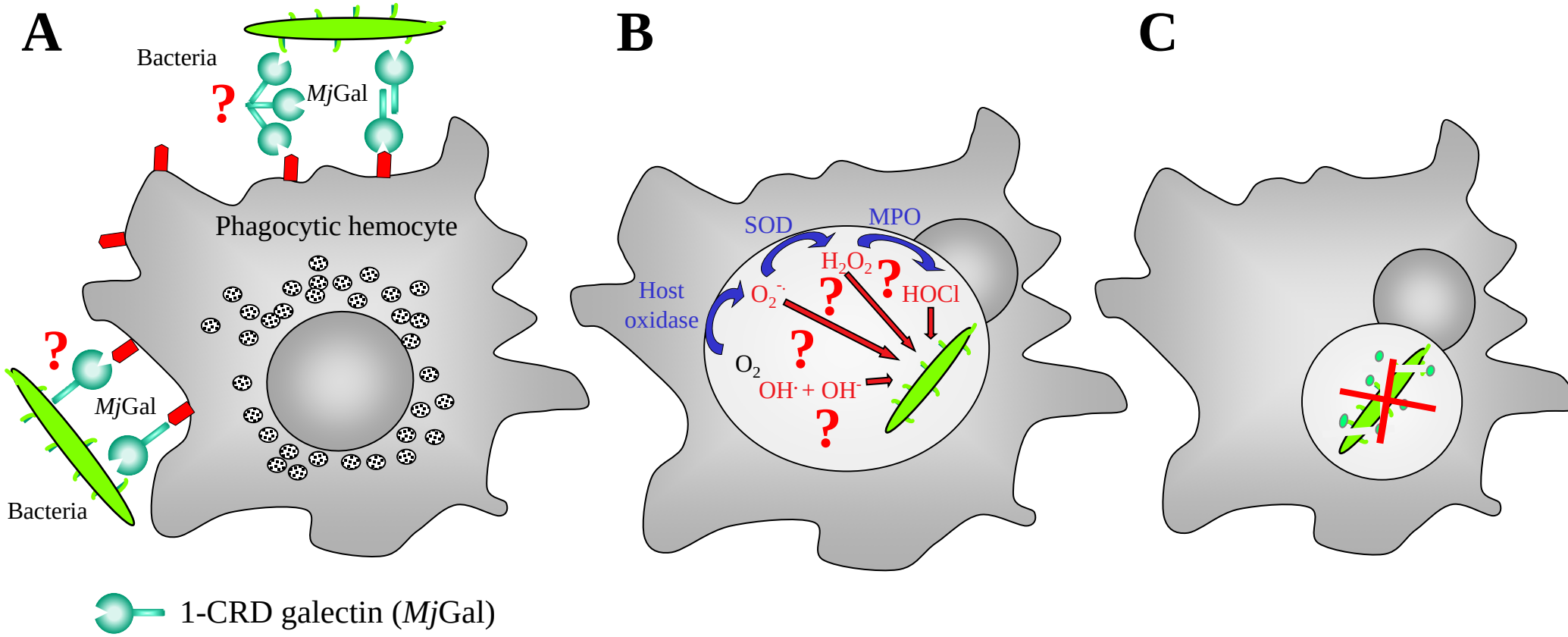

A
Bacteria
?
MjGal
Phagocytic hemocyte
?
MjGal
Bacteria
B
SOD
MPO
Host
oxidase
O2-·
H2O2
?
?
HOCl
?
O2
OH· + OH-
?
C
1-CRD galectin (MjGal)

