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An Emerging Role for Galectins in Tuning the Immune Response: Lessons from Experimental Models of Inflammatory Disease, Autoimmunity and Cancer

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Abstract

Inflammation is a critical process for eliminating pathogens, but can lead to serious deleterious effects if left unchecked. Identifying the endogenous factors that control immune tolerance and inflammation is a key goal in the field of immunology. Galectins, a family of endogenous lectins with affinity for β-galactoside-containing oligosaccharides, are expressed by several cells of the immune system and tissue-resident stromal cells. According to their architecture, this family of glycan-binding proteins is classified in those containing one-carbohydrate-recognition domain (CRD) (proto-type), those containing two-CRD joined by a linker non-lectin domain (tandem-repeat) and those that have one-CRD attached to an N-terminal peptide (chimera-type). Accumulating evidence indicates that galectins play critical regulatory roles in immune cell response and homeostasis. In this review, we summarize recent developments in our understanding of the galectins' roles within different immune cell compartments, and in the broader context of the inflammatory microenvironments. In particular we illustrate the immunoregulatory role of three representative members of each galectin subfamily: galectin-1, -3 and -9. This body of knowledge, documenting the coming of age of galectins as potential immunosuppressive agents or targets for anti-inflammatory drugs, represents a sound basis to further explore their potential as novel therapies for autoimmune diseases, chronic inflammation and cancer.

Introduction

The immune system has a remarkable set of mechanisms that work in concert to maintain self-tolerance and homeostasis. Several mechanisms act together to ensure self-tolerance, including activation-induced cell death, anergy, expansion of regulatory T cells and cytokine deviation. Lack of activation-induced cell death, an improper balance of T helper (Th)-1, Th2 and Th-17-derived cytokines, or the absence of regulatory T cells can result in the loss of immune cell tolerance, the breakdown of immune homeostasis and the subsequent appearance of exacerbated inflammatory conditions and autoimmune diseases.

Regulated glycosylation control critical immune process, including T-cell activation, homing and survival, by creating or masking ligands for endogenous lectins [1]. Galectins, a family of evolutionarily conserved glycan-binding proteins has recently attracted the attention of immunologists as novel regulators of the inflammatory response [2–7]. As the identification of discoidin-1 in the cellular slime mold *Dictyostelium discoideum* and electrolectin in the electric organ tissue of the electric eel early during 1979s [2, 4], the family of β-galactoside-binding lectins or galectins have received increasing attention. However, it was only in the late 1990s that a growing body of experimental evidence emerged, illuminating a novel role for galectins in the regulation of physiological or pathological processes, particularly in the control of immune cell homeostasis and inflammation [2, 7].

Galectins are defined by a conserved carbohydrate-recognition domain (CRD) with affinity for β-galactosides [2–4]. To date, 15 mammalian galectins have been identified, which can be subdivided into three groups: the single-CRD proto-type galectins (including galectins-1, -2, -5-, -7, -10, -13, -14 and -15); the tandem-repeat galectins are those that have two-CRD joined by a linker

peptide of variable length (galectins-4, -6, -8-, -9 and -12), and the unique 'chimera-type' galectin-3, which contains a single CRD fused to unusual tandem repeats of short amino acid stretches [2, 4]. Of these 15 members it should be highlighted that the inclusion of Gal-11, which was first characterized as a lens-specific protein called GRIFIN (galectin-related interfibre protein) remains controversial. In fact, Gal-11 lacks two of the seven key amino acid residues conserved in most galectin CRD and does not display β-galactoside-binding activity [4].

Many galectins are either bi- or multivalent with regards to their carbohydrate-binding activities – some one-CRD (e.g. galectin-1) galectins exist as dimers; two-CRD galectins have two carbohydrate-binding sites in tandem, and galectin-3 forms oligomers when it binds to multivalent carbohydrates [5]. Cross-linkage of cell-surface receptors by galectins can trigger transmembrane signalling events through which diverse processes, such as proliferation, cytokine secretion and differentiation are modulated [6]. Remarkably, the responsiveness of cells to individual members of the galectin family can fluctuate depending on the repertoire of potentially glycosylated molecules expressed on the cell surface and the activities of specific glycosyltransferases that are responsible for generating galectin ligands. These variables can dramatically change according to the differentiation and activation state of the cells [7].

