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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☆

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This article is dedicated to the memory of Barbara Giomarelli, Ph.D.

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ABSTRACT

The infectious hematopoietic necrosis virus (IHNV; *Rhabdoviridae*, Novirhabdovirus) infects teleost fish, such as salmon and trout, and is responsible for significant losses in the aquaculture industry and in wild fish populations. Although IHNV enters the host through the skin at the base of the fins, the viral adhesion and entry mechanisms are not fully understood. In recent years, evidence has accumulated in support of the key roles played by protein-carbohydrate interactions between host lectins secreted to the extracellular space and virion envelope glycoproteins in modulating viral adhesion and infectivity. In this study, we assessed *in vitro* the potential role(s) of zebrafish (*Danio rerio*) proto type galectin-1 (Drgal1-L2) and a chimera galectin-3 (Drgal3-L1) in IHNV adhesion to epithelial cells. Our results suggest that the extracellular Drgal1-L2 and Drgal3-L1 interact directly and in a carbohydrate-dependent manner with the IHNV glycosylated envelope and glycans on the epithelial cell surface, significantly reducing viral adhesion.

Abbreviations: IHNV, infectious hematopoietic necrosis virus; Drgal1-L2, zebrafish galectin-1; Drgal3-L1, zebrafish galectin-3; PRR, pattern recognition receptors; CRD, carbohydrate-recognition domain; FBS, fetal bovine serum; MOI, multiplicity of infection; PBS, phosphate-buffered saline; BME, β-mercaptoethanol; HRP, horseradish peroxidase; LacNAc, *N*-acetyllactosamine; GlcNAc, *N*-acetylglucosamine; DSA, *Datura stramonium* agglutinin; Galβ(1–3)GalNAc, galactose-β(1–3)-*N*-acetylgalactosamine; Galβ(1–4)GlcNAc, galactose-β(1–4)-*N*-acetylglucosamine.

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1. Introduction

Viral infections cause severe diseases in farmed and wild fish populations and have a detrimental impact in the aquaculture industry and the environment. Both rhabdoviruses, such as the infectious hematopoietic necrosis virus (IHNV), snakehead rhabdovirus (SHRV) and viral hemorrhagic septicemia virus (VHSV), and birnaviruses such as the infectious pancreatic necrosis virus (IPNV) are responsible for massive losses of fish in both wild and farmed salmon and trout species by highly pathogenic local and systemic infections that lead to significant morbidity and mortality (Wolf, 1988; Crane and Hyatt, 2011; Purcell et al., 2012).

Rhabdoviruses are characterized by a single-stranded, negative-sense RNA genome, and an envelope consisting of trimers of a glycoprotein that displays multiple N-linked oligosaccharides and plays an integral role in the pathogenesis of viral infection. IHNV (family *Rhabdoviridae*, genus Novirhabdovirus type species) infects teleost fish, such as salmon and trout, and is responsible for catastrophic losses in the aquaculture industry. The genome of IHNV encodes for six gene products, the nucleocapsid (N), phosphoprotein (P), matrix protein (M), envelope glycoprotein (G), non-virion protein (NV) and polymerase or large protein (L), and is organized in the following order: 3′-leader-N-P-M-G-NV-L-trailer-5′. Three major genetic groups of North American IHNV isolates have been defined based on their envelope protein sequence similarity, designated as the U, M, and L genogroups. The M genogroup is endemic in the rainbow trout farming region in Idaho, where phylogenetically distinct sub-groups, designated MA–MD have been reported. The MB, MC, and MD sub-groups are the three most prevalent and widely distributed types of IHNV in the virus-endemic region, and they have been shown to co-circulate in the field for over 20 years (Troyer and Kurath, 2003).

IHNV infections spread throughout fish populations by waterborne transmission (Batts et al., 1991), as the virus shed by the infected animals into the water enters the new host, and soon infections can be detected in gills, esophagus and cardiac stomach (Chilmonczyk and Monge, 1980; Helmick et al., 1995). The primary infection site(s) of IHNV have not been rigorously identified but it has been reported that the virion enters the host through the skin at the base of the fins (Harmache et al., 2006). Skin has also been identified as a main entry site for the koi herpesvirus in *Cyprinus carpio* (Costes et al., 2009). The viral adhesion and entry mechanisms, however, are not fully understood but a unique fibronectin has been identified as an IHNV attachment factor present at the cell surface (Bearzotti et al., 1999; Liu and Collodi, 2002).