Figure 6

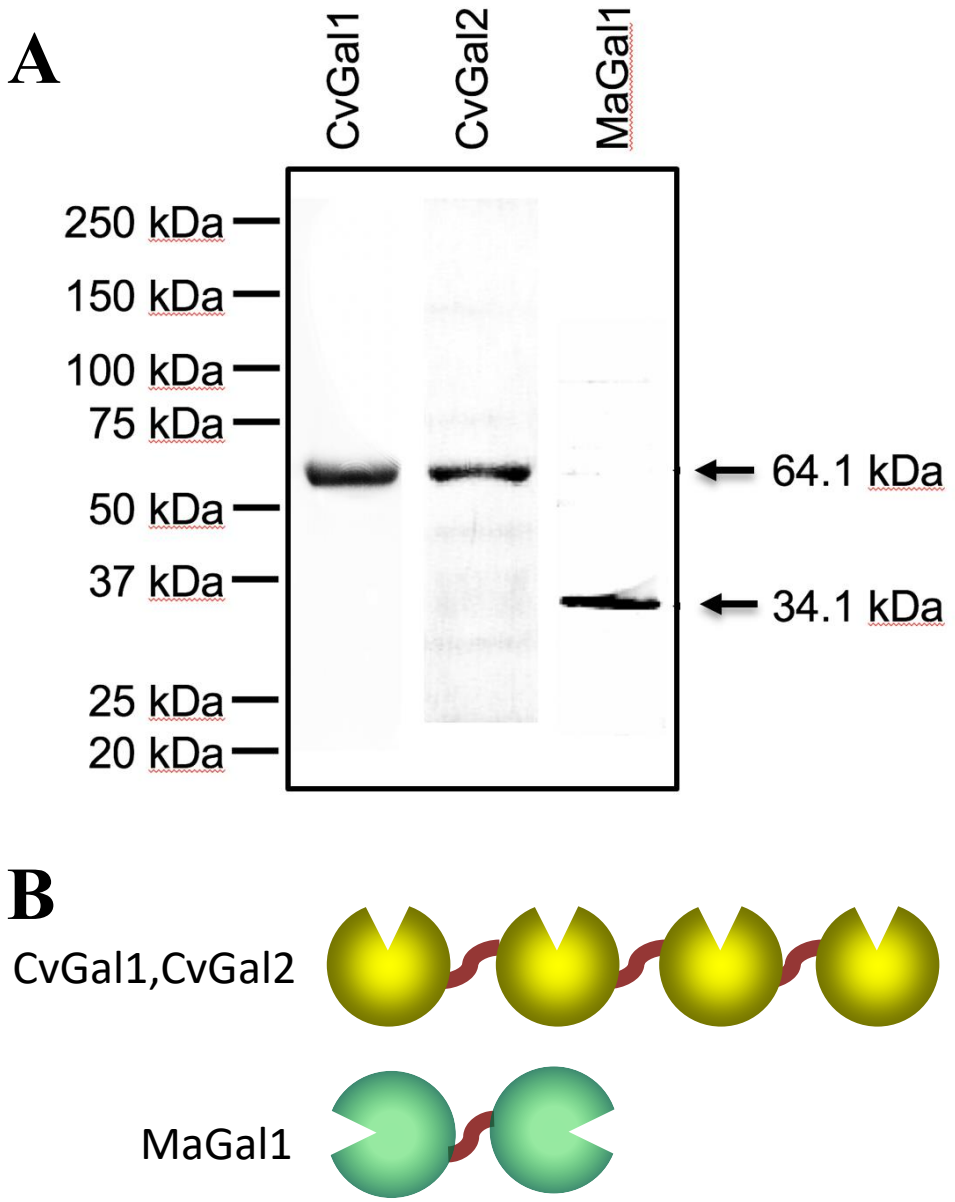

A
CvGal1
CvGal2
MaGal1
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
64.1 kDa
34.1 kDa
B
CvGal1,CvGal2
MaGal1