Although galectins do not contain signal peptides to direct them through the classical endoplasmic reticulum–Golgi apparatus secretory system, they can be secreted by non-classical secretory pathways [4]. Once outside the cell, galectins bind to, and cross-link multiple glycoconjugates found on the cell surface or in the extracellular matrix (ECM) [4]. Although most mammalian galectins bind preferentially to glycoconjugates containing the ubiquitous disaccharide N-acetyllactosamine [Galβ1-3GlcNAc or Galβ1-4GlcNAc], binding to individual lactosamine units is of relatively low affinity (Kd$\sim$1 mM), and arrangement of lactosamine disaccharides in repeating chains (polylactosamine) increases binding avidity. Moreover, detailed structural analysis of the CRD suggests subtle differences in carbohydrate-binding specificities of individual members of this family [5]. Whether differences in saccharide specificity might be responsible for distinct biological effects in response to individual galectin binding, still remains to be established.

Some galectins are distributed in a wide variety of tissues, where as others have a more restricted localization [7]. Within the immune system, galectins are found in activated macrophages, activated B cells, dendritic cells and activated T cells [7–10]. Moreover, recent studies using gene expression arrays have indicated elevated expression of galectins in $CD4^+$ $CD25^+$ regulatory T cells [11–14]. The expression of galectins is regulated during the activation and differentiation of immune cells and may be significantly altered under several pathological conditions [15]. In addition, as will be discussed later expression of galectins can be modulated by several cytokines, chemokines, hormones and differentiating agents [3, 6]. Although, several regulatory elements have been identified in promoter regions of galectin genes, the molecular mechanisms involved in the regulated expression of most galectins are not well understood.

Accumulating evidence has shown that galectins play a role in the initiation and resolution phases of inflammatory responses by promoting anti- or pro-inflammatory effects (Fig. 1). In this regard, it has been recently hypothesized that the same galectin may exert pro- or anti-inflammatory effects depending on multiple factors, such as the concentration reached in inflammatory foci, the extracellular microenvironment and the particular target cells impacted [15]. It has been suggested that multivalency of individual members of the galectin family, their biochemical and biophysical properties, and their cross-linking activity might determine different biological responses by inducing aggregation of specific cell surface glycoreceptors, which in many cases, are associated with different signal transduction events [5].

Here, we focus on the role of three members of the galectin family (galectin-1, -3 and -9), each representative of a different subfamily, in the regulation of autoimmune and inflammatory responses and discuss their potential role as selective anti-inflammatory agents or targets for anti-inflammatory drugs.

Galectin-1

Functions of galectin-1 demonstrated *in vitro*

Impact of galectin-1 in T-cell growth and survival

Galectin-1 can control T-cell growth and apoptosis of human and murine T cells during the development in the thymus and after stimulation in the periphery [9, 16–19]. Different cell surface glycoconjugates appear to be primary receptors for galectin-1, such as CD45, CD43, CD2, CD3 and CD7 [20–23]. Interestingly, galectin-1 binding to T cells results in a marked redistribution of these glycoproteins into segregated membrane microdomains [22]. Furthermore, it has been demonstrated that specific glycosylation of these glycoproteins is a critical determinant factor that affects the T-cell response to galectin-1. For example, CD45 can positively or negatively regulate galectin-1-induced T-cell death, depending on its glycosylation status [21]; $CD45^+$ T cells lacking the core 2β-1,6 N-acetylglucosaminyltransferase (C2GnT), an enzyme responsible for creating branched structures on O-glycans, are resistant to galectin-1-