In recent years, evidence has accumulated in support of the key roles played by protein-carbohydrate interactions between host lectins and virion envelope glycoproteins in modulating viral adhesion and infectivity (Ouellet et al., 2005; St-Pierre et al., 2011; Yang et al., 2011; Toledo et al., 2014). Glycans on the viral surface can be recognized by host lectins that function as pattern recognition receptors (PRRs) that signal to activate and regulate the appropriate innate and adaptive immune responses (Barrionuevo et al., 2007; Jeon et al., 2010). However, multiple examples reveal that co-evolution of host-pathogen consortia has led to subversion of the immune recognition roles of lectins to facilitate adhesion and entry of the pathogens into the host cells (Kamhawi et al., 2004; Ouellet et al., 2005; Okumura et al., 2008; Yang et al., 2011). Galectins are good examples of these opposing recognition functions of lectins that may be either beneficial for the host in their roles as PRRs, but also detrimental by functioning as facilitators of viral, bacterial, and parasitic infection (Tasumi and Vasta, 2007; Nieminen et al., 2008; Stowell et al., 2008; Vasta, 2009; St-Pierre et al., 2011; Yang et al., 2011; Vasta, 2012).

Galectins comprise an evolutionary conserved family of β-galactoside binding proteins, ubiquitous in mammals and other vertebrate taxa, invertebrates, and fungi. Galectins are defined by a unique sequence motif in their carbohydrate-recognition domains (CRDs), and are classified into three major structural types: (i) proto-type galectins, which contain one CRD and form homodimers; (ii) chimera-type galectins, which have a single CRD and can oligomerize forming trimers and pentamers; (iii) tandem-repeat-type galectins, which are characterized by two CRDs joined by a linker peptide (Vasta and Ahmed, 2008). Since their discovery in the 1970s, over a dozen galectin subtypes (galectins 1–14, numbered in the order of their discovery) have been identified in mammals. Our understanding of their biological roles, initially limited to the recognition of cell surface glycans in embryogenesis and early development (Camby et al., 2006), has expanded in recent years by the discovery of their immunoregulatory activities (Rabinovich and Toscano, 2009). In this regard, galectin 1 largely displays anti-inflammatory activity, whereas galectin 3 is endowed with pro-inflammatory properties (Di Lella et al., 2011). A gradual paradigm shift has taken place in the past few years through the realization that galectins also bind glycans on the surface of potentially pathogenic microbes, and function as recognition and effector factors in innate immunity (Stowell et al., 2008). Galectins can function as PRRs, recognizing various pathogen-associated molecular patterns (PAMPs) such as glycans, like lipopolysaccharides and peptidoglycan on the surface of pathogenic microbes, parasites, and fungi (Vasta, 2012). Most galectins are either bivalent or multivalent with regard to their carbohydrate-binding activities, which enable the simultaneous recognition of multiple binding partners ("self" or "non self"). This suggests that galectins can act as bridging molecules between immune cells such as phagocytes and their targets, acting as opsonins (Karlsson et al., 2009) or between microbes and other host cells modulating the immune response (Rabinovich and Toscano, 2009; Vasta, 2009; Davicino et al., 2011). Galectins of all three structural types have been identified and characterized in various tissues, plasma and mucus of teleost fish, and their roles in recognition of bacterial pathogens has been reported for multiple fish species (Vasta et al., 2011). Among these, the zebrafish offers multiple advantages over mammalian models for the elucidation of developmental and immune processes (Patton and Zon, 2001; Jesuthasan, 2002). The value of the zebrafish as a model system is buttressed by the observation that many orthologous genes, including galectins (Ahmed et al., 2004, 2009), are shared with mouse and man. Thus, the use of zebrafish for addressing basic questions about host-pathogen interactions and infectious disease has expanded dramatically in recent years (Yoder et al., 2002). All three galectin types (proto, chimera and tandem repeat) are present in zebrafish, and although its galectin repertoire is less complex than that of mammals, subtype isoforms are expressed, most likely resulting from genome duplications in this species (Ahmed et al., 2004). Thus, for our initial studies aimed at assessing *in vitro* the potential roles of zebrafish galectins in viral adhesion to epithelial cells, we selected members of the "proto" and "chimera" galectin types (galectin 1, and galectin 3, respectively) that in mammals: (a) are secreted to the extracellular space and bind to viral, bacterial, and fungal pathogens and parasites (Vasta, 2009), and (b) have opposite roles in the regulation of immune responses (Di Lella et al., 2011). Further, to facilitate this initial study, the subtypes/isoforms selected (DrGal1-L2 and DrGal3-L1) were those that are most abundantly expressed in zebrafish. Our results suggest that the extracellular Drgal1-L2 and Drgal3-L1 interact directly with the IHNV glycosylated envelope in a carbohydrate-dependent manner, as well as with galactosyl moieties on the epithelial cell surface, significantly reducing viral adhesion. A preliminary glycosylation study of the IHNV envelope glycoprotein identified