REVIEW

Endogenous galectins and the control of the host inflammatory response

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Abstract

A new era of research is being devoted to deciphering endogenous mediators and mechanisms that are in place to resolve the inflammatory response. Accruing evidence indicates that galectins fall into this category of immunoregulatory mediators signifying their use as prospective novel anti-inflammatory agents. The focus of this review is to depict the immunoregulatory bioactivities of three members of the galectin superfamily, Galectin (Gal)-1, Gal-3 and Gal-9. Emphasis is given to the studies investigating the properties of these endogenous lectins. Gal-1, Gal-3 and Gal-9 are emerging as pertinent players in the modulation of acute and chronic inflammatory diseases, autoimmunity and cancer, and thus being increasingly recognised as molecular targets for innovative drug discovery.

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Galectins – generalities; biochemistry; cell sources

Members of the galectin family of proteins are classified by their ability to bind β-galactosides and by a conserved sequence of approximately 130 amino acids within their carbohydrate recognition domains (CRDs) (Barondes *et al.* 1994). To date, 15 members have been identified, which based on their structure and number of CRDs, are subdivided into one of three groups (Fig. 1). The prototype galectins contain a single CRD and can form homodimers, whereas the tandem-repeat galectins consist of two non-identical CRDs joined by a short peptide, and the unique chimera-type galectin-3 contains a single CRD with an extended N-terminus (Barondes *et al.* 1994).

In contrast to the selectins, galectin binding to carbohydrates is calcium independent (Hughes 2001). Galectins bind to *N*-acetyllactosamine (Galβ1, 3GlcNAc or Galβ1, 4GlcNAc), a common disaccharide found on many *N*- or *O*-linked glycans (Elola *et al.* 2005). The mere presence of galactose residues in glycoconjugates is not sufficient to promote high-affinity binding, and a fine specificity in binding is evident by the limited set of glycoconjugates to which they bind. In addition, many galectins can bind to carbohydrates in a bivalent or multivalent style, allowing cross-linking and redistribution of cell surface glycoproteins (Yang *et al.* 2008). Galectins can also bind their ligands in a carbohydrate-independent manner. This is often the case intracellularly, where ligand binding occurs predominantly through protein–protein interactions (see Liu *et al.* 2002 for review). An intriguing aspect of galectin biology is that, although these proteins lack a signal peptide and therefore do not exit the cell via the classical secretory pathway, they are known to be actively secreted from cells (Cho & Cummings 1995). Various models for exportation of the different members of the galectin family have been proposed (reviewed by Elola *et al.* 2007).

Galectins have been isolated from a number of species ranging from vertebrates to sponges, suggesting that they perform essential roles in basic cellular function (Cooper & Barondes 1999, Houzelstein *et al.* 2004). Nuclear localisation of Gal-1 and Gal-3 is possibly connected with a role in the regulation of pre-mRNA splicing (Vyakarnam *et al.* 1997, Wang *et al.* 2004), while extracellular location indicates functions in cell–cell and cell–matrix interactions (Hughes 2001, reviewed by Elola *et al.* (2007)). A diverse range of biological functions involved in immune and inflammatory responses and tumour development have been reported for galectins over the last decade including roles in cellular adhesion, migration and survival (see Elola *et al.* 2007, Yang *et al.* 2008 for recent reviews).

Within the immune system, Gal-1 is specifically localised in lymphoid organs (Baum *et al.* 1995*b*), T cells (Blaser *et al.* 1998, Fuertes *et al.* 2004), activated macrophages (Rabinovich *et al.* 1998) and endothelial cells (Lotan *et al.*

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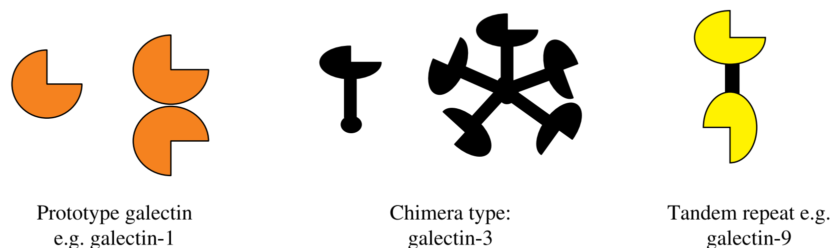

Figure 1 Subtypes of the galectin family. Galectins can be divided into three subtypes based on their structure. Schematic examples of prototype galectins: Gal-1 has one carbohydrate recognition domain and can function as a monomer or homodimer; Gal-3 is the only chimeric galectin with a carbohydrate recognition domain and an extended N-terminus through which it can form pentamers; tandem-repeat galectins (e.g. Gal-9) consist of two non-identical CRDs joined by a short peptide.

1994, Baum *et al.* 1995*b*). Furthermore, expression of Gal-1 in endothelial cells can be modulated by several inflammatory agents, supporting its role in inflammatory incidences (Baum *et al.* 1995*b*). Gal-3 is expressed by virtually all immune cell types, including endothelial cells, lymphocytes (Lotan *et al.* 1994, Baum *et al.* 1995*b*), neutrophils (Truong *et al.* 1993), monocytes and macrophages (Liu *et al.* 1995), mast cells (Craig *et al.* 1995) and dendritic cells (Flotte *et al.* 1983). Gal-3 expression has been found to be increased in neutrophils upon adhesion to the endothelium (Gil *et al.* 2006*b*), which also coincided with a relocalisation of Gal-3 to the plasma membrane in endothelial cells (Gil *et al.* 2006*b*). Such relocalisation to the membrane of endothelial cells has also been observed upon adhesion of tumour cells (Glinsky *et al.* 2001). Gal-9 is also distributed in certain cells fundamental to the inflammatory response: endothelial cells (Imaizumi *et al.* 2002), T cells (Matsumoto *et al.* 1998) and fibroblasts (Asakura *et al.* 2002). Knockout mice have been generated for these three galectins and have provided a tool for researchers to investigate their roles under inflammatory conditions. Table 1 outlines the phenotypes of these mice.

Finally, the keen reader could refer to recent excellent reviews on galectins' biology (Elola *et al.* 2007, Yang *et al.* 2008). Here, we will dwell on recent understanding of the impact of galectins, mainly Gal-1, Gal-3 and Gal-9, on the immune response.

Inflammation and anti-inflammation: a balancing act!

During an inflammatory response, individuals experience the cardinal signs of inflammation; pain, fever, redness and swelling and in chronic conditions, this can ultimately lead to loss of function. These symptoms are a result of a complex set of microscopic events that take place both at the site of inflammation and systemically. Inflammatory reactions are generally protective and serve to maintain tissue homeostasis, although if uncontrolled they become deleterious to the host. In nearly all cases, the fundamental cause of tissue damage is leukocyte accumulation. Leukocyte recruitment in both homeostatic and inflammatory situations is a highly regulated process that requires specific and sequential molecular interactions between leukocytes and the vascular endothelium. Insights into the cellular and molecular processes involved in each step of the cascade have been provided by a range of experimental approaches performed both *in vitro* and *in vivo*. These include antibody inhibition studies, static adhesion assays, parallel-plate flow chamber models, as well as using intravital microscopy of small animals to visualise live interactions of leukocytes with the vessel wall. These studies have helped to elucidate that i) initial leukocyte–endothelial interactions (capture and rolling) are instigated primarily by a family of molecules called selectins along with their oligosaccharide ligands, and ii) firm adhesion and transmigration are mediated by leukocyte integrins interacting with the endothelial immunoglobulin superfamily of adhesion molecules.