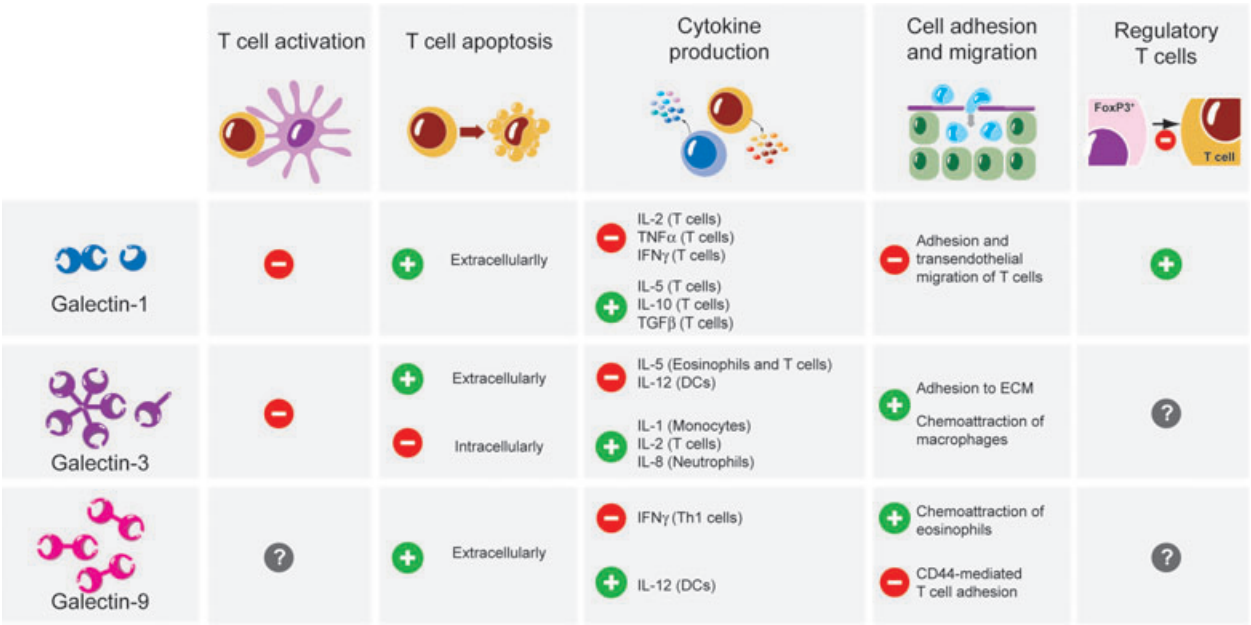

Figure 1 Role of galectin-1, -3 and -9 in the development and resolution of inflammatory responses. This scheme illustrates the influence of individual members of the galectin family in different immune processes, including immune cell activation, survival, cytokine production, cell adhesion and migration and the function of regulatory cells. Galectins are represented according to their biochemical structure: Galectin-1 is a prototype (one-CRD) galectin which can dimerize; galectin-9 belongs to the tandem-repeat family and has two distinct CRD in tandem, connected by a linker of up to 70 amino acids and galectin-3 consists of unusual tandem repeats of proline-and glycine-rich short stretches fused onto the CRD.

induced death [21]. In addition, it has been demonstrated that T-cell susceptibility to galectin-1-induced cell death can be negatively regulated by the ST6Gal1 sialyltransferase which selectively modifies N-glycans on CD45 to reduce galectin-1 binding to the cells and abrogate signalling through the CD45 intracellular domain [24]. Therefore, critical factors that determine the responsiveness of cells to galectin-mediated signals include both the repertoire of glycoproteins expressed on the cell surface and the activities of specific glycosyltransferases that are responsible for glycosylating these proteins and generating galectin ligands. These variables can change according to the state of activation and differentiation of lymphoid cells.

The signal transduction events that lead to apoptosis induced by galectin-1 involve several intracellular mediators of apoptosis, including, in some cases, induction of specific transcription factors (i.e. AP-1) and the modulation of BCL2 protein production [25], the activation of caspases and cytochrome *c* release [26], and the involvement of proximal signals, such as $p56^{lck}$ and ZAP70 [27]. Interestingly, recent evidence indicates that acid sphingomyelinase-mediated release of ceramide is essential to trigger the mitochondrial pathway of apoptosis induced by galectin-1 [28]. However, another work has shown that galectin-1 induced T-cell death can occur in a caspase-independent fashion and can proceed in the absence of *de novo* protein synthesis, implying that expression of transcription factors is not required for all T cells to be susceptible to galectin-1 [29].

Interestingly, Endharti *et al.* [30] demonstrated that, in contrast to the pro-apoptotic role of galectin-1 on activated T cells, secretion of this protein by stromal cells is capable of supporting the survival of naïve T cells without promoting proliferation. Thus, it seems apparent that galectin-1 might trigger different signals (i.e. apoptosis or survival) and even different apoptosis end points depending on a number of factors including the activation state of the cells, the spatiotemporal expression of specific glycosyltransferases, the general context of the cell culture, biochemical properties of purified galectin-1 (monomeric versus dimeric forms) and/or the nature of the target cell (activated, differentiated or resting peripheral T cells). These apparent discrepancies remain to be elucidated in future work by addressing the potential role of endogenous galectin-1 in the regulation of apoptosis *in vivo* and the effects of different microenvironments in the apoptotic and immunoregulatory activities of this carbohydrate-binding protein.