Figure 7

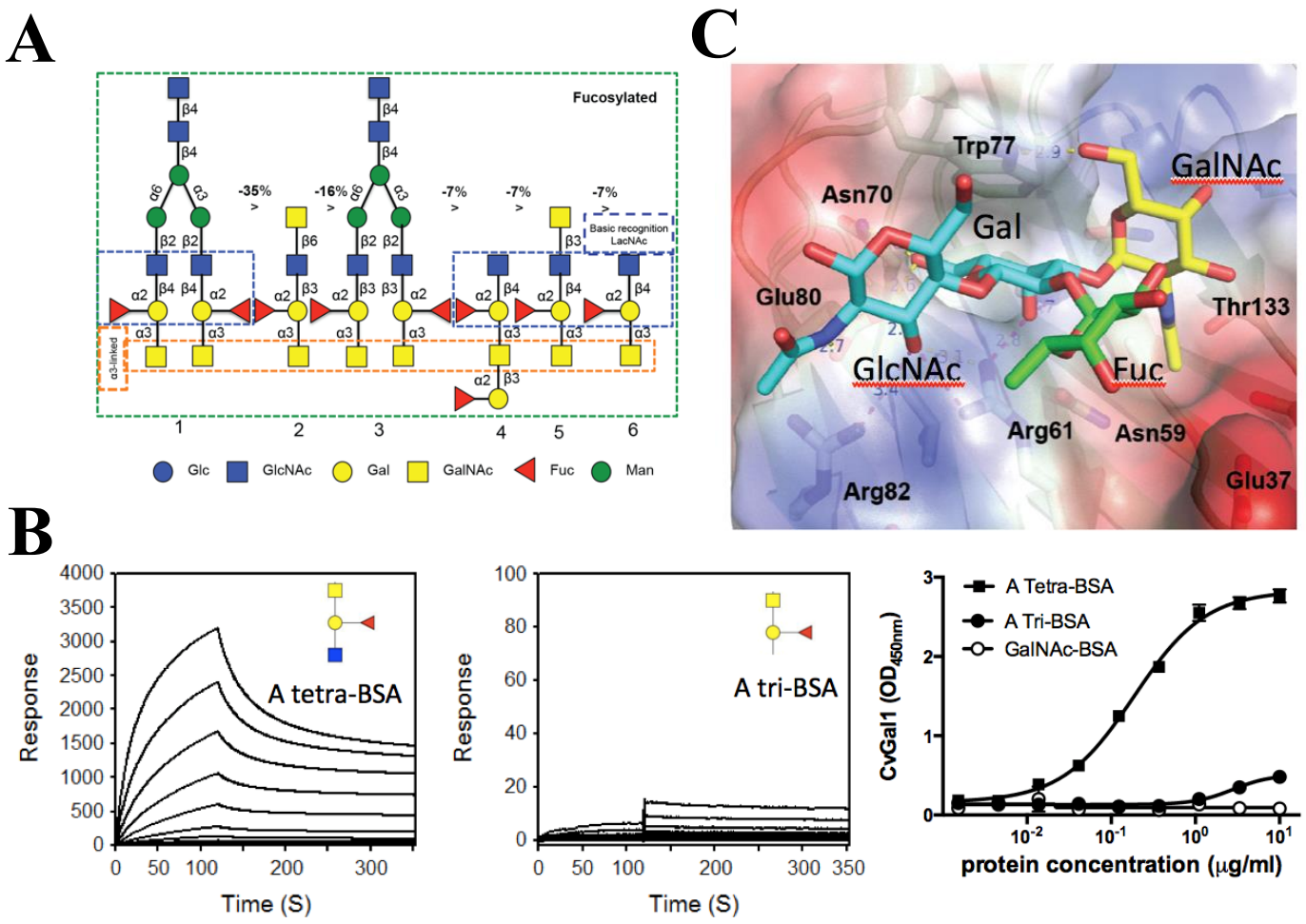
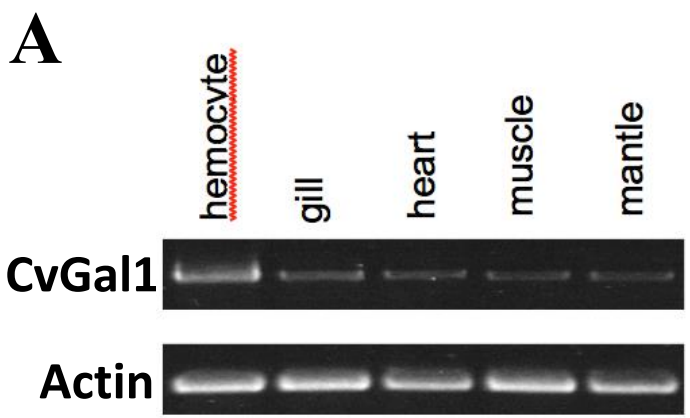