The reparative and resolving phase of inflammation is not merely a passive process as once believed, but actively takes place. While an array of pro-inflammatory mediators exist to initiate inflammation, a repertoire of anti-inflammatory mediators and mechanisms operate in the host to promote and control the phase of resolution, by inhibiting leukocyte migration and promoting clearance of inflammatory cells (Gilroy *et al.* 2004, Serhan *et al.* 2007). Accumulating evidence indicates that galectins fall into this category of immunoregulatory mediators signifying their potential use as novel anti-inflammatory agents. Their actions on cells of the vascular system are outlined in Fig. 2. We will summarise now the current knowledge on the properties of endogenous Gal-1, Gal-3 and Gal-9 as evidenced from integrated system biology analyses.

Anti-inflammatory and pro-inflammatory galectins

Generally, Gal-1 is known to bestow a range of anti-inflammatory effects on various cells types, inhibiting cell trafficking, inducing apoptosis and modulating the release of

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Table 1 Phenotype of galectin null mice

	Disease model/inflammogen	Phenotype	References
Null mouse			
Galectin-1	Peritonitis	Increased neutrophil recruitment	www.functionalglycomics.org/
	IL1B-inflamed cremaster	Increased leukocyte adhesion and emigration	Cooper *et al.* (2008)
	Experimental allergic encephalomyelitis	Increased susceptibility, 'hyper' Th1 and Th17 responses	Toscano *et al.* (2007)
	Delayed-type hypersensitivity	Increased oedema and lymphocyte infiltration to the inflamed paw	Norling *et al.* (2008)
Galectin-3	Diabetes	Accelerated glomerulopathy in a model of streptozotocin-induced diabetes with pronounced increases in circulating and renal/glomerular AGE levels	Pugliese *et al.* (2001)
	Peritonitis	Reduced neutrophil recruitment	Colnot *et al.* (1998*a*,*b*)
	Endotoxic shock	Increased susceptibility, with increased production of pro-inflammatory cytokines and NO	Li *et al.* (2008)
	Bacterial infection	Reduced neutrophil recruitment to the lungs following *S. pneumoniae* infection	Nieminen *et al.* (2008)
	Parasite infection	Decreased inflammation following *T. Gondii* infection, higher Th1 response with increased levels of IFNG and IL12. Reduced granuloma formation following infection with Schistosomiasis	Bernardes *et al.* (2006) and Breuilh *et al.* (2007)
	Prion infection	Increased survival, following intracerebral and peripheral scrapie infection	Mok *et al.* (2007)
Galectin-9	Arthritis	Enhanced incidence, increased numbers of TIM-3^{+}CD4^{+}T cells	Seki *et al.* (2008)
	Endotoxic shock	Increased mortality	Tsuboi *et al.* (2007)

mediators. By contrast, Gal-3 is widely pro-inflammatory provoking leukocyte activation, whereas Gal-9 is most commonly known for its chemotactic activity towards eosinophils, and has more recently been revealed as a negative regulator of Th1 cells. Specific effects of Gal-1, -3 and -9 will be addressed in succession.

Actions of exogenous galectin-1

Application of exogenous Gal-1 (LGALS1) has shown immunosuppressive and anti-inflammatory efficacy in various experimental models of inflammation and autoimmunity, including colitis (Santucci *et al.* 2003), concanavalin A-induced hepatitis (Santucci *et al.* 2000), arthritis (Rabinovich *et al.* 1999*b*), diabetes (Perone *et al.* 2006), experimental autoimmune encephalomyelitis (EAE) (Offner *et al.* 1990), myasthenia gravis (Levi *et al.* 1983) and uveitis (Toscano *et al.* 2006).

Using gene or protein therapy strategies, Gal-1 has been shown to attenuate paw swelling, clinical score and histopathological symptoms of collagen-induced arthritis (Rabinovich *et al.* 1999*b*). Investigation into the molecular mechanisms involved in this process revealed that Gal-1 treatment increases T-cell susceptibility to activation-induced apoptosis and promotes a shift from a T-helper cell type 1 (Th1) to a Th2-polarised immune response, characterised by an increase in IL5 and a concomitant reduction in IL2 and IFNG levels (Rabinovich *et al.* 1999*b*). In a model of hepatitis, Gal-1 pre-treatment (40 μg; 30min) prevented liver injury and tissue infiltration of T cells. These effects were associated with apoptosis of activated T cells and inhibition of concanavalin A-induced TNF and IFNG secretion (Santucci *et al.* 2000). Indeed, several studies have implicated Gal-1 to modulate the T cell cytokine repertoire. Low concentrations of Gal-1 (10–100 nM) can inhibit IFNG and TNF production by IL2-activated T cells *in vitro* (Rabinovich *et al.* 1999*a*) and production of cytokines such as TNF, IL1B, IL12 and IFNG *in vivo* (Santucci *et al.* 2003). Additionally, treatment of T cells with Gal-1 is associated with increased mRNA and protein expression of IL10 (van der Leij *et al.* 2004), and an inhibition of IL2 secretion (van der Leij *et al.* 2007).

The anti-inflammatory and immunosuppressive effects of Gal-1 in models of T cell-driven pathologies are often deemed to be due to the pro-apoptotic nature of this lectin, and thus these studies are complemented by a much larger series of *in vitro* studies. Regulation of cell death by apoptosis is vital for normal cell turnover and maintenance of homeostasis. Apoptosis occurs during T cell maturation in the thymus to remove potentially autoaggressive cells, as failure to do so may lead to various autoimmune diseases if these cells escape to the periphery. In relation to this, early studies showed Gal-1 synthesis by thymic epithelial cells caused apoptosis of immature thymocytes (Baum *et al.* 1995*a*). Together, these results suggest a functional role of Gal-1 in the process of positive and/or negative selection in the thymus (Perillo *et al.* 1997). Indeed, current investigations have highlighted that Gal-1 can selectively promote negative selection and oppose positive selection by reducing and enhancing the TCR signalling threshold respectively (Liu *et al.* 2008). In addition, activated mature T cells undergo apoptosis to prevent an overactive immune response.

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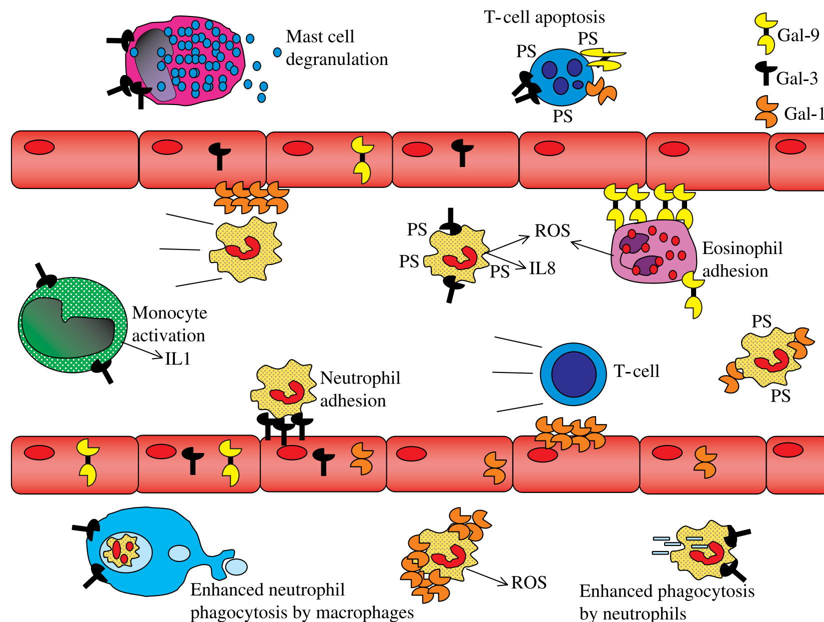