Galectin-1 in the regulation of T-cell receptor-dependent signalling and cytokine production

Galectin-1 has been shown to affect early T-cell receptor (TCR)-dependent signals during T-cell activation. Vespa *et al.* [31] found that galectin-1 inhibits TCR-induced

interleukin-2 (IL-2) production and proliferation in a murine T-cell hybridoma clone and freshly isolated mouse thymocytes. Interestingly, the same group further demonstrated that galectin-1 antagonizes TCR signals that require costimulation such as IL-2 production, while allowing TCR responses that only require partial TCR signals, such as CD69 upregulation and apoptosis. The authors showed that galectin-1 can modulate the immunological synapse by blocking TCR/costimulator-dependent lipid raft clustering at the TCR constant site [32].

Although the precise mechanisms still remain to be elucidated, different members of the galectin family have been shown to positively or negatively influence the production of a wide range of anti- or pro-inflammatory cytokines. We have reported that recombinant galectin-1, at low concentrations (approximately 0.01–01 μM), can inhibit the secretion of pro-inflammatory cytokines, such as tumour necrosis factor-α and interferon-γ (IFN-γ) by activated T cells without inducing T-cell apoptosis [33]. Interestingly, this concentration is significantly lower than that required for galectin-1 to dimerize (approximately 7 μM). In addition, we have shown that galectin-1 inhibits the allogeneic T-cell response through apoptotic and non-apoptotic mechanisms [19]. Interestingly, in this study we found selective inhibition of Th1 cytokine production in the viable non-apoptotic T-cell population [19], suggesting that several galectin-1-mediated mechanisms may operate to achieve immunosuppression *in vivo* (see below). Furthermore, van der Leij *et al.* [34] reported a marked increase in IL-10 mRNA and protein levels in non-activated and activated $CD4^+$ and $CD8^+$ T cells following exposure to recombinant galectin-1. In addition, the authors generated leucine-zipper based stable galectin-1 homodimers and recently found that this stable dimeric galectin-1 can efficiently induce apoptosis, increase IL-10 and decrease IL-2 secretion at 100-fold lower concentrations compared with wild type recombinant galectin-1 [34].

Certainly, one of the most consistent findings among the literature is the ability of galectin-1 to skew the balance from a Th1- toward a Th2-polarized immune response in different experimental models of chronic inflammation, autoimmunity and cancer [35–41]. Investigation of cytokine balance in draining lymph nodes and spleens from mice treated with recombinant galectin-1 revealed decreased amounts of IFN-γ and IL-2 and high levels of IL-5, IL-10 and TGF-β production.

Galectin-1 and regulatory T cells

In addition to activation-induced cell death and induction of anergy, avoidance of collateral damage to the host is also achieved by active immune suppression mediated by T regulatory cell populations. We found that treatment with recombinant galectin-1 in the efferent phase of autoimmune ocular inflammation results in increased IL-10 and transforming growth factor-β (TGF-β) production and expansion of regulatory T-cells *in vivo* [38]. Adoptive transfer of regulatory T cells obtained from galectin-1 treated mice prevented the development of autoimmune disease in naïve recipient mice [38]. Interestingly, recent studies demonstrated by DNA microarray analysis that *Lgals1* mRNA (the transcript encoding galectin-1 protein) is overexpressed in naturally-occurring regulatory T cells [11, 12], and that blockade of galectin-1 significantly reduced the suppressive effects of human and mouse $CD4^+$ $CD25^+$ regulatory T cells [12]. These findings indicate that expression of galectin-1 is an important component of the suppressive activity of T regulatory cells, thus providing another potential mechanism for the immunoregulatory potential of this glycan-binding protein. Importantly, further studies are needed to establish a definitive role for galectin-1 in the immunosuppressive capacity of regulatory T cells *in vivo* and the mechanisms responsible for its overexpression on regulatory versus effector T cells.

Influence of galectin-1 in lymphocyte adhesion and migration

Adhesion and migration of immune cells across blood-vessel walls and through ECM barriers are instrumental processes in maintaining homeostasis during ongoing inflammatory responses. We found that exposure to galectin-1 inhibited T-cell adhesion to ECM glycoproteins, such as fibronectin and laminin [33]. In addition, galectin-1 present on the surface of the ECM reduced the ability of T cells to migrate through the matrix; this effect required CD43 clustering but was independent of the presence of core 2 O-glycans [42]. Hence, it is becoming increasingly apparent that apoptosis may only partially explain the immunosuppressive properties of galectin-1; T cells that are refractory to apoptosis may be subject to suppression of pro-inflammatory cytokine secretion, inhibition of their migratory capacity and targeting for phagocytic removal. The cross-talk between different biological effects mediated by galectin-1 still remains to be investigated.