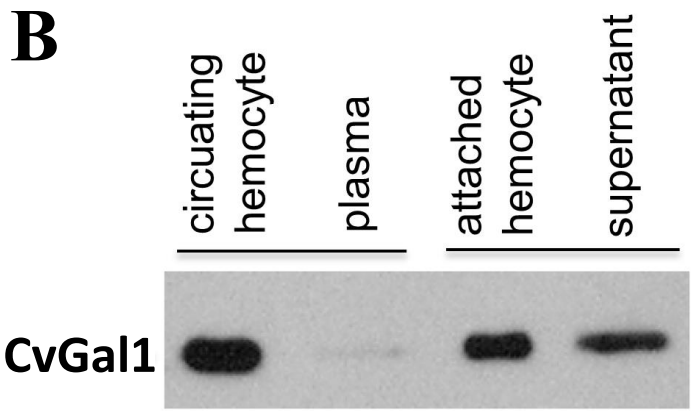
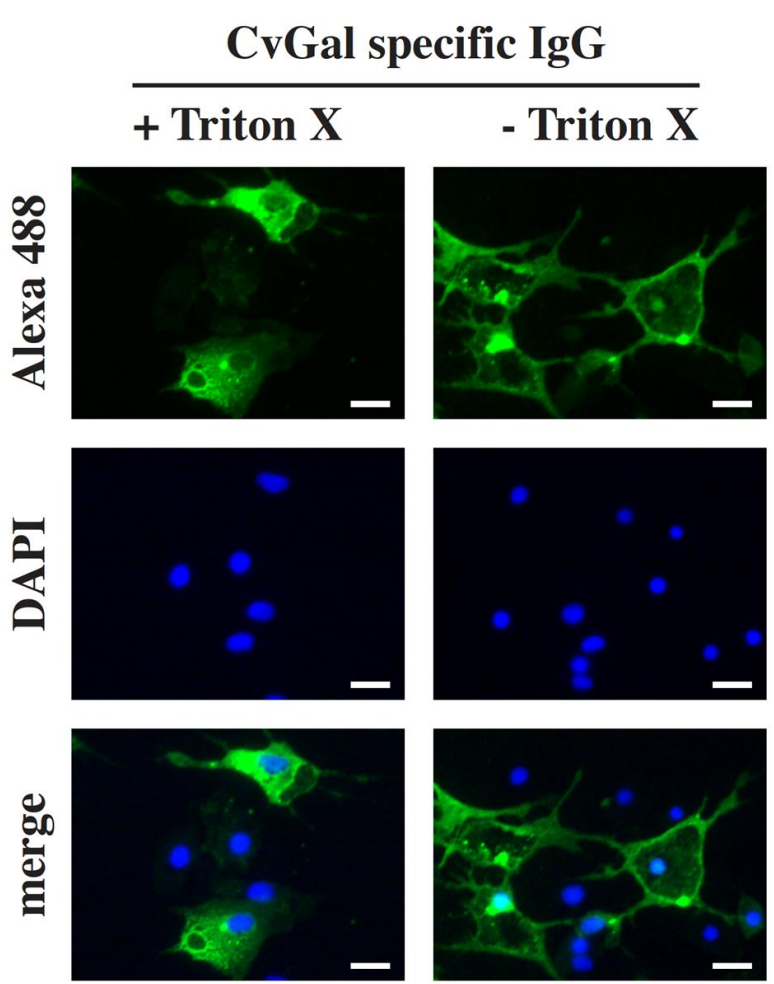
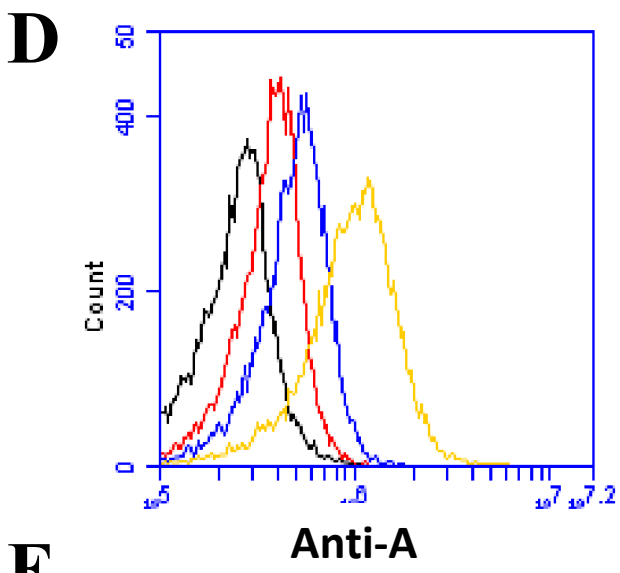