Figure 2 Events controlled by Gal-1, Gal-3 and Gal-9 on the blood and vascular cells during inflammation. Gal-1, Gal-3 and Gal-9 have been reported to have numerous effects on the cells of the vascular system. Gal-1 has been reported to inhibit PMN adhesion and T-cell adhesion and emigration, while Gal-3 promotes PMN adhesion both to endothelial cells and components of the extracellular matrix. Gal-9 has been found to promote eosinophil recruitment. All three galectins induce T-cell apoptosis, while Gal-1 and Gal-3 also induce phosphatidylserine exposure on PMN in the absence of apoptosis. The pro-inflammatory actions of Gal-3 are further substantiated by its ability to induce IL1 production by monocytes, mast cell degranulation and ROS and IL8 generation by PMN. Gal-9 has also been found to induce ROS generation by eosinophils. Gal-1 appears to have concentration-dependent effects; at high concentrations, Gal-1 induces ROS generation by exudated PMN. Gal-3 may also play a role in the resolution of inflammation by enhancing phagocytosis of apoptotic PMN by macrophages and phagocytosis of bacteria by PMN.

Various studies have indicated that exogenous Gal-1 induces apoptosis of mature and activated, but not resting, T cells (Blaser *et al.* 1998, Rabinovich *et al.* 1998), by specific recognition of the differentially glycosylated CD45RO isoform of PTPRC displayed by memory T cells (Perillo *et al.* 1995). These results may well explain the *in vivo* efficacy of Gal-1; however, it must be noted that relatively high concentrations (10 μM) are often used to attain these effects, and that the apoptotic effect is dependent on cross-linking specific cell surface glycoproteins (Symons *et al.* 2000). Whether Gal-1 levels could be so high *in vivo* is questionable and further studies are required to elucidate the mode of Gal-1 actions, although it could be postulated that these concentrations might be reached within specific intracellular and paracellular microenvironments.

Aside from its apoptotic role, it has been documented that Gal-1 also exerts its anti-inflammatory effects via apoptotic-independent mechanisms. At concentrations below its apoptotic threshold (10–100 nM), Gal-1 inhibits T-cell adhesion to extracellular matrix (ECM) glycoproteins (Rabinovich *et al.* 1999*a*) and TNF and IFNG secretion by activated T cells (Rabinovich *et al.* 1999*b*).

Interestingly, Gal-1 also plays a pivotal role in the innate immune response-promoting resolution of acute inflammation. First, experimental evidence was seen in a rat model of paw oedema induced by bee venom phospholipase A_2 (Rabinovich *et al.* 2000). Local administration of Gal-1 repressed the inflammatory response in a dose-dependent manner. This effect was not abrogated when Gal-1 was pre-incubated with 100 mM lactose, but could be reversed with Gal-1 anti-serum, showing a specific yet carbohydrate-independent effect. Immunohistochemical assessment of the inflamed paws showed a dramatic reduction in PMN infiltration, degranulated mast cells and overall tissue damage with a 30 min pre-treatment of Gal-1. To investigate the mechanism of its anti-inflammatory properties, tests were performed *in vitro* on LPS-stimulated macrophages, and showed that Gal-1 inhibits arachadonic acid and PGE_2 secretion, in a dose-dependent and carbohydrate-independent fashion (Rabinovich *et al.* 2000). Further investigations to elucidate the anti-inflammatory activities of Gal-1 on activated rat peritoneal macrophages showed that this protein inhibits inducible nitric oxide synthase expression and potentiates the

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arginase pathway of L-arginine metabolism, thus inducing an 'alternative activation' of these macrophages (Correa *et al*. 2003).

Unlike its effects on T cells, Gal-1 does not induce neutrophil apoptosis, although it does cause exposure of phosphatidylserine on the cell membrane, which significantly promotes phagocytosis of apoptotic neutrophils by mouse macrophages (Dias-Baruffi *et al*. 2003). This study highlights an important facet of Gal-1 in leukocyte turnover, hence possessing the ability to aid in the resolution of inflammation.

Not all of Gal-1s actions are anti-inflammatory, at high concentrations (≥40 uM range), it activates the NAD(P)H oxidase and subsequently superoxide generation in extravasated (but not peripheral) neutrophils (Almkvist *et al*. 2002), indicating that the activated leukocyte might expose Gal-1 receptors. Moreover, a recent paper has revealed a unique function of Gal-1 as a platelet activator (Pacienza *et al*. 2008).

Research from our laboratory has shown that Gal-1 inhibits the initial interactions of PMNs with endothelial cells of the post-capillary venule in an experimental model of inflammation (La *et al*. 2003). Mice treated with a low dose (0.3 µg corresponding to ∼21 pmol) of hr-Gal-1 showed a potent reduction in the effect of IL1B on cell flux, cell adhesion and emigration of PMNs. *In vitro* assays further confirmed that incubation of PMNs with hr-Gal-1 inhibited IL8-induced PMN chemotaxis and transendothelial migration (La *et al*. 2003). We have also demonstrated that incubation of PMNs with low concentrations of hr-Gal-1 (27–270 nM) results in a significant inhibition in their capture, rolling and adhesion on endothelial cells under conditions of shear stress (Cooper *et al*. 2008). Furthermore, the reverse effect is observed when Gal-1 protein levels are knocked down using small interference RNA (siRNA) in HUVEC, with a marked increase (∼90%) in cell recruitment.

Of great interest, these effects could be mimicked also when peripheral lymphocytes were flown over the endothelial monolayers, both in terms of pharmacological effect of added hr-Gal-1 and physiological properties revealed when endothelial Gal-1 levels were markedly reduced with siRNA (Norling *et al*. 2008).

Collectively, these results are strongly suggestive that endothelial Gal-1 is present on the membrane to mitigate an overzealous recruitment of lymphocytes (Norling *et al*. 2008). Incidentally, this inhibitory effect is also evident in static conditions, where decreased lymphocyte transmigration was observed when endothelial Gal-1 was overexpressed in response to prostate cancer cell-conditioned media (He & Baum 2006). This effect was not associated with cell death, and could be inhibited by antiserum to Gal-1. These findings illustrate a potent inhibitory action for exogenous Gal-1 on lymphocyte recruitment, an additional property for Gal-1 that may underscore its efficacy in models of immune-mediated inflammation. Studies describing the actions of administration of recombinant Gal-1 *in vivo* are outlined in Table 2.

Gal-1 and the in vivo *immune response*

The exact role of Gal-1 with regards to inflammatory and immune functions *in vivo* is currently unclear as targeted disruption of the Gal-1 gene in knockout mice produces animals that develop normally and are viable and fertile. The absence of major inflammatory phenotypic abnormalities under physiological conditions suggests that other proteins may potentially compensate for Gal-1 (Poirier & Robertson 1993). Utilisation of Gal-1 null mice has proved to be an important tool for assessing its function in immune responses, and has provided further evidence that this protein plays a key role in inflammation.

The functional relevance of endogenous Gal-1 was recently demonstrated in a model of peritonitis, whereby a 30 min pre-treatment of anti-Gal-1 serum prior to administration of carrageenin augmented the neutrophil influx into the peritoneum at 48 h (Gil *et al*. 2006*a*). It has also been documented on the Functional Glycomics Consortium that Gal-1 null mice display an increased neutrophil recruitment into the inflamed peritoneum 72 h post-injection with peptone (www.functionalglycomics.org/).

