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. 1995b), T cells (Blaser et al. 1998, Fuertes et al. 2004), activated macrophages (Rabinovich et al. 1998) and endothelial cells (Lotan et al. 2002–0795/09/0201–169 © 2009 Society for Endocrinology Printed in Great Britain

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1994, Baum et al. 1995b). Furthermore, expression of Gal-1 in endothelial cells can be modulated by several inflammatory agents, supporting its role in inflammatory incidences (Baum et al. 1995b). Gal-3 is expressed by virtually all immune cell types, including endothelial cells, lymphocytes (Lotan et al. 1994, Baum et al. 1995b), 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. 2006b), which also coincided with a relocalisation of Gal-3 to the plasma membrane in endothelial cells (Gil et al. 2006b). 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 superfamilly 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...
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. 1999b), 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. 1999b). 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. 1999b). 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. 1999a) 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. 1995a). 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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**Table 1 Phenotype of galectin null mice**

<table>
<thead>
<tr>
<th>Null mouse</th>
<th>Disease model/inflammogen</th>
<th>Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin-1</td>
<td>Peritonitis</td>
<td>Increased neutrophil recruitment</td>
<td><a href="http://www.functionalglycomics.org/Cooper">www.functionalglycomics.org/Cooper</a> et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>IL1B-inflamed cremaster</td>
<td>Increased leukocyte adhesion and emigration</td>
<td>Toscano et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Experimental allergic encephalomyelitis</td>
<td>Increased susceptibility, ‘hyper’ Th1 and Th17 responses</td>
<td>Norling et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Delayed-type hypersensitivity</td>
<td>Increased oedema and lymphocyte infiltration to the inflamed paw</td>
<td>Pugliese et al. (2001)</td>
</tr>
<tr>
<td>Galectin-3</td>
<td>Diabetes</td>
<td>Accelerated glomerulopathy in a model of streptozotocin-induced diabetes with pronounced increases in circulating and renal/glomerular AGE levels</td>
<td>Colnot et al. (1998a,b)</td>
</tr>
<tr>
<td></td>
<td>Peritonitis</td>
<td>Increased susceptibility, with increased production of pro-inflammatory cytokines and NO</td>
<td>Li et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Endotoxic shock</td>
<td>Reduced neutrophil recruitment to the lungs following <em>S. pneumoniae</em> infection</td>
<td>Nieminen et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Bacterial infection</td>
<td>Decreased inflammation following <em>T. Gondii</em> infection, higher Th1 response with increased levels of IFNG and IL12. Reduced granuloma formation following infection with Schistosomiasis</td>
<td>Bernardes et al. (2006) and Breuilh et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Parasite infection</td>
<td>Increased survival, following intracerebral and peripheral scrapie infection</td>
<td>Mok et al. (2007)</td>
</tr>
<tr>
<td>Galectin-9</td>
<td>Arthritis</td>
<td>Enhanced incidence, increased numbers of TIM-3+CD4+ T cells</td>
<td>Seki et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Endotoxic shock</td>
<td>Increased mortality</td>
<td>Tsuboi et al. (2007)</td>
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</tbody>
</table>
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. 1999a) and TNF and IFNG secretion by activated T cells (Rabinovich et al. 1999b).

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 A2 (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 arachidonic acid and PGE2 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
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-1’s 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 IL1β 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. 2006a). 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 IL1β-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-1 null mice compared with their WT counterparts, as assessed 5 h post-challenge (Norling et al. 2008).

Additionally, 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
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 T cells. 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).