Role of galectin-1 in the regulation of B-cell physiology

Whereas compelling evidence has been accumulated regarding the effects of galectin-1 on T-cell fate, limited information is available on how galectin-1 may impact on B lymphocytes. A pioneer study by Gauthier *et al.* [43] demonstrated that galectin-1 expressed by stromal cells acts as a ligand of the pre-B-cell receptor (BCR) implicated in synapse formation between pre-B and stromal cells. The authors found that pre-BCR binding to stromal cells depends upon galectin-1 anchoring to glycosylated counter-receptors and these complexes relocalize

at the contact zone to form the immunological synapse [43]. The authors recently extended their findings showing that $\alpha_4\beta_1$ (VLA-4), $\alpha_5\beta_1$ (VLA-5), and $\alpha_4\beta_7$ integrins are the major receptors for galectin-1 during pre-BCR relocalization, activation and signalling [44]. Thus, galectin-1 is critical during B-cell progenitor development in the bone marrow compartment. At the peripheral level, a significant increase in galectin-1 expression was found in stimulated B cells receiving signals via cross-linking of the BCR and CD40 [10]. Interestingly, no changes in B-cell survival were found following exposure of galectin-1 *in vitro* independently of the activation state of these cells [10]. In search for genes differentially transcribed in anergic B cells, Clark *et al.* [45] recently found, using representational difference analysis that *Lgals1 and Lgals3*, the genes encoding galectin-1 and -3 are significantly overexpressed in anergic B cells tolerized by self antigens. These findings prompt further exploration of the role for galectins as regulators of B-cell tolerance and homeostasis.

Impact of galectin-1 on cells of the myelomonocytic lineage

Previous results from our laboratory showed that galectin-1, similarly to Th2- and Th3-type cytokines, inhibits nitric oxide synthesis, favouring instead the expression of arginase (the alternative metabolic pathway of L-arginine) in activated peritoneal macrophages [46]. In addition, we have recently shown that galectin-1 can differentially regulate the expression and function of critical regulatory molecules (i.e. FcγRI and major histocompatibility class II) on human monocytes and mouse macrophages through a non-apoptotic ERK1/2-mediated pathway [47]. This effect was clearly observed in macrophages recruited in response to inflammatory stimuli following treatment with recombinant galectin-1 and further confirmed in cells obtained from galectin-1-deficient (*Lgals1*$^{-/-}$) mice [47]. This result together with our previous observations that galectin-1 favours arginase activity [44], suggests that this endogenous lectin might promote a state of 'alternative activation' or 'deactivation' in elicited macrophages. However, the pathophysiological role of galectin-1-stimulated macrophages in pathological settings including autoimmune processes, infection and cancer still remains to be established.

Concerning the influence of galectins on dendritic cell function, Lee's group reported the ability of exogenous galectin-1 to augment dendritic cell secretion of pro-inflammatory cytokines, and to influence dendritic cell migration through the ECM [48] Similarly, it has been demonstrated that dendritic cells engineered to overexpress galectin-1 are highly activated; these transgenic dendritic cells can stimulate naïve T cells and induce apoptosis of activated T cells [49], consistent with findings described in previous sections.

In addition to its role in adaptive immune response, galectin-1 has been shown to affect innate immunity and attenuate the acute inflammatory response [50, 51]. We have shown that galectin-1 ameliorates phospholipase A_2-induced oedema by blocking neutrophil extravasation *in vivo* [50]. In addition, La *et al.* [51] found that galectin-1 inhibits transendothelial migration and chemotaxis of neutrophils. Furthermore, Stowell *et al.* [52] reported that galectins-1, -2 and -4 can induce phosphatidylserine exposure in activated human neutrophils without affecting their survival. Further studies are warranted to dissect the precise role of individual members of the galectin family in neutrophil-mediated immune functions and the regulation of innate immunity.