Using intravital microscopy of the mouse cremaster, an increase in leukocyte adhesion and emigration in Gal-1 null mice was observed following IL1B-induced inflammation (Cooper *et al*. 2008). Moreover, recent unpublished data from our laboratory visualising the PAF-inflamed cremaster demonstrated enhanced leukocyte emigration in Gal-1 null mice compared with controls during a 2 h time course, indicating that the heightened leukocyte infiltration in these null mice is not stimulus or tissue site specific. Short-term homing assays further implicate Gal-1 as a negative regulator of leukocyte recruitment during homeostatic and inflammatory conditions. Gal-1 null mice displayed a significant increase in the proportion of labelled splenocytes within the mesenteric lymph nodes under naive conditions. Under inflammatory conditions, increased numbers of methylated BSA-sensitised lymphocytes were recruited to the inflamed paw in Gal-1null mice compared with their WT counterparts, as assessed 5 h post-challenge (Norling *et al*. 2008). Altogether, we believe that endogenous Gal-1 acts as a break signal in counteracting the extent of leukocyte trafficking in the early stages of inflammation; its source and localisation is the endothelium, but it is yet unclear whether these effects result from a direct inhibition on the leukocyte or are indirectly determined by a non-genomic alteration of the phenotype of the endothelial cell.

Aside from its role in leukocyte recruitment, Gal-1 has recently been demonstrated as an important factor for angiogenesis. Current research has shown that knockdown of endothelial Gal-1, using specific antisense oligonucleotides, inhibits endothelial proliferation and migration (Thijssen *et al*. 2006). Additionally, Gal-1 null mice display impaired tumour progression due to decreased neovascularisation (Thijssen *et al*. 2006). In this respect, endothelial Gal-1 may be a novel way of targeting various cancers for

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Table 2 *In vivo* actions of exogenous galectins

Disease model/species	Inflammogen	Treatment	Effect	References
Paw oedema rat	Phospholipase A_2	Pre-treatment of hr-Gal-1 (30 min; 5–160 ng) or co-injection (40–80 ng) with PLA_2	Inhibited oedema. Reduced infiltration of PMN and mast cell degranulation.	Rabinovich *et al.* (2000)
Peritonitis mouse	IL1B	Co-injection of hr-Gal-1 (0.3–3μg) with IL1B	Reduced PMN migration into the peritoneum	La *et al.* (2003)
Colitis mouse	Trinitrobenzene sulphonic acid	Prophylactic: hr-Gal-1 (0.04–4 mg/kg) daily i.v. for 7 days. Therapeutic: hr-Gal-1 i.v. daily for 7 days, 2 weeks after colitis induction	Prevented/reverted wasting syndrome. Inhibition of pro-inflammatory cytokine production (TNF, IL1B, IL12, IFNG).	Santucci *et al.* (2003)
Hepatitis mouse	Concavalin A	Pre-treatment of hr-Gal-1 (30 min; 5–40 μg)	Prevents liver injury. Inhibits T-cell infiltration. Inhibition of TNF and IFNG production	Santucci *et al.* (2000)
Nephritis rat	Rabbit anti-glomerular BM serum	Gal-1 (1 mg/kg) i.p. on alternate days for 2 weeks	Reduced crescent formation, proliferation of glomerular cells and macrophage infiltration	Tsuchiyama *et al.* (2000)
Autoimmune uveitis mouse	Interphotoreceptor retinoid-binding protein	50 μg Gal-1 i.p. during afferent (days 2,4,6) or efferent (days 14,16,18) phases	Prevents ocular pathology. Decreases leukocyte infiltration	Toscano *et al.* (2006)
Pneumonia mouse	*S. Pneumoniae*	5 μg of Gal-3 intratracheally at time of infection	Decreased lung injury and bacteraemia. Reduced levels of IL6 and TNF in BAL fluid.	Farnworth *et al.* (2008)
Arthritis mouse	Collagen type II	Gal-9 (10 μg) i.v. daily from second immunisation at day 21	Increased number of apoptotic cells in joint. Reduced clinical score and cellular infiltrate	Seki *et al.* (2007)
Experimental allergic encephalomyelitis mouse	Myelin oligodendrocyte glycoprotein	Stable Gal-9 (100 μg) i.p. daily from day 3 to 7	Decreased antigen-specific IFNG producing Th1 cells. Reduced mortality and disease severity	Zhu *et al.* (2005)
Asthma mouse	*Dermatophagoides farinae* allergen	Stable Gal-9 (10–100 μg) i.v. 24 h or 1 h before and 8 h after intranasal antigen challenge	Inhibited Th2 cell infiltration into the lung. Reduced airway hyperresponsiveness	Katoh *et al.* (2007)
Nephritis rat	Rabbit anti-glomerular BM serum	Gal-9 (1 mg/kg) i.p. on alternate days for 2 weeks	Induced apoptosis of $CD8A^+$T cells. Inhibited macrophage infiltration and crescent formation	Tsuchiyama *et al.* (2000)
Skin transplant mouse	Allogeneic skin grafts	Gal-9 (100 μg) daily post-transplant	Induced apoptosis of $CD8A^+$T cells. Prolonged skin graft survival	Wang *et al.* (2007)

therapeutic applications (Thijssen *et al.* 2007). This report supports the notion that endothelial Gal-1 should be suppressed in tumours, whereas hr-Gal-1 or Gal-1 mimetics should be targeted to the endothelium during chronic inflammation to prevent over-reactive immune responses. Recent studies have shown that Gal-1 null mice display an attenuated response in a model of chronic hypoxia-induced pulmonary hypertension, highlighting a likely role for Gal-1 in vascular remodelling (Case *et al.* 2007).

An intriguing critical role for Gal-1 has recently been demonstrated in fetomaternal tolerance, with Gal-1 null mice displaying increased foetal loss. Gal-1 was shown to induce the development of tolerogenic dendritic cells early on in successful pregnancies therefore promoting expansion of IL10-producing regulatory T cells (Blois *et al.* 2007). Relevantly, elevated placental Gal-1 levels have been demonstrated in patients with severe pre-eclampsia, implicating a role for Gal-1 in fetomaternal tolerance in humans (Than *et al.* 2008).

Following antigen-induced activation of murine T cells, Gal-1 synthesis is upregulated and consequently inhibits antigen-induced proliferation of naive and memory

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CD8A^{+}T cells, thus acting as an autocrine negative feedback loop on T-cell reactivity (Blaser *et al*. 1998). Further analysis clarified that Gal-1 arrests cell cycle progression between the S and G2/M phases, thereby switching off T-cell effector functions (Allione *et al*. 1998). This mechanism may be in place to ensure that the immune response mounted declines appropriately after antigen is cleared. It is therefore likely that the adaptive immune response would be overactive in Gal-1 null mice. Supporting this concept, a recent study indicates that Gal-1 modulates the Th1 and Th17 but not the Th2 life span, thus Gal-1 null mice exhibit 'hyper' Th1 and Th17 responses thereby making them more susceptible to an experimental model of multiple sclerosis (Toscano *et al*. 2007). Additionally, emerging data have illuminated an important suppressive function of Gal-1 in regulatory T cells, thus acting as a negative regulator of the adaptive immune response (Garin *et al*. 2007). The phenotype of the Gal-1 null mice is outlined in Table 1.