A
Fucosylated
-35%
-16%
-7%
-7%
-7%
Basic recognition LacNAc
α3-linked
1
2
3
4
5
6
Glc
GlcNAc
Gal
GalNAc
Fuc
Man
C
Trp77
GalNAc
Asn70
Gal
Glu80
Thr133
GlcNAc
Fuc
Arg61
Asn59
Glu37
Arg82
B
A tetra-BSA
Response
Time (S)
A tri-BSA
Response
Time (S)
A Tetra-BSA
A Tri-BSA
GalNAc-BSA
CvGal1 (OD450nm)
protein concentration (μg/ml)

Figure 8


A
hemocyte
gill
heart
muscle
mantle
CvGal1
Actin


B
circuating hemocyte
plasma
attached hemocyte
supernatant
CvGal1


C
CvGal specific IgG
+ Triton X
- Triton X
Alexa 488
DAPI
merge


D
Count
Anti-A

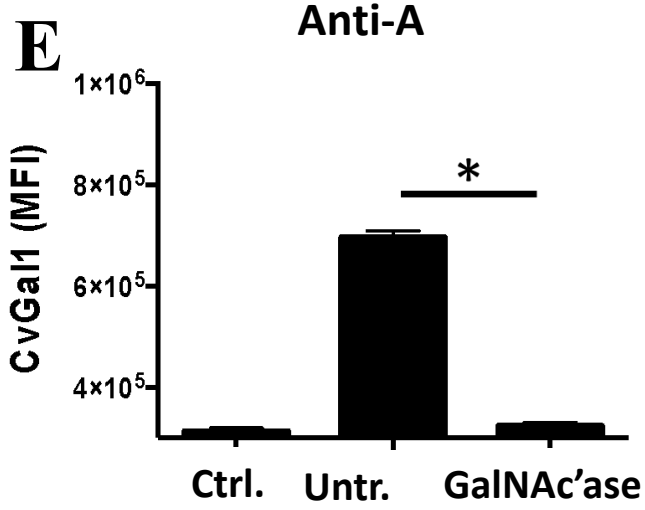

E
CvGal1 (MFI)
Ctrl.
Untr.
GalNAc'ase
*

Figure 9

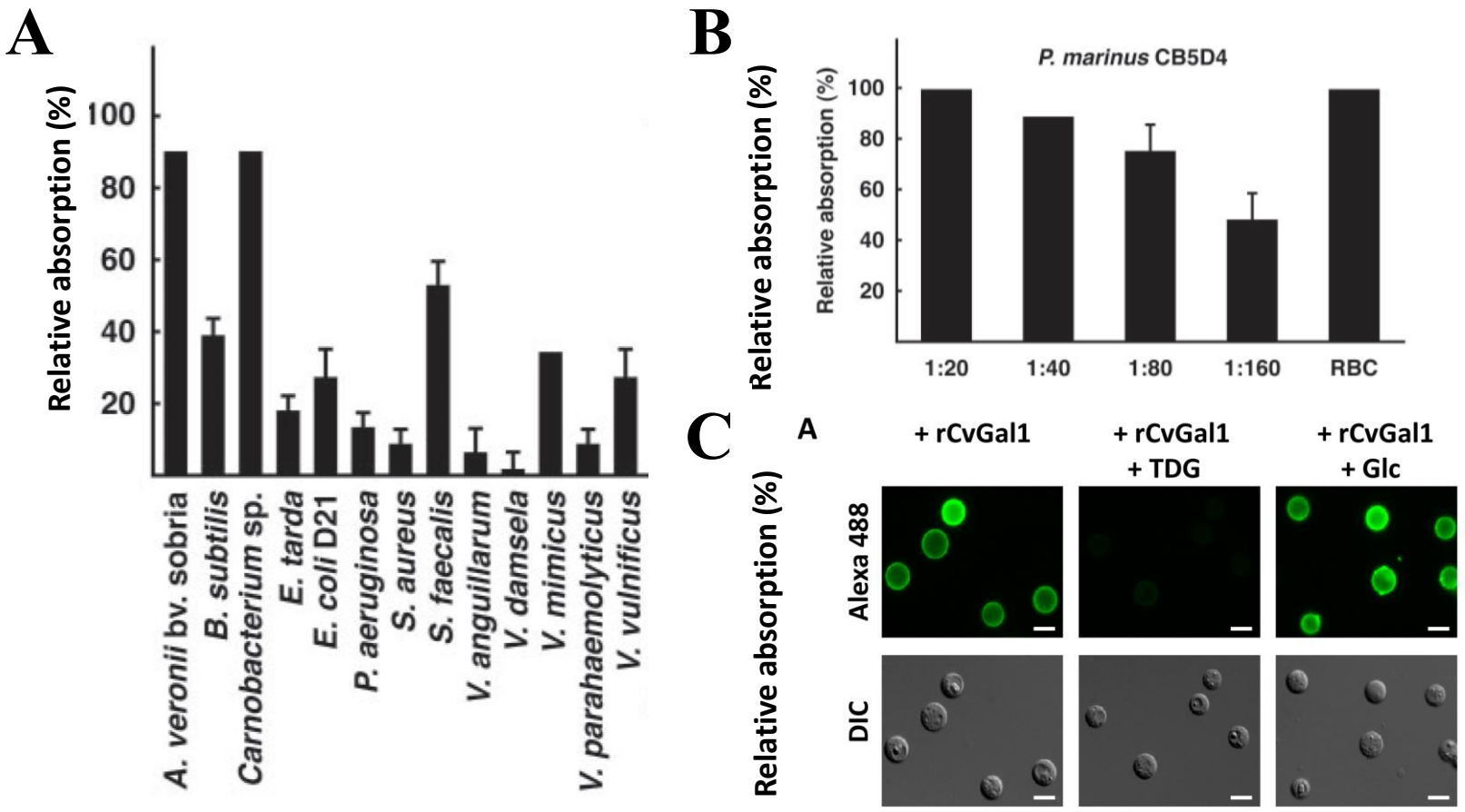

A
Relative absorption (%)
100
80
60
40
20
A. veronii bv. sobria
B. subtilis
Carnobacterium sp.
E. tarda
E. coli D21
P. aeruginosa
S. aureus
S. faecalis
V. anguillarum
V. damsela
V. mimicus
V. parahaemolyticus
V. vulnificus
B
Relative absorption (%)
P. marinus CB5D4
Relative absorption (%)
100
80
60
40
20
1:20
1:40
1:80
1:160
RBC
C
Relative absorption (%)
A
+ rCvGal1
+ rCvGal1
+ TDG
+ rCvGal1
+ Glc
Alexa 488
DIC