Effects of galectin-1 demonstrated *in vivo*

Regulation of autoimmunity and chronic inflammation

Galectin-1 has been proposed to be, in general, a negative regulator of inflammatory and autoimmune responses *in vivo* [7]. Early in the 1980s, Levi *et al.* [53] reported the preventive and therapeutic effects of electrolectin, a galectin-1 homologue purified from the fish *Electrophorus electricus*, in an experimental model of autoimmune myasthenia gravis in rabbits. As then, the anti-inflammatory properties of galectin-1 have been evaluated in several models of chronic inflammation and autoimmunity including experimental autoimmune encephalomyelitis (EAE) [54], collagen-induced arthritis (CIA) [35], concanavalin A-induced hepatitis [36], hapten-induced colitis [37], interphotoreceptor-binding protein-induced uveitis [38], autoimmune diabetes [39] and graft versus host disease (GvHD) [40].

Offner *et al.* demonstrated that galectin-1 prevented the development of clinical and histopathological signs of EAE in Lewis rats [54]. Although the mechanisms of action of galectin-1 were not investigated in this study, the authors proposed that galectin-1 might block the sensitization and activation of encephalitogenic T cells.

In 1999, our group demonstrated that a single cell injection of syngeneic fibroblasts engineered to secrete galectin-1 on the day of the disease onset abrogated clinical and histopathological manifestations of CIA, an experimental model of rheumatoid arthritis (RA) in DBA/1 mice [35]. This effect was also observed in response to daily injection of recombinant galectin-1. Insights into the mechanisms involved in this immunoregulatory effect revealed a critical role for galectin-1 in skewing the balance from Th1- toward Th2-polarized immune responses. In addition, lymph node cells from mice engaged in the galectin-1 gene therapy protocol had increased susceptibility to antigen-induced apoptosis [35].

Similarly, Santucci *et al.* [36, 37] found that galectin-1 treatment prevented tissue injury and T-cell mediated

inflammation in animal models of concanavalin A-induced hepatitis and hapten-induced colitis. Here, galectin-1 treatment induced a reduction in the number of antigen-activated mucosal T-cells and a decreased secretion of pro-inflammatory and Th1 cytokines.

The immunoregulatory effects of galectin-1 have also been investigated in experimental autoimmune uveitis (EAU), a T-cell mediated model of retinal disease [38]. Treatment with galectin-1 either early or late during the course of EAU was sufficient to suppress clinical ocular pathology and to counteract pathogenic Th1 cells. Administration of galectin-1 ameliorated retinal inflammation by skewing the uveitogenic response towards non-pathogenic Th2- or T regulatory-mediated anti-inflammatory responses [38]. These results highlight the ability of this endogenous lectin to counteract Th1 mediated responses through different, but potentially overlapping anti-inflammatory mechanisms. Remarkably, a striking correlation was observed between the levels of anti-retinal galectin-1 autoantibodies in sera from uveitic patients and the severity of autoimmune retinal inflammation [55]. However, further work remains to be performed to elucidate the pathophysiological role of these autoantibodies and their clinical significance as disease markers.

In addition, recent evidence indicates that dendritic cells engineered to overexpress galectin-1 can delay the onset of autoimmune diabetes and insulitis when targeted to inflammatory sites [39]. Interestingly, this therapeutic effect was accompanied by increased percentage of apoptotic T cells and reduced number of IFN-γ-secreting $CD4^+$ T cells in pancreatic lymph nodes [39].

Finally, regarding the immunosuppressive activity of galectin-1 in a transplantation setting, Baum *et al.* investigated the efficacy of galectin-1 treatment in a murine model of GvHD and found that 68% of galectin-1-treated mice survived, compared with 3% of vehicle-treated mice [40]. Similar to findings in autoimmune models, Th1 cytokines were markedly reduced, while production of Th2 cytokines was similar between galectin-1-treated and control animals [40]. Thus, galectin-1 can restore immune cell tolerance in several autoimmune, transplantation and inflammation settings by acting as an anti-inflammatory and immunoregulatory cytokine. From a therapeutic standpoint, these findings suggest the potential use of galectin-1 for the selective treatment of Th1 and Th-17-mediated inflammatory disorders.