Actions of exogenous galectin-3

Gal-3 (LGALS3 antigen, IGE-binding protein, carbohydrate binding protein-35, epsilon BP, HL-29, RL-29) was first identified as an antigen expressed on the surface of murine thioglycollate-elicited macrophages (Ho & Springer 1982). In contrast to Gal-1, Gal-3 has been largely purported to have a pro-inflammatory role with its increased expression in a host of inflammatory/immune disorders underscoring its potential roles in inflammation.

Increased levels of Gal-3 have been detected in: bronchoalveolar lavage (BAL) fluid in OVA-challenged mice with macrophages being major cell type containing Gal-3 (Zuberi *et al*. 2004), in prion-infected brain tissue (Mok *et al*. 2007), in thymus following *Trypanosoma cruzi* infection (Silva-Monteiro *et al*. 2007) as well as synovial tissue from RA patients (Ohshima *et al*. 2003). Levels as high as 50 μg/ml have been detected in the BAL fluid of mice after infection with *Streptococcus pneumoniae* (Farnworth *et al*. 2008). In addition, Gal-3 expression has been linked to increased malignancy in a number of tumours (Inohara *et al*. 2008, Saussez *et al*. 2008) and may be of use as a marker for determining stages of certain tumours (Balasubramanian *et al*. 2008, Matsuda *et al*. 2008). The role of Gal-3 in innate immunity is supported by studies showing that neutrophil and macrophage recruitment is attenuated in *in vivo* models of peritonitis conducted in mice nullified for this lectin (Colnot *et al*. 1998*b*, Hsu *et al*. 2000). This is further corroborated by *in vitro* studies in which recombinant Gal-3 promotes neutrophil–endothelial interactions (Sato *et al*. 2002).

During inflammation Gal-3 is released into the extracellular space where it may activate inflammatory cells or contribute to their retention by increasing cellular interactions with extracellular matrix glycoproteins. In line with a pro-inflammatory role, exogenous Gal-3 has been demonstrated to activate numerous cell types involved in the inflammatory/immune response; namely, inducing mast cell degranulation (Frigeri *et al*. 1993, Suzuki *et al*. 2008), IL1 and superoxide production in monocytes (Jeng *et al*. 1994, Liu *et al*. 1995) and superoxide and IL8 generation and L-selectin shedding in neutrophils (Yamaoka *et al*. 1995, Nieminen *et al*. 2005, Farnworth *et al*. 2008). Indication for a positive loop at the level of the neutrophil has emerged: Gal-3 increases cellular expression of CEACAM1 and CEACAM8, which then act as receptors for transducing Gal-3-mediated activation of NAD(P)H oxidase activity (Feuk-Lagerstedt *et al*. 1999, Fernandez *et al*. 2005). Interestingly, primed neutrophils are then capable of deactivating Gal-3 by causing its cleavage mainly *via* the serine protease elastase (Nieminen *et al*. 2005).

As well as promoting cellular activation, exogenous Gal-3 also promotes cellular adhesion. Administration of hr-Gal-3 promotes adhesion of neutrophils to laminin in a carbohydrate-dependent, calcium-independent manner, while in the presence of divalent cations Gal-3 activates neutrophils increasing their adhesion to other ligands such as fibronectin (Kuwabara & Liu 1996). Gal-3 also promotes neutrophil adhesion to endothelial cells *in vitro* and may play an important role in beta-2 integrin-independent neutrophil extravasation *in vivo* (Sato *et al*. 2002). These results, along with a decreased cellular infiltrate observed in numerous *in vivo* models of inflammation performed in Gal-3 null mice, provide evidence for a role for this galectin in mediating leukocyte recruitment during an inflammatory response (Colnot *et al*. 1998*a*,*b*, Bernardes *et al*. 2006, Nieminen *et al*. 2008)

With regards to apoptosis, Gal-3 appears to function differently in relation to its localisation, i.e. whether it is inside or outside the cell. Intracellular Gal-3 would inhibit apoptosis, which may then lead to persistence of blood-borne cells at the sites of inflammation. Inhibition of apoptosis is thought to be due to Gal-3 localising to the mitochondria, preventing cytochrome c release (Moon *et al*. 2001, Yu *et al*. 2002). This protective effect of intracellular Gal-3 appears to function in numerous cell types and in response to a wide range of apoptosis-inducing agents. Overexpression of Gal-3 in human leukaemic T cells conferred resistance to apoptosis induced by anti-FASN antibody and staurosporine (Yang *et al*. 1996), while overexpression in breast carcinoma cells increased resistance against cisplatin and free radical-induced apoptosis (Akahani *et al*. 1997, Moon *et al*. 2001). Accordingly, cells that lack intracellular Gal-3 are more susceptible to apoptosis as shown by increased apoptosis of peritoneal macrophages from Gal-3 null mice and increased UVB-induced apoptosis of Gal-3 null keratinocytes (Saegusa *et al*. 2008), an effect thought to be consequent to suppression of *Erk* phosphorylation and enhancement of *Akt* activation.

Like Gal-1, exogenous Gal-3 induces phosphatidylserine exposure and apoptosis of T cells (Fukumori *et al*. 2003, Stillman *et al*. 2006, Stowell *et al*. 2008). Gal-1 and Gal-3 both bind numerous receptors on T cells with some overlap, although while CD7 has been linked to Gal-1-induced apoptosis (Pace *et al*. 2000), Gal-3 does not bind this receptor. It does however, interact with ITGB1, SPN, PTPRC and TFRC, all of which have been linked – in various ways – to

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apoptosis. Stillman *et al.* (2006) found, however, that ITGB1 and SPN were not required for Gal-3-induced apoptosis, while cells lacking PTPRC did not respond to Gal-3 application with apoptosis. TFRC also appears to play a role with clustering of this receptor observed in all apoptotic cells. Following infection with *T. cruzi* recombinant, Gal-3 induced increased levels of death in cortical immature thymocytes while thymuses from Gal-3 null mice did not show cortical depletion after parasite infection *in vivo* (Silva-Monteiro *et al.* 2007). Treatment with hr-Gal-3 ($\geq$100 nM for 18–44 h) can induce apoptosis of mast cells in a carbohydrate, RAGE and caspase-3 dependent manner (Suzuki *et al.* 2008). The effect of Gal-3 on neutrophil apoptosis is not fully defined with one report showing that hr-Gal-3 enhances the apoptotic rate of this cell type (Fernandez *et al.* 2005), and more recent studies have reported that Gal-3, similar to Gal-1, induces phosphatidylserine exposure on neutrophils without inducing apoptosis (Stowell *et al.* 2008): in fact Farnworth *et al.* (2008) found that Gal-3 could delay neutrophil apoptosis. It is plausible that these differences may be due to the concentration and treatment duration of Gal-3 used in these assays, with low concentrations for a short pre-incubation period (0.4 μg/ml, 15 min) enhancing apoptosis (Fernandez *et al.* 2005) and higher concentrations for more prolonged incubation periods (30 μg/ml, 18 h) delaying it (Farnworth *et al.* 2008).