Figure 10

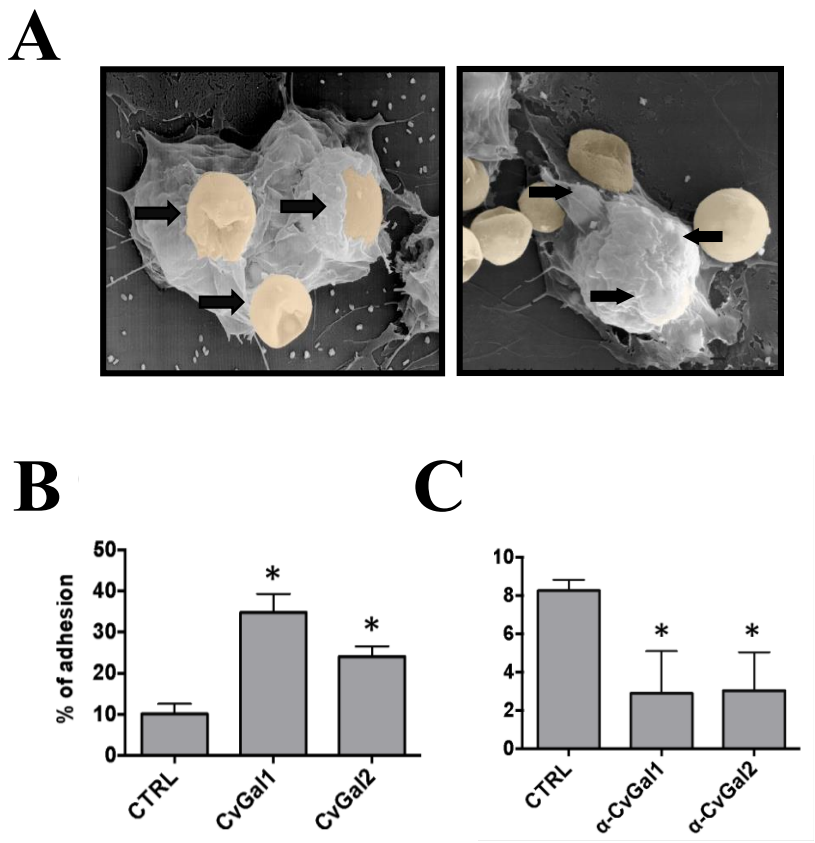
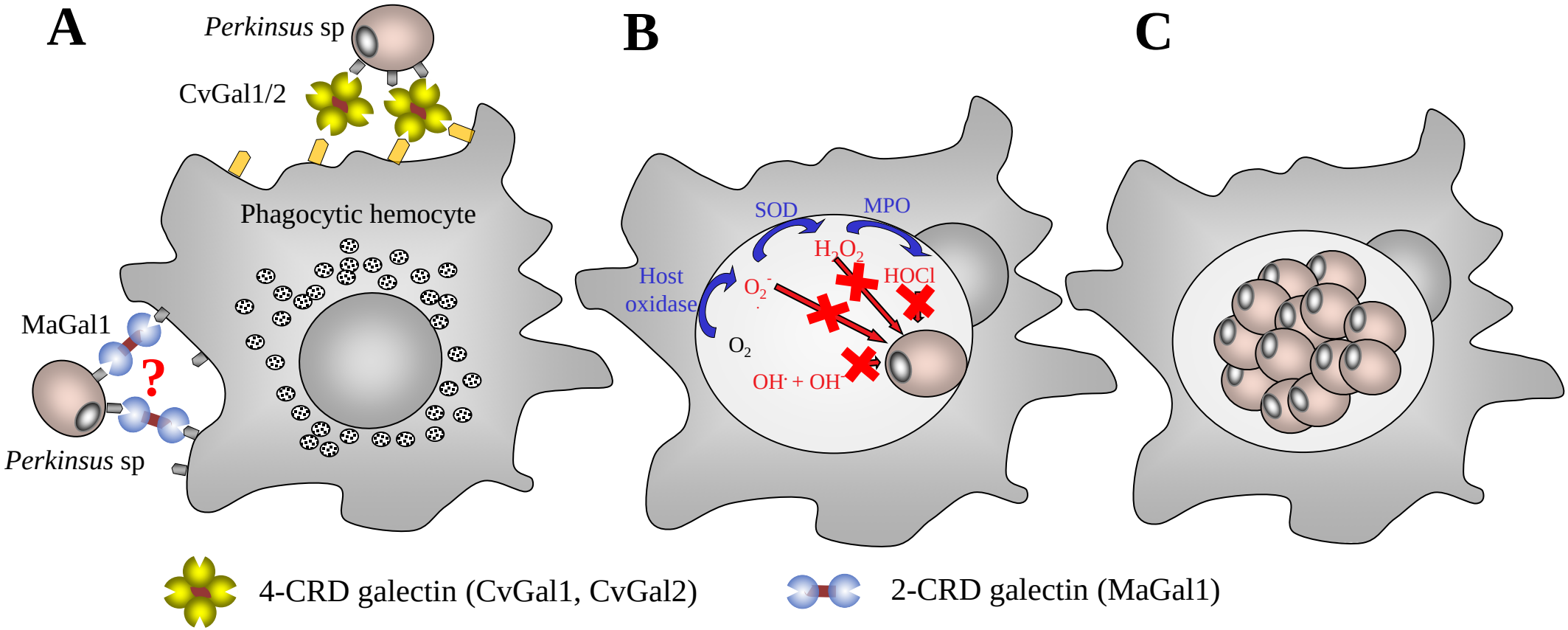

A
B
% of adhesion
50
40
30
20
10
0
*
*
CTRL
CvGal1
CvGal2
C
10
8
6
4
2
0
*
*
CTRL
α-CvGal1
α-CvGal2

Figure 11


A
Perkinsus sp
CvGal1/2
Phagocytic hemocyte
MaGal1
?
Perkinsus sp
B
SOD
MPO
H2O2
HOCl
Host
oxidase
O2-
O2
OH· + OH-
C
4-CRD galectin (CvGal1, CvGal2)
2-CRD galectin (MaGal1)