Galectin-1 in tumour immunity

Interestingly, expression of galectin-1 (as well as other galectins) in cancer cells and cancer-associated stroma positively correlates with the aggressiveness of different tumour types [6]. Using a combination of *in vitro* and *in vivo* strategies, we have demonstrated a role for galectin-1 in tumour-induced immunosuppression [41]. Blockade of the immunosuppressive and pro-apoptotic activity of galectin-1 within tumour tissue resulted in heightened T-cell mediated tumour rejection with increased survival of IFN-γ-producing Th1 cells [41]. Supporting our findings, Le *et al.* [56] have recently identified galectin-1 as a molecular link between tumour hypoxia and tumour-immune privilege. The authors found a strong inverse correlation between galectin-1 expression and the presence of T cells in human tumour sections corresponding to head and neck squamous cell carcinoma patients [56]. In addition, it has been recently shown that endothelial cell expression of galectin-1 induced by prostate cancer cells inhibit T-cell transendothelial migration [42]. Taken together, these results support the concept that galectin-1 in tumours and in tumour-associated stroma contributes to immune privilege of tumours by negatively regulating the survival or migration of effector T cells. Given its potent immunosuppressive effects, galectin-1 may be a useful target for therapeutic intervention in cancer.

Galectin-3

Biochemical aspects and cell biology

Galectin-3 is the only member of the galectin family with an N-terminal region composed of tandem repeats of short amino-acid segments (approximately 120 amino acids) connected to a C-terminal CRD. Like other galectins, galectin-3 lacks a signal sequence required for secretion through the classical secretory pathway. Nevertheless, the protein is released into the extracellular space through a yet undefined mechanism [57] and was found to be secreted by cultured cells and detectable in extracellular fluid under various inflammatory conditions [58]. Lukyanov *et al.* [59] showed that galectin-3 is able to bind to membrane lipids and penetrate lipid bilayers. It is possible that the protein is released by the cell through this mechanism. More recently, Delacour *et al.* [60] found that galectin-3 is present in vesicles containing glycoproteins destined for the plasma membrane at the apical side of the cell. The protein may be exported to outside the cells together with these glycoproteins.

Galectin-3 can oligomerize in the presence of multivalent carbohydrate ligands and is capable of crosslinking glycans on the cell surface, thereby initiating transmembrane signalling events and affecting various cellular functions [58, 61, 62]. Additionally, a number of intracellular functions have been reported for galectin-3 and, in some cases, intracellular binding partners have been identified [63].

Functions of galectin-3 demonstrated *in vitro*

Regulation of cellular homeostasis by galectin-3

Exogenously added galectin-3 has been shown to influence the growth of many different cell types, including immune cells [58]. The function of endogenous galectin-3 as a positive growth regulator of T cells was established by demonstrating that cells treated with galectin-3-specific antisense oligonucleotides, and thus containing lower levels of the protein, had a reduced growth rate [64]. In contrast, exogenously added galectin-3 has a negative effect on T cell growth, as it inhibits mitogen-induced proliferation of peripheral blood T cells [65].

Like galectin-1, galectin-3 has been shown to induce apoptosis in T cells, including human T leukaemia cell lines, human peripheral blood mononuclear cells (PBMC), and activated mouse T cells [20, 66]. One study found that CD7 and CD29 mediate galectin-3's effect [66], but another showed that CD45 and CD71, but not CD29 and CD43, are involved [20]. In addition, galectin-3 has also been shown to induce apoptosis in neutrophils, although the specific receptors on this cell types have not been identified [67].

While exogenously-added galectin-3 induces cell death, endogenous galectin-3 has anti-apoptotic function. This has been shown in a number of cell types and with diverse apoptotic stimuli, by using gene transfection or anti-sense nucleotide methods [63]. Among the cells studied are immune cells, including B lymphoma cells [68], the T cell lines Jurkat [69] and CEM [66] and macrophages [70]. In the setting of juvenile idiopathic arthritis, the degree of apoptosis of mononuclear cells found in synovial tissue is inversely correlated with the expression level of galectin-3, consistent with the anti-apoptotic function of the protein [71]. A large body of studies suggest that endogenous galectin-3 confers resistance to apoptosis by functioning inside the cells and engaging apoptosis-regulation pathways [63, 72] or modulating mitochondrial homeostasis [73].

It is to be noted, however, that suppression of galectin-3 expression in human peripheral blood T cells resulted in decreased activation-induced apoptosis and increased proliferation [65], suggesting that endogenous galectin-3 promotes apoptosis, as well as suppresses cell growth. It remains to be determined whether under these conditions, the observed effect primarily reflects that of the secreted protein, which is known to be apoptosis-inducing in this cell type.