### Table 2 In vivo actions of exogenous galectins

<table>
<thead>
<tr>
<th>Disease model/species</th>
<th>Inflammonogen</th>
<th>Treatment</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paw oedema rat</td>
<td>Phospholipase A2</td>
<td>Pre-treatment of hr-Gal-1 (30 min; 5–160 ng) or co-injection (40–80 ng) with PLA2</td>
<td>Inhibited oedema. Reduced infiltration of PMN and mast cell degranulation.</td>
<td>Rabinovich et al. (2000)</td>
</tr>
<tr>
<td>Peritonitis mouse</td>
<td>IL1B</td>
<td>Co-injection of hr-Gal-1 (0.3–3 μg) with IL1B</td>
<td>Reduced PMN migration into the peritoneum</td>
<td>La et al. (2003)</td>
</tr>
<tr>
<td>Colitis mouse</td>
<td>Trinitrobenzene sulphonic acid</td>
<td>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</td>
<td>Prevented/reverted wasting syndrome. Inhibition of pro-inflammatory cytokine production (TNF, IL1B, IL12, IFNG).</td>
<td>Santucci et al. (2003)</td>
</tr>
<tr>
<td>Hepatitis mouse</td>
<td>Concavalin A</td>
<td>Pre-treatment of hr-Gal-1 (30 min; 5–40 μg)</td>
<td>Prevents liver injury. Inhibits T-cell infiltration. Inhibition of TNF and IFNG production</td>
<td>Santucci et al. (2000)</td>
</tr>
<tr>
<td>Nephritis rat</td>
<td>Rabbit anti-glomerular BM serum</td>
<td>Gal-1 (1 mg/kg) i.p. on alternate days for 2 weeks</td>
<td>Reduced crescent formation, proliferation of glomerular cells and macrophage infiltration</td>
<td>Tsujiyama et al. (2000)</td>
</tr>
<tr>
<td>Autoimmune uveitis mouse</td>
<td>Interphotoreceptor retinoid-binding protein</td>
<td>50 μg Gal-1 i.p. during afferent (days 2,4,6) or efferent (days 14,16,18) phases</td>
<td>Prevents ocular pathology. Decreases leukocyte infiltration</td>
<td>Toscano et al. (2006)</td>
</tr>
<tr>
<td>Pneumonia mouse</td>
<td>S. Pneumoniae</td>
<td>5 μg of Gal-3 intratracheally at time of infection</td>
<td>Decreased lung injury and bacteraemia. Reduced levels of IL6 and TNF in BAL fluid.</td>
<td>Farnworth et al. (2008)</td>
</tr>
<tr>
<td>Arthritis mouse</td>
<td>Collagen type II</td>
<td>Gal-9 (10 μg) i.v. daily from second immunisation at day 21</td>
<td>Increased number of apoptotic cells in joint. Reduced clinical score and cellular infiltrate</td>
<td>Seki et al. (2007)</td>
</tr>
<tr>
<td>Experimental allergic encephalomyelitis mouse</td>
<td>Myelin oligodendrocyte glycoprotein</td>
<td>Stable Gal-9 (100 μg) i.p. daily from day 3 to 7</td>
<td>Decreased antigen-specific IFNG producing Th1 cells. Reduced mortality and disease severity</td>
<td>Zhu et al. (2005)</td>
</tr>
<tr>
<td>Asthma mouse</td>
<td>Dermatophagoides farinae allergen</td>
<td>Stable Gal-9 (10–100 μg) i.v. 24 h or 1 h before and 8 h after intranasal antigen challenge</td>
<td>Inhibited Th2 cell infiltration into the lung. Reduced airway hyperresponsiveness</td>
<td>Katoh et al. (2007)</td>
</tr>
<tr>
<td>Nephritis rat</td>
<td>Rabbit anti-glomerular BM serum</td>
<td>Gal-9 (1 mg/kg) i.p. on alternate days for 2 weeks</td>
<td>Induced apoptosis of CD8A+T cells. Inhibited macrophage infiltration and crescent formation</td>
<td>Tsujiyama et al. (2000)</td>
</tr>
<tr>
<td>Skin transplant mouse</td>
<td>Allogeneic skin grafts</td>
<td>Gal-9 (100 μg) daily post-transplant</td>
<td>Induced apoptosis of CD8A+T cells. Prolonged skin graft survival</td>
<td>Wang et al. (2007)</td>
</tr>
</tbody>
</table>
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. 1998b, 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 (Suzuki et al. 2008), IL1 and superoxide production in monocytes (Jeng et al. 1994, Liu et al. 1995) and superoxide and IL8 generation and 1-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. 1998a,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 phosphatidylycerine 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...
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 thymocytes 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 (≥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 (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 vivo; 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). 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 latices 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. 1998a), indeed Gal-1/Gal-3 double knockouts are also viable (Colnot et al. 1998a). 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.
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. 1998b) 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 (Breuhl 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).
Galectin-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 (Tsukiyama 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.

**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 p56Lck (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 p56Lck, 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 p56Lck 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 downregulated 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.
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 bioactivities. 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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