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The interaction of host and nematode galectins influences the outcome of gastrointestinal nematode infections

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Review

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Abstract

Galectins are a family of proteins that bind β-galactosides and play key roles in a variety of cellular processes including host defence. They have been well studied in hosts but less so in gastrointestinal nematodes. Both host and parasite galectins are present in the gastrointestinal tract following infection. Parasite galectins can both bind antibody, especially highly glycosylated IgE and be bound by antibody. Parasite galectins may act as molecular sponges that soak up antibody. Host galectins promote mast cell degranulation while parasite galectins inhibit degranulation. Host and parasite galectins can also bind mucins and influence mucus viscosity. As the protective response against gastrointestinal nematode infection is partly dependent on IgE mediated mast cell degranulation and mucus, the interactions between host and parasite galectins play key roles in determining the outcome of infection.

Introduction

Galectins belong to an evolutionary ancient family of glycan-binding proteins which bind β-galactosides, such as lactose and N-acetyllactosamine, either in free form or as components of glycoproteins or glycolipids (Massa *et al.*, 1993). Galectins have been isolated from fungi, invertebrates, fish, amphibians, birds and mammals. Galectins are involved in important intracellular processes; they regulate signalling pathways and cell interactions (Vasta, 2012). They also mediate host defence by acting as pattern recognition receptors (Vasta, 2009), killing microbes (Stowell *et al.*, 2010) and by regulating immune responses. Various aspects of galectins have recently been reviewed by a number of groups (Shi *et al.*, 2018; Modenutti *et al.*, 2019) but the interaction of nematode galectins and host galectins has not been explored.

Structure of host galectins

There are 17 distinct galectins identified in mammals and they are labelled gal-1 to gal-17. Generally, galectins with the same name in mammals are homologous; gal-1 in humans is homologous to gal-1 in mice. However, gal-11 and gal-15, both in sheep, are probably variants of the same protein. There are two different proteins called galectin-14; one from sheep and one in humans. The sheep molecule was the first protein to be called galectin-14 (Dunphy *et al.*, 2002). In comparison with the human galectins, the ovine gal-14 sequence is most similar to galectin-9 with 57% amino acid identity. Functionally, ovine gal-14 is most similar to human galectin-10 because both gal-10 and gal-14 function similarly in eosinophils (Young *et al.*, 2009). However, amino acid similarity with gal-10 is only 25% (Dunphy *et al.*, 2002). The second protein to be called gal-14 was originally called placental protein 13-like because of its similarity to placental protein 13 (Yang *et al.*, 2001*a*). It is part of a cluster of 5 galectin genes on human chromosome 19 (Than *et al.*, 2009). Gal-5 is found in rat erythrocytes but has not been reported in humans (Gitt *et al.*, 1995). Galectin-6 is only found in mice and is a recent duplication of gal-4 (Gitt *et al.*, 1998; Cooper, 2002). Galectins in other classes of vertebrates with the same name are not necessarily homologous; gal-1 in humans need not correspond to gal-1 in a fish species.

There is considerable diversity among galectins in their structure and function. Most galectins show less than 40% amino acid identity to each other (Fig. S1). Some galectins occur in the cytoplasm while others are secreted by the cell and act extracellularly. However, no galectin contains a secretion signal peptide (Cooper and Barondes, 1999). Galectin synthesis occurs on free polyribosomes prior to export through a noncanonical Golgi-independent pathway (Lindstedt *et al.*, 1993). Some galectins such gal-1 and gal-3 are expressed in cells of the myeloid and lymphoid lineages while other galectins like gal-7 and gal-12 are only expressed in specific tissues.

Galectins vary in size from 14 to 39 KDa. Structurally, galectins are classified into three types: prototype, chimaeric or tandem repeat. Figure 1 shows an example of each type of galectin. Gal-1, gal-2, gal-7 (Saussez and Kiss, 2006), gal-10 (Ackerman *et al.*, 2002), gal-11, gal-13 (Than *et al.*, 2009) ovine gal-14, human gal-14, gal-16 and gal-17 are prototype galectins that each have one carbohydrate recognition domain (CRD). They are usually synthesised as monomers and non-covalently homodimerize.

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The only chimaeric galectin is gal-3 which is produced as a 35KDa monomer with 250 amino acids and one CRD (Fig. 1). The monomers can polymerize to form oligomers after binding of the CRD (Modenutti *et al.*, 2019).