In line with its effects on cellular activation and adhesion, Gal-3 also promotes chemotaxis of monocytes *in vivo* and macrophages *in vitro* (1 μM) (Sano *et al.* 2000) as well as eosinophils in OVA-induced asthma (Zuberi *et al.* 2004). One facet of inflammation where Gal-3 appears to have beneficial effects is phagocytosis. Phagocytosis is necessary to clear pathogens, foreign bodies and cellular debris, thus allowing inflammation to resolve. Gal-3 has been found to play a critical role in macrophage phagocytosis with Gal-3 null macrophages demonstrating decreased phagocytosis of IgG-opsonised erythrocytes and thymocytes *in vitro*; moreover, Gal-3 null mice display reduced phagocytosis of red blood cells by kupffer cells in a model of haemolytic anaemia (Sano *et al.* 2003). Treatment with hr-Gal-3 increases phagocytosis of apoptotic neutrophils by monocyte-derived macrophages (Karlsson *et al.* 2008), and this is in agreement with the fact that Gal-3 null macrophages demonstrate reduced phagocytosis of apoptotic neutrophils (Farnworth *et al.* 2008). As well as increasing macrophage phagocytosis, Gal-3 also enhances the phagocytic capabilities of neutrophils, a fact that may in part account for the protective role of Gal-3 in infections such as *S. pneumoniae* (Farnworth *et al.* 2008).

The anti-inflammatory nature of Gal-1 is thought to be due, at least in part, to its ability to skew the Th1/Th2 balance in favour of a Th2-type response. By contrast, Gal-3 suppresses type-2-mediated inflammation by inhibiting IL5 production by eosinophils and antigen-specific T-cell lines, suggesting a potential role in allergic inflammation (Cortegano *et al.* 1998). In line with this, gene therapy experiments have shown that treatment of asthmatic rats with a plasmid encoding Gal-3 improves the eosinophil count in these animals and normalises airway hyper-responsiveness to methacholine (Lopez *et al.* 2006). However, a previous study in Gal-3 null mice suggested a pro-inflammatory role for Gal-3 with increased levels of IFNG and decreased levels of IL4 in OVA-challenged mice, which is indicative of a higher Th1 response; these mice also had lower eosinophilic infiltration and airway hyper-responsiveness (Zuberi *et al.* 2004). The differences between these two studies may result from differing effects of the endogenous protein when compared with Gal-3 overexpression or compensatory mechanisms in the Gal-3 null mice by other members of the galectin family. Gal-3 has also been shown to modulate T-cell behaviour; inhibition of Gal-3 using antisense technology blocks proliferation of TCR-stimulated T cells (Joo *et al.* 2001). The strongest evidence has arisen from mice deficient in mannosyl (alpha-1,6-)-glycoprotein beta-1,6-*N*-acetyl-glucosaminyltransferase (*Mgat5*); these mice show increased TCR activation, susceptibility to autoimmune disease and an enhanced Th1 response, all attributable to inefficient formation of multivalent lattices of Gal-3 and *N*-glycans in the TCR complex (Demetriou *et al.* 2001).

One area of Gal-3 biology that could potentially be exploited positively is during the resolution of inflammation. Alternative activation of macrophages drives resolution and occurs when macrophages are stimulated with the Th2 cytokines IL4 or IL13. Such activation has been implicated in a number of pathologies including host response to parasitic infections, asthma, wound repair and fibrosis in granulomatous disease. Gal-3 appears to be required for alternative activation of macrophages as siRNA depletion of Gal-3 blocks IL4-mediated alternative activation as measured by arginase activation and alternative marker expression; classical activation induced by IFNG/LPS was not affected (MacKinnon *et al.* 2008). Furthermore, alternative activation with IL4 and IL13 stimulates Gal-3 expression and release while classical activation with IFNG/LPS inhibits Gal-3 expression. Studies describing the actions of administration of recombinant Gal-3 *in vivo* are outlined in Table 2.

Galectin-3 and the in vivo *immune response*

Gal-3 null mice, like their Gal-1 null counterparts, develop normally and are viable and fertile (Colnot *et al.* 1998*a*), indeed Gal-1/Gal-3 double knockouts are also viable (Colnot *et al.* 1998*a*). It has recently been found, however, that Gal-3 null mice spontaneously develop pathological changes in the liver at 6 months of age typical of non-alcoholic fatty liver disease. These changes may be due to the function of Gal-3 as a receptor for advanced glycation end products (AGEs), with levels of AGE and the AGE receptor RAGE increased in Gal-3 null mice (Nomoto *et al.* 2006). Numerous studies have, however, now been carried out using these mice and comparisons between wild-type and Gal-3 null mice have supported the concept that this lectin plays a predominantly pro-inflammatory role *in vivo*.

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Several studies have shown that Gal-3 null mice exhibit a reduced inflammatory response compared with wild-type mice, thus emphasising the pro-inflammatory nature of this protein. Lower numbers of neutrophils are recruited to the peritoneum following injection of thioglycollate (Colnot *et al*. 1998*b*) and to the lungs following *S. pneumoniae* infection (Nieminen *et al*. 2008). Neutrophil recruitment in *S. pneumoniae* is independent of β2 integrin whereas the β2 integrin-dependent recruitment in *Escherichia Coli* infection was not affected by the lack of Gal-3 (Nieminen *et al*. 2008). Increased survival of Gal-3 null mice has been observed following intracerebral and peripheral scrapie infection (Mok *et al*. 2007), while Gal-3 null mice have reduced granuloma formation following infection with Schistosomiasis (Breuilh *et al*. 2007), a disease normally characterised by a Th2-driven response. Gal-3 null mice, however, mounted a biased Th1 response as demonstrated by increased IFNG and IgG2b levels. Gal-3 appears to alter strength of immune response triggered by DCs. Mature DCs from null mice induced increased proliferation as well as enhanced production of IFNG and *Il4* by T cells. Infection of Gal-3 null mice with another parasite, *T. Gondii* again resulted in decreased inflammation and a higher Th1 response evident by increased levels of IFNG and IL12 (Bernardes *et al*. 2006). Decreased survival of null mice when the parasite was given an i.p. injection was associated with a deficient influx of PMN and macrophages into the peritoneal cavity.

Although Gal-3 appears to play a deleterious role in a host of inflammatory and immune conditions, the opposite appears to be the case in conditions such as diabetes where advanced glycation end products (AGEs) play a role in disease pathogenesis (Pugliese *et al*. 2001, Iacobini *et al*.2004). AGEs are formed as a result of hyperglycaemia and are known to be pathogenic mediators of most complications that result from diabetes (Peppa *et al*. 2003). Gal-3 has been identified as an AGE receptor (AGE-R3) (Vlassara *et al*. 1995) that binds AGEs with high affinity leading to their internalisation and degradation. Thus, the Gal-3–AGE R pathway is believed to act as a protective mechanism toward AGE-induced injury (Pugliese *et al*. 2001). In support of this, Gal-3 null mice develop accelerated glomerulopathy in a model of streptozotocin-induced diabetes with pronounced increases in circulating and renal/glomerular AGE levels (Pugliese *et al*. 2001). Gal-3 may also influence expression of other AGE-binding proteins, this being supported by the observation that non-diabetic Gal-3 null mice have reduced renal/glomerular levels of AGE-R1 (DDOST) and MSR1 (implicated in AGE removal) and increased AGE-R2 (PRKCSH) and RAGE (AGER) (mediate cell activation). As a result, the cell's ability to remove AGEs may be compromised. Not all of the effects of Gal-3 in diabetes occur as a result of its function as an AGER. Canning *et al*. (2007) showed significantly less diabetes-mediated inner blood-retinal barrier dysfunction in Gal-3 null mice than wild-type counterparts at 2 weeks, a time point at which AGE levels are comparable with non-diabetic controls, it was therefore suggested that Gal-3 may alter vascular cell function independently of AGE binding due to its numerous pro-inflammatory actions. Suppression of angiogenesis during diabetes is a recognised phenomenon. Retinal ischaemia and neovascularisation were studied in a murine model of oxygen-induced proliferative retinopathy in wild-type and Gal-3 null mice after perfusion of preformed AGEs. Ablation of Gal-3 abolished the AGE-mediated increase in ischaemia and restored the neovascular response to that seen in controls (Stitt *et al*. 2005). Independent of AGE binding, Gal-3 has been shown to increase angiogenesis (Nangia-Makker *et al*. 2000); therefore, it may only be anti-angiogenic in a diabetic environment.