Regulation of cellular response by galectin-3

Galectin-3 binds to the ECM proteins in a carbohydrate-dependent manner and can influence cell adhesion to extracellular matrices [6]. It also interacts with integrins and can modulate cell adhesion through binding to these molecules [6]. Conceivably, galectin-3 can affect immune and inflammatory responses by modulating cell adhesion of various immune cell types [74].

Despite our knowledge of these possible interactions, how galectin-3 might affect cell adhesion is difficult to predict. Conceptually, galectin-3 can bridge cells to cells or cells to extracellular matrices by binding simultaneously to glycans on two partners (as galectin-3 can form pentamers upon binding to glycans). It can also promote cell adhesion by binding to and activating cell adhesion molecules, such as integrins. On the other hand, it may interfere with cell adhesion by binding to some other molecules involved in cell adhesion (because of steric hindrance). Exogenously added galectin-3 was found to attenuate interaction of thymocytes with thymic nurse cells *in vitro* [75]. The authors suggested that galectin-3 may influence the development, survival and migration of thymocytes by modulating their adhesion to surrounding cells in the thymus. Furthermore, recombinant galectin-3 was found to promote adhesion of human neutrophils to laminin [76] and to endothelial cells [77]. In the case of adhesion of neutrophils to laminin, galectin-3 was in fact shown to both serve as a bridge between the cell and the ECM and activate the cell through binding to cell surface glycans.

Whether endogenous galectin-3 functions in the ways demonstrated by using recombinant galectin-3 has been addressed. Thus, the involvement of endogenous galectin-3 in adhesion of T cells to dendritic cells and macrophages is supported by the finding that the adhesion is inhibitable by a known galectin-3 sugar ligand and anti-galectin-3 antibody [78]. Additionally, endogenous galectin-3 was shown to participate in homotypic aggregation of monocytes induced by antibody against CD13 [79], as this aggregation was inhibited by both anti-galectin-3 antibodies and lactose.

Galectin-3 has been shown to induce cell activation through its multivalent lectin activity. These include immunoglobulin E (IgE) production by B cells [80] as well as IL-2 production [81] and extracellular calcium ion uptake [82] by T cells. Galectin-3 was described to be associated with the TCR complex [83]. Treatment of T cells with lactose, which inhibits galectin-3 binding to TCR, increased TCR clustering as well as signalling through the receptor. This suggests that galectin-3 may serve as a negative regulator of TCR-initiated signal transduction [83].

Galectin-3 was previously shown to bind to IgE (hence the name IgE-binding protein) and IgE receptor (FcϵRI) [84]. Recombinant galectin-3 induces mediator release in both IgE-sensitized and non-sensitized mast cells [84], possibly by cross-linking FcϵRI-bound IgE, FcϵRI, or both. In addition, galectin-3 induces superoxide anion production [85] and potentiates lipopolysaccharide

(LPS)-induced IL-1 production [86] in human peripheral blood monocytes. A number of studies have shown that galectin-3 induces oxidative responses in human peripheral blood neutrophils primed with various agents [58]. The protein also induces L-selectin shedding and IL-8 production in both naïve neutrophils and those primed with cytochalasin B [87], and promotes phagocytic activity of this cell type [67]. Finally, galectin-3 can induce migration of human monocytes/macrophages and alveolar macrophages [88].

Galectin-3 can also exert a suppressive effect on myeloid cells, as exemplified by its inhibition of IL-5 production in human eosinophils, an eosinophilic cell line, human PBMC, and an antigen-specific T-cell line, because of downregulation of IL-5 mRNA levels [89].

The function of endogenous galectin-3 in phagocytosis has been demonstrated by comparing macrophages from galectin-3-deficient (*Lgals3*$^{-/-}$) mice and wild type mice [90]. *Lgals3*$^{-/-}$ macrophages are defective in phagocytosis of opsonized erythrocytes and apoptotic thymocytes. Interestingly, under the phagocytic situations, galectin-3 is present in phagocytic cups and concentrated around phagosomes. Additional studies suggested that the protein mediates this function by acting intracellularly.

Studies of mast cells in *Lgals3*$^{-/-}$ mice have revealed a role for galectin-3 in the regulation of mast cell functions. *Lgals3*$^{-/-}$ mast cells produced lower levels of granular mediators and cytokines, when activated by cross-linkage of cell surface IgE receptor, compared with wild type cells [91]. Additional studies provided evidence that galectin-3 regulates these responses by acting inside the cells