Galectin-4, −6, −8, −9 and −12 are tandem repeat galectins with two distinct CRDs joined by a linker peptide. The CRDs in the same molecule differ in their binding affinities (Gitt *et al.*, 1998; Cooper, 2002; Huflejt and Leffler, 2004). Galectin-8 has a high affinity for 3-O-sulfated or 3-O-sialylated glycoconjugates and a Lewis X-containing glycan and this affinity is largely due to the N-terminal CRD (Ideo *et al.*, 2003, 2011). The linker of gal-9 is of various sizes in humans. The long linker has 58 amino acids, the medium linker has 26 amino acids and the short linker is only 14 amino acids (Hirashima *et al.*, 2002). Alternative splicing creates at least 5 splice variants (Heusschen *et al.*, 2014). Gal-12 has an unusual C-terminal domain and many usually conserved residues are absent (Yang *et al.*, 2001*b*). Uniquely among galectins, gal-12 preferentially recognises 3-fucosylated structures (Maller *et al.*, 2020).

Structurally, the CRDs of galectins are arranged in a tightly folded conserved beta-sandwich structure formed by a six-stranded sheet (S1–S6) and a five stranded sheet (F1–F5). Figure 1 shows the structure of gal-1 (Kishor *et al.*, 2018), gal-3 (Saraboji *et al.*, 2012) and gal-4 (Rustiguel *et al.*, 2016). The carbohydrate-binding amino acids are in strands S4–S6 (Rini and Lobsanov, 1999; Loris, 2002) and they have been highlighted (Fig. 1).

Figure 2 shows the alignment of the prototypic human galectins gal-1, 2, −7, −10, −13 and −14. There is considerable diversity in the amino acid sequences. The amino acids implicated in binding to carbohydrate in galectin-1 (Kishor *et al.*, 2018) have been identified as His44, Asn46, Arg48, His52, Asn61, Trp68, Glu71 and Arg73. Of these eight sites, only Trp68 is conserved among all the prototypic galectins. Clearly, the amino acids involved in binding carbohydrate are not identical in each galectin and these differences presumably influence the specificity of carbohydrate-binding (Modenutti *et al.*, 2019).

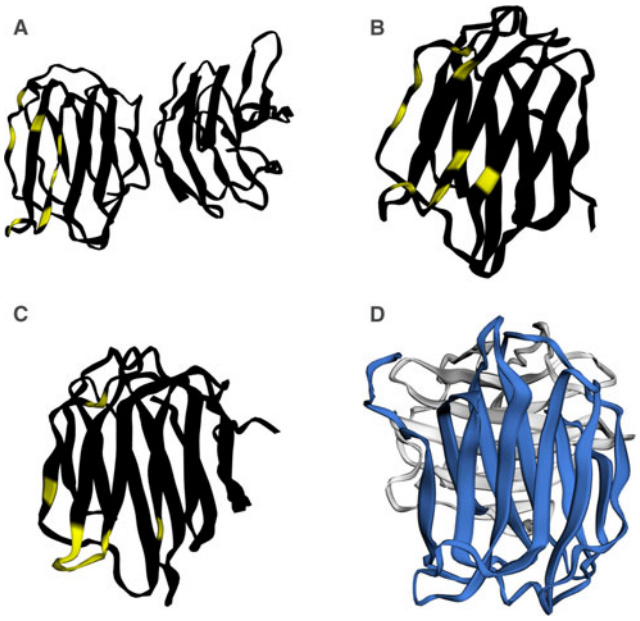

Fig. 1. Molecular structure of human and parasitic galectins showing the similarity in structure. (A) The prototypic human galectin 1(PDB 2 km^2) shown as a dimer. (B) The chimaeric human galectin 3 (PDB 3ZSL). (C) The tandem repeat human galectin 4. (D) The predicted structure of the tandem repeat galectin 1 from *T. circumcincta* (O01410) using Phyre2 (Kelley *et al.*, 2015). The human galectin structures were determined but the parasite galectin is a predicted structure. To emphasize this difference, the parasite galectin has been coloured differently, the N terminal CRD is shown in blue while the C terminal CRD is shown in grey. In all three figures of human galectin, the amino acids responsible for binding beta-galactosides have been highlighted but the amino acids responsible for binding glycans have not been determined for the parasite galectin. The structures were visualised in EzMol (Reynolds *et al.*, 2018).