In contrast to its pro-inflammatory nature, Gal-3 null mice are more susceptible to endotoxic shock than wild-type mice with increased production of pro-inflammatory cytokines and NO (Li *et al*. 2008). Gal-3 has been found to bind LPS of numerous bacteria including *Klebsiella pneumoniae*, *Salmonella typhimurium* and *E. Coli* (Mey *et al*. 1996). These interactions are thought to occur via both the CRD and N-terminal domain of Gal-3. Gal-3 null macrophages had elevated LPS-induced signalling and cytokine generation compared with wild-type cells that was inhibited by recombinant Gal-3, while blocking Gal-3 with a neutralising Ab in wild-type cells increased their production of cytokines in response to LPS. By contrast, Gal-3 was found to favour salmonella survival (Li *et al*. 2008). Gal-3 null mice developed an increased Th1 response in response to salmonella infection, which might have contributed to its reduced replication in the Gal-3 null mice. The phenotype of the Gal-3 knockout mice is outlined in Table 1.

As well as binding LPS, Gal-3 also interacts with *Candida albicans* through β-1,2 mannosides. Gal-3 was found to localise at the level of phagocytic cups formed around yeasts and at the periphery of ingested yeasts (Jouault *et al*. 2006). The data suggest that macrophages differentially sense *C. albicans* and *S. cerevisiae* through a mechanism involving TLR2 and Gal-3, which were shown to be associated in differentiated macrophages following incubation with *C. albicans*.

Actions of exogenous galectin-9

Gal-9 (LGALS9) was originally identified as a potent eosinophil chemoattractant produced and released by antigen-stimulated T cells (Matsumoto *et al*. 1998). Furthermore, this lectin was also shown to act directly on eosinophils, inducing aggregation, superoxide production and prolonging their survival (Matsumoto *et al*. 2002). Yet, the role of Gal-9 in allergic inflammation has yet to be fully characterised. There are some discrepancies regarding the relationship between Gal-9 and asthma, since this galectin seemed not to be involved in the pathology of airway hypersensitivity using a guinea pig model (Yamamoto *et al*. 2007), while, in murine, mite allergen-induced asthma Gal-9 was shown to reduce airway hyper-responsiveness and lung inflammation, an effect thought to be therapeutically linked to its ability to modulate T-cell infiltration into the airway (Katoh *et al*. 2007).

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Gal-9 has been shown to be a potent activator of dendritic cell maturation and hence an initiator of the adaptive immune response. Comparative to LPS, Gal-9 caused an upregulation of maturation markers and co-stimulatory molecules on DC, and induced IL12 secretion in a dose-dependent manner, eliciting the secretion of Th1 cytokines by allogeneic $CD4^{+}$T cells (Dai *et al*. 2005). Similarly to Gal-1, Gal-9 is also known to induce apoptosis of thymocytes (Wada *et al*. 1997) and peripheral T cells (Kashio *et al*. 2003), implicating a role in both T-cell maturation and in the modulation of T-cell-mediated immune reactions. However, these two galectins require different ligands and utilise distinct intracellular cell death pathways to induce apoptosis, due to their distinct structural features (Bi *et al*. 2008).

Gal-9-mediated apoptosis has been demonstrated in a nephrotoxic serum nephritis animal model (Tsuchiyama *et al*. 2000) and a model of diabetic nephropathy (Baba *et al*. 2005), producing efficacious outcomes in both models. Daily administration of Gal-9 improved survival of allogeneic skin grafts in mice, which was proposed to be due to apoptosis of host cytotoxic $CD8A^{+}$T cells (Wang *et al*. 2007). A beneficial effect of Gal-9-induced apoptosis was also demonstrated in a model of collagen-induced arthritis, suppressing pannus formation, bone erosion and inflammatory infiltrate (Seki *et al*. 2007). Indeed, apoptotic cells were identified within RA synovial tissue implanted into SCID mice following Gal-9 treatment. Gal-9 was shown to preferentially induce apoptosis of fibroblast-like synoviocytes isolated from RA compared with OA patients, indicating a potential mechanism for the suppression of RA (Seki *et al*. 2007).

A novel binding partner for Gal-9 was identified in 2005 by Zhu and colleagues; T-cell immunoglobulin and mucin-domain-containing protein-3 (HAVCR2; previously known as TIM3), shown to be expressed on terminally differentiated Th1 cells (Zhu *et al*. 2005). In a HAVCR2-dependent manner, Gal-9 triggered calcium entry, aggregation and apoptosis of Th1 cells. Pathological relevance was demonstrated with exogenous administration of Gal-9, which decreased disease severity and mortality in an experimental allergic encephalitis model (Zhu *et al*. 2005). Importantly, Gal-9 is involved in a negative feedback loop, whereby IFNG that is known to induce Gal-9 (Asakura *et al*. 2002, Imaizumi *et al*. 2002) consequently suppresses Th1 cells, thus preventing prolonged inflammation and allowing efficient resolution. An exciting recent report has revealed that Gal-9 specifically induces the formation of regulatory T cells, while simultaneously repressing the generation of pro-inflammatory Th17 cells in a model of collagen-induced arthritis (Seki *et al*. 2008). Treatment with Gal-9 significantly decreased the formation of pro-inflammatory IL17, IL12 and IFNG within the joint, and lowered the percentage of peripheral blood $CD4^{+}$ $HAVCR2^{+}$T cells. Studies describing the actions of administration of recombinant Gal-9 *in vivo* are outlined in Table 2.

Galectin-9 and the in vivo *immune response*

Only a limited number of studies have been performed utilising the Gal-9 null mouse to date. Strikingly, Gal-9-deficient mice have an enhanced incidence of developing collagen-induced arthritis; most notably, this phenotype is evident in a C57BL/6J strain, which is normally resistant to CIA. These null mice have increased numbers of $HAVCR2^{+}CD4^{+}$T cells compared with wild-type mice, substantiating the role of Gal-9 in the modulation of *Havcr2*-positive cells *in vivo* (Seki *et al*. 2008). Functionality of Gal-9 during infection has recently been highlighted in LPS-induced inflammation. Mice treated with Gal-9 had greatly improved survival rates, whereas Gal-9 null mice were prone to increased mortality and died within 72 h of LPS induction. The proposed target cell for Gal-9-mediated suppression in this model is PMN, because neutropenic mice were no longer protected from the beneficial effects of Gal-9 (Tsuboi *et al*. 2007). The phenotype of the Gal-9 null mice is outlined in Table 1.

Targets (receptors) for Gal-1, Gal-3 and Gal-9

The extracellular matrix (ECM) consists of numerous components including collagen, glycosaminoglycans, laminin, fibronectin and many other glycoproteins. Its classical function is to provide structural support for tissues, but it is also shown to play a more active role in regulating the behaviour of cells that contact it (Streuli 1999). Two main ways in which this is achieved is by direct cell–ECM interactions and by its association with growth factors (Taipale & Keski-Oja 1997). Several ECM components have been identified as ligands for Gal-1 and Gal-3, including laminin and fibronectin (Zhou & Cummings 1993, Ozeki *et al*. 1995, Kuwabara & Liu 1996). Association of Gal-1 with ECM proteins causes a direct reduction in leukocyte adhesion, as well as inhibiting T-cell migration through the ECM (He & Baum 2006), whereas Gal-3 localisation with ECM enhances leukocyte adhesion. Gal-9 has also been shown to reduce tumour cell adhesion by preventing binding to ligands on the endothelium and ECM (Nobumoto *et al*. 2008).

Other acceptors/ligands for galectins include membrane proteins such as integrins, lysosome-associated membrane proteins (LAMPs) and even certain gangliosides. The Gal-1 ligand ganglioside GM1 has recently been identified as important for endocytosis of Gal-1 in Jurkat cells, a process mediated by clathrin and lipid raft-dependent mechanisms, although the reason for internalisation remains to be established (Fajka-Boja *et al*. 2008). Gal-1 binds to a number of leukocyte cell surface molecules including CD4, CD7, SPN and PTPRC (Perillo *et al*. 1995, Hernandez & Baum 2002, Stillman *et al*. 2006). However, the precise carbohydrate structures on these macromolecules, which are recognised by galectins, are not well defined. Studies utilising biotinylated

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galectins have illustrated binding partners for Gal-1 and Gal-3 on the lymphocyte. Pre-incubation of lymphocytes with Gal-3 results in a partial displacement in binding of Gal-1 (Stillman *et al.* 2006), suggesting some binding sites are Gal-1 specific, or alternatively that Gal-1 has a higher affinity for these sites than Gal-3. Indeed, it is well known that the galectins display differing oligosaccharide-binding specificity due to the subtle differences in their CRDs (Hirabayashi *et al.* 2002).

The study of He & Baum (2006) illustrated that Gal-1 clusters SPN on the T-cell surface, which is thought to retard SPN redistribution to the trailing edge during transmigration and hence inhibit this process. During lymphocyte–endothelial interactions, lymphocytes become polarised due to chemokine activation and form a cellular projection at the rear referred to as a uropod where certain proteins are relocated including SPN, CD44 and PTPRC (del Pozo *et al.* 1997). It is therefore intriguing to hypothesise that due to Gal-1 preventing the relocalisation of these bulky glycoproteins to the uropod, this could result in steric hindrance to molecules such as LFA1 from interacting with endothelial adhesion molecules (Manjunath *et al.* 1995).

It is also possible, however, that Gal-1 could signal through one/or both of these receptors to decrease lymphocyte recruitment. Of particular interest, a parallel was found between upregulated PTPRC expression on lymphocytes that have rolled over the activated endothelium and the degree of binding of biotGal-1, suggesting that the two events are closely interlinked (our unpublished observations). This raises important questions about the downstream signalling events transmitted by Gal-1 binding. PTPRC is the prototype tyrosine phosphatase expressed on T cells, and regulates the activity of $p56^{Lck}$ (LCK) kinase by dephosphorylating the negative regulatory tyrosine residue (Y505). PTPRC activity itself can be regulated through autoinhibition by dimerisation (Mustelin *et al.* 2005). Reports indicating that binding of Gal-1 to PTPRC causes clustering and a decrease in its intrinsic protein tyrosine phosphatase (PTP) activity (Walzel *et al.* 1999, Fouillit *et al.* 2000, Amano *et al.* 2003) suggest that Gal-1 would function to decrease LCK activity. Indeed, pre-treatment of lymphocytes with an LCK kinase inhibitor mimicked the effects of exogenous hr-Gal-1 in inhibiting lymphocyte recruitment within the flow chamber. Combining the two treatments of hr-Gal-1 and the LCK kinase inhibitor were not additive, suggesting sharing of the same or similar pathway(s). These inhibitor studies suggest that Gal-1 acts on PTPRC to possibly cause inhibition of the Src kinase $p56^{Lck}$, thereby bringing about its inhibitory effect on lymphocyte adhesion. It is also possible to hypothesise that Gal-1 would bind to another receptor, and then activate a signalling cascade that would impact on PTPRC activity. Notably, the Src kinase $p56^{Lck}$ is a key determinant for a high-affinity state of VLA4 on circulating lymphocytes, allowing rapid response to chemokines on the endothelium (Feigelson *et al.* 2001).

An opposing mechanism has been proposed for Gal-3, which is thought to cause redistribution of the large MUC1 antigen on cancer cells, thus allowing topological accessibility of ligands on these cancer cells to interact with endothelial counter receptors, enhancing adhesion (Yu *et al.* 2007). Further evidence for this mechanism was demonstrated by pre-treatment of HUVEC with anti-E-selectin or anti-CD44H antibodies, which caused a reduction in Gal-3-mediated cell adhesion (Yu *et al.* 2007).

Of interest, studies by Katoh and colleagues have demonstrated efficacy of Gal-9 in a model of mite allergen-induced asthma, an effect therapeutically linked to its ability to modulate CD44-mediated functions. Gal-9, but not other members of the galectin family, has been shown to directly inhibit CD44 from binding to its ligand hyaluronan, in a carbohydrate-dependent fashion (Katoh *et al.* 2007). CD44 is normally expressed in an inactive form on naive lymphocytes, which lacks ligand-binding activity, and can be converted to an active form upon lymphocyte activation (English *et al.* 1998). This receptor on activated lymphocytes mediates rolling on hyaluronate, and functions as an additional mechanism to the canonical selectin-mediated rolling during inflammation. (DeGrendele *et al.*1996, 1997).

Galectin research to date has largely focused on the role of these proteins in animal models of disease such as collagen-induced arthritis, EAE, diabetes and infection by various parasitic organisms. To date, clinical data are restricted to the expression of these proteins in human tissue biopsy samples with increased expression of Gal-3 and Gal-9 detected in synovium taken from rheumatoid arthritis patients compared with the less inflamed osteoarthritic synovium (Ohshima *et al.* 2003, Seki *et al.* 2007), while Gal-1 expression is down-regulated in synovium from patients with juvenile idiopathic arthritis (Harjacek *et al.* 2001). Expressions of Gal-1 and Gal-9 have also been observed in numerous tumours and may be linked to malignancy. Due to the immune modulatory properties of Gal-1, Gal-3 and Gal-9, it is likely that all three have some role in malignancy (for an extensive review on galectins in tumour progression, see Liu & Rabinovich 2005).

From patho-physiology to pharmacology, opportunities for new anti-inflammatory therapeutics

As outlined in this review, overwhelming experimental evidence demonstrates that galectins play key roles in immune, infectious and inflammatory reactions, by providing stimulatory or inhibitory signals. The temporal and spatial expression of Gal-1, -3 and -9 during inflammatory episodes is likely to be in place to co-ordinate and finely regulate the host response. The individual galectins discussed here have distinct biological actions due to their unique structural features, and hence their binding preferences for different ligands. The same galectin may also display differing effects depending on cellular compartmentalisation, concentration in the local milieu and differentiation status of the target cell.

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Indeed, the regulated expression of glycosyltransferases leads to creation (or masking) of different galectins ligands during differentiation and activation of T cells, accordingly Gal-1, -3 and -9 act on different subsets demonstrating lineage-specific recognition and bioactions. It is plausible that cell-to-cell crosstalk might lead to the identification of a *galectin network* in inflammation such that, for instance, Gal-9 expression might be under the control of other members of the galectin family. In any case, deciphering how specific galectins exert their biological effects should provide insights into how they can be exploited for therapeutic interventions, and potentially have major clinical implications for the treatment of immune and inflammatory conditions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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