Function of host galectins

Galectins bind glycans on glycoproteins and glycolipids and mediate a wide variety of cellular processes including cellular interactions, intracellular signalling and host defence against infection (Nielsen *et al.*, 2018). Of particular relevance to parasitologists, several galectins play key roles in immune regulation (Sato *et al.*, 2009). Gal-1 mediates T lymphocyte apoptosis (Perillo *et al.*, 1995) as does gal-2 (Sturm *et al.*, 2004). Gal-2 has also been shown to inhibit the development of the parasitic nematode *Ascaris suum* as well as the free-living nematode *Caenorhabditis elegans* by binding to the galactoseβ1-4fucose epitope (Takeuchi *et al.*, 2019).

Gal-3 binds IgE and promotes mast cell degranulation (Dumic *et al.*, 2006; Henderson and Sethi, 2009) while gal-9 has a high affinity for IgE, which is the most heavily glycosylated immunoglobulin.

Gal-11 has only been reported in three species of Bovidae (cattle, sheep and goats). It is an inducible galectin present in the cytoplasm and nucleus of epithelial cells of the ovine gastrointestinal tract during infection with *H. contortus*; uninfected animals lack gal-11 (Dunphy *et al.*, 2000). Protein production is restricted to the epithelial cells lining the gastrointestinal system, as no changes in gal-11 expression level are noticed after infection with the bovine lungworm *Dictyocaulus viviparus* (Hoorens *et al.*, 2011). Increased expression of gal-11 is observed 2–7 days after challenge infection with *H. contortus* (Robinson *et al.*, 2011). Increased levels of gal-11 were observed in the late phase of infection or after repeated infection of animals (da Souza *et al.*, 2015). Gal-11 binds to molecules from the L4 and adult stages of *H. contortus*, but not to the L3 stage and is associated with inhibited larval development of *H. contortus* (Preston *et al.*, 2015). Sheep possess two gal-11 variants (Sakthivel *et al.*, 2020). Only one variant is able to form polymers and only this variant reduced the development of L3–L4 (Sakthivel *et al.*, 2020). It also damaged L4 during *in vitro* exposure (Sakthivel *et al.*, 2020). The targets of gal-11 and ovine gal-14 have been investigated by affinity chromatography combined with mass spectrometry and over 30 molecules from adult *H. contortus* have been identified for each galectin (Sakthivel *et al.*, 2018).

Ovine galectin-14 is produced by eosinophils in ovine gastrointestinal mucus (Young *et al.*, 2009). It is homologous and functionally similar to human galectin-10 (Ackerman *et al.*, 2002). Ovine gal-14 levels are increased 7 days after *H. contortus* infection and are temporally associated with parasite expulsion (Robinson *et al.*, 2011). In addition, the level of abomasal ovine gal-14 is correlated with the parasitic burden in sheep infected with *H. contortus* (da Souza *et al.*, 2015); animals with low parasite burdens express lower levels of gal-14. Ligands for gal-11 and gal-14 have been identified in L4 and adult stage extracts (Sakthivel *et al.*, 2015). Increased levels of gal-15 are observed in the abomasal mucosa of the resistant Canarian Hair Breed compared to the susceptible Canarian Sheep breed (Guo *et al.*, 2016). Proteomic analysis of the gastric mucosal wash showed production of Gal-14 and Gal-15 in sheep challenged with *T. circumcinta* but not in uninfected animals (Athanasiadou and Huntley, 2008).

Mucus is a poorly understood aspect of host immunity. Nematodes encounter mucus very early in infection and lie within the superficial mucus during the parasitic phase. The properties of mucus are determined by the high molecular weight heavily

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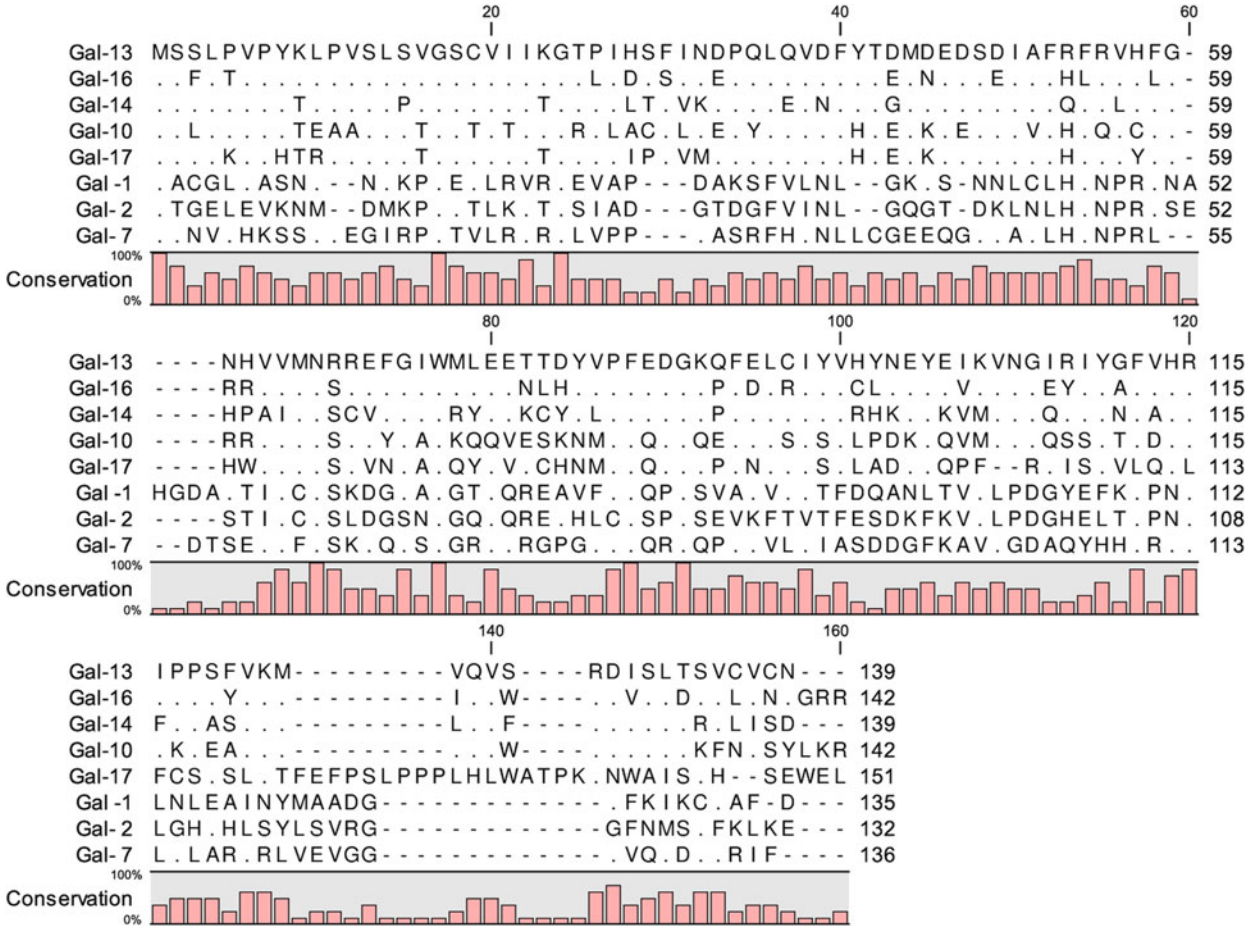

Fig. 2. Multiple sequence alignment of prototypic human galectins that show differences in the amino acid sequences among galectins. The accession numbers were gal-1 (P09382), gal-2 (P05182), gal-7 (P47929), gal-10 (Q05315), gal-13 (Q9UHV8), gal-14 (Q8TCE9), gal-16 (A8MUM7) and gal-17 (Q6ZW74).

glycosylated mucins which make up about 10% of the weight of the mucus (Miller, 1987). Other constituents include proteins, glycolipids and DNA (Miller, 1987). Interactions among large molecules and with water determine mucus viscosity. Mucus viscosity increases following nematode infection and this may also be influenced by the addition of immunoglobulins and other molecules (Miller, 1987) and of increased sulphation (Hasnain *et al.*, 2017). Disulphide bridges can affect the gel-like nature of mucus (Miller, 1987). Interestingly, mice that lack the mucin Muc5a (Hasnain *et al.*, 2011) or the sulphate transporter Sat1 (Hasnain *et al.*, 2017) are unable to expel *Trichuris muris*. The production of gal-11 corresponds with increased mucus stickiness (Robinson *et al.*, 2011); possibly gal-11 is cross-linking the glycans on mucins as shown for gal-1 (Wasano and Hirakawa, 1997) and this would impede nematode motility.

Nematode galectins

The model non-parasitic nematode *C. elegans* has 12 galectins encoded by genes *lec-1* to *lec-12* although other galectins may exist (Nemoto-Sasaki *et al.*, 2008). A genome search found 38 sequences with one or more galectin-like domains in *C. elegans* but only 13 sequences were found in the transcriptome (Bauters *et al.*, 2017). Fewer galectins have so far been identified among the parasitic nematodes. The nematode galectins are not orthologous to the mammalian galectins (Houzelstein *et al.*, 2004). However, they do appear similar in structure (Fig. 1).

Figure 3 shows a tree of the nematode galectins. The tree was created in Geneious Prime as a neighbour-joining tree from the protein sequences using Jukes-Cantor genetic distances. There are no close homologues to some of the *C. elegans* galectins such as lec-6, −9 and −11. Nomenclature is not comparable within the parasitic nematodes; e.g. gal-1 from *Teladorsagia circumcincta* (Greenhalgh *et al.*, 1999) is not orthologous to gal-1 from *Toxocara canis* (Fig. 3).

Interactions between host and nematode galectins

After exsheathing in the sheep rumen, incoming *H. contortus* larvae produce numerous galectin-like proteins (Hewitson *et al.*, 2009). Similarly, galectins and members of the venom allergen family are major components of excretory-secretory products of incoming *T. circumcincta* larvae in sheep (Craig *et al.*, 2006). Several mammalian galectins are also upregulated in parasitic infections: gal-1, gal-3, gal-9, gal-11, ovine gal-14 and gal-15. The simultaneous appearance of both host and parasite galectins following gastrointestinal nematode infection provides an opportunity for these molecules to interact. Nematode galectins may have evolved not only to regulate cellular processes but also to mimic host galectin (Tang *et al.*, 2014).

The nematode *Strongyloides ratti* has seven identified galectins named after the *C. elegans* galectins (Ditgen *et al.*, 2018). Sr gal-1 is predominantly expressed in free-living adult females while Sr gal-3 is predominantly expressed in parasitic females. Both galectins may bind to host mucosal cells and trigger the release of the Th2 cytokines thymic stromal lymphopoietin and IL-22 (Ditgen *et al.*, 2018). Galectin from *Trichinella spiralis* has also been implicated in the invasion of host mucosal cells (Xu *et al.*, 2018).

Vaccination with a combination of recombinant rHco-gal-m/f proteins (from male and female) can confer partial protection to

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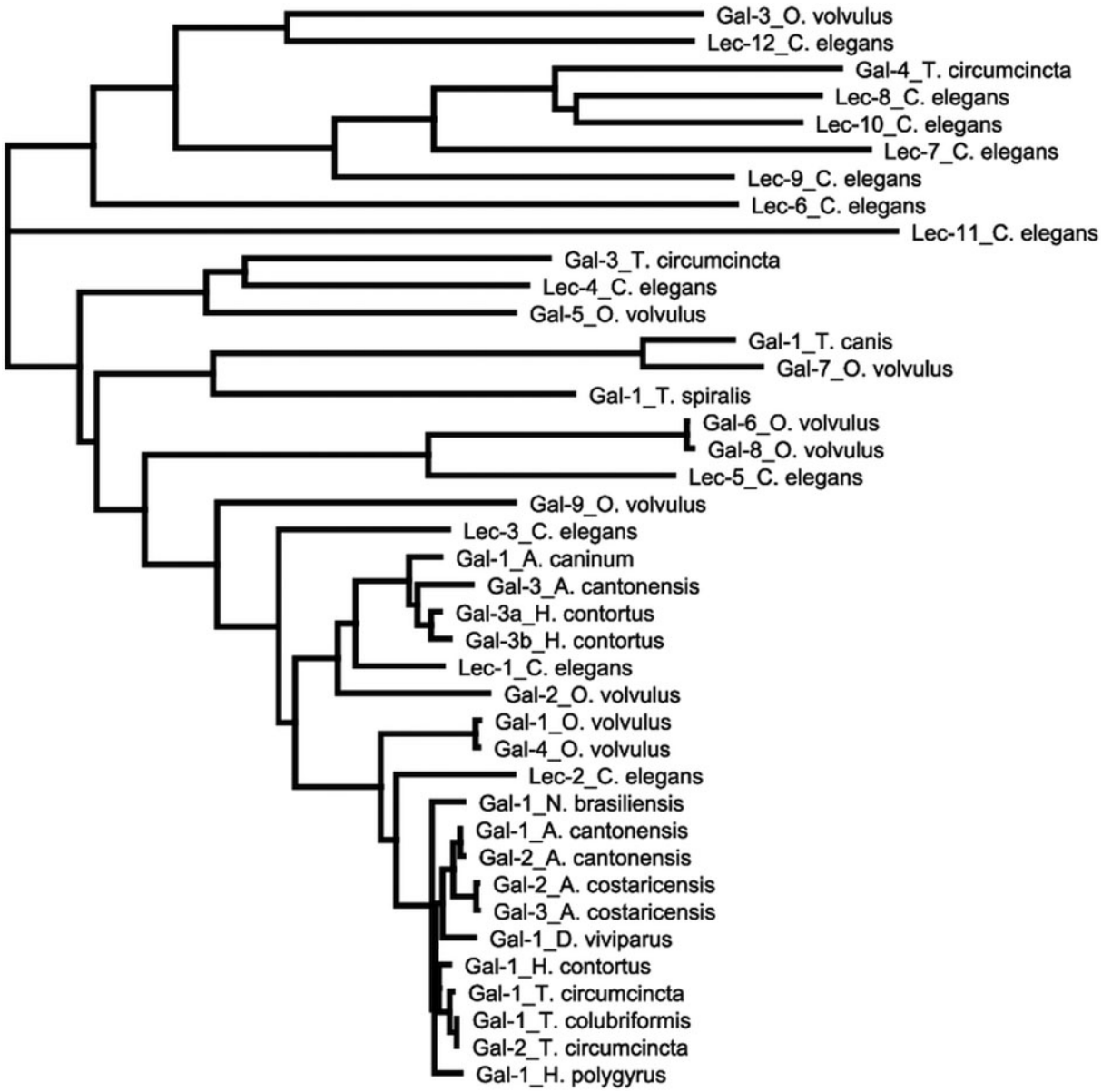

Fig. 3. Phylogenetic tree of nematode galectins created by using Jukes-Cantor distances and the neighbour-joining method. The tree demonstrates that the nematode galectins fall into multiple groups with varying degrees of similarity to the *C. elegans* galectins. The accession numbers were *A. caninum* gal-1 (AOA368GH13), *A. cantonensis* gal-1 (AOA158PB17) gal-2 (G1EUS2), gal-3 (G1EUS1), *A. costaricensis* gal-1 (A0A0R3PHH7), gal-2 (A0A3P7H3L8), *D. viviparus* gal-1 (AOAOD8Y2F3), *H. contortus* gal-1 (O76646), gal-3a (Q9NJV1), gal-3b (O44126), *H. polygyrus* gal-1 (AOA3P8DGW1), *N. brasiliensis* gal-1 (A0A0N4Y6Y9), *O. volvulus* gal-1 (Q25597), gal-2 (A0A044VHG4), gal-3 (A0A044UJD3), gal-4 (A0A044QNW3), gal-5 (A0A044STF4), gal-6 (A0A044VFH7), gal-7 (A0A044R2B2), gal-8 (O96928), gal-9 (A0A044VGC2) *T. canis* gal-1 (A0A0B2VDY3), *T. circumcincta* gal-1 (O01410), gal-2 (O01412), gal-3 (A0A2G9UTC0), gal-4 (A0A2G9V3L5), *T. colubriformis* gal-1 (Q7KPD1), *T. spiralis* (A0A0V1B757), *C. elegans* Lec-1 (P36573), Lec-2 (Q20684), Lec-3 (I2HAG1), Lec-4 (Q18625), Lec-5 (G5EFI4), Lec-6 (Q9N384), Lec-7 (Q09605), Lec-8 (Q09610), Lec-9 (G5EC10), Lec-10 (G5EBV4), Lec-11 (Q94215) Lec-12 (Q5ZR27).

homologous infection in goats (Yanming *et al*., 2007). However, vaccination with Hco-gal- 2 does not provide strong protection in sheep (Newlands *et al*., 1999). The eosinophil-specific chemokinetic activity of *H. contortus* infective L3 larvae is mediated by nematode galectins in a carbohydrate-dependent manner and may mimic the activity of host galectin 9 (Vasta, 2009). In mice, vaccination against *Angiostrongylus cantonensis* galectin inhibits the immune response to subsequent infection with *A. cantonensis* (Yan *et al*., 2018). *Angiostrongylus cantonensis* gal-1 causes apoptosis of macrophages by binding to Annexin A2 and activating the apoptotic signalling pathway (Shi *et al*., 2020).

The changes in mucus following infection are at least partly host-mediated and widely assumed to benefit the host (Knight *et al*., 2011; Hasnain *et al*., 2017). However, *H. contortus* produces a mucin-like molecule and vaccination with parasite-derived extracts containing this molecule significantly enhances protection (Piedrafita *et al*., 2012). This mucin exists on the external surface of *H. contortus* L3 and interacts with host mucus. This molecule is hypothesized to increase the viscosity of mucus and vaccination against this molecule is expected to reduce mucus viscosity.

Teladorsagia circumcincta galectins strongly bind to or are bound by sheep antibody (McCririe *et al*., 1997). Similarly, galectin from *Onchocerca volvulus* has been shown to bind IgE (Klion and Donelson, 1994). The binding of heavily glycosylated immunoglobulin IgE by both host and parasite galectin will influence mast cell degranulation. Mast cell degranulation is mediated by cross-linking IgE bound to the high-affinity IgE receptor-FcεRI present on the mast cell surface (Turner and Kinet, 1999). Upon degranulation, mast cells release vasoactive mediators and constrict blood vessels, contract gut smooth muscle, increase mucus production and upregulate proinflammatory

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factors such as histamine, tryptase, chymase, prostaglandins and leukotrienes, matrix metalloproteinase 9, platelet-activating factor and cytokines mainly IL-4, IL-10 and TNF-a, that expel the adult nematodes (Pennock and Grencis, 2006). Resistant sheep with higher concentrations of degranulated mast cells (globule leucocytes) had lower numbers of *T. circumcincta* following reinfection (Stear *et al.*, 1995). Mast cells from Gal-3 deficient mice release less Interleukin 4 and less histamine during mast cell degranulation (Chen *et al.*, 2006).

Most research has assumed that modulating the induction of immune responses is the key mechanism. However, in many nematode infections, hosts are continually exposed to reinfection. Consequently, most nematodes enter hosts that have already mounted an anti-nematode immune response. Here there is little value in trying to prevent an immune response being mounted but the enormous value in modulating the effector mechanisms such as IgE-mediated mast cell degranulation.

Parasite galectin might also influence the improvement in autoimmune disorders observed during nematode infections. The number of mast cells increases in intestinal tissues and mast cells are important sources of inflammation in Inflammatory Bowel Disease (Hamilton *et al.*, 2011; Stasikowska-Kanicka *et al.*, 2012). In addition, mast cells regulate the permeability of the intestinal epithelium due to their release of granule proteases (Scudamore *et al.*, 1995; McDermott *et al.*, 2003). Nematodes may suppress IgE mediated mast cell responses (Pritchard, 1993) and protect the host against atopic and autoimmune disorders. *In vivo* studies are needed to help us better understand the complex interplay between host galectins (especially gal-3 and gal-9), parasite galectins and mast cells.

In conclusion, both host and parasite galectins are present in the gastrointestinal tract following infection and they both influence protective immune responses against nematodes. Their interaction plays an important role in modulating the host immune response, influencing the outcome of gastrointestinal nematode infection. Galectins from some nematodes have been shown to bind IgE. The control of IgE activity and mast cell degranulation is of considerable value to many parasites so it seems plausible that nematode galectin acts as a molecular sponge that soaks up immunoglobulin IgE and inhibits mast cell degranulation. In addition, nematode galectin may interfere with the binding of host galectin to glycans on gastrointestinal mucins and affect mucus viscosity which will influence nematode survival. Parasite galectin might be also immunomodulate autoimmune disorders.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003118202100007X.

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Conflicts of interest. The authors declare there are no conflicts of interest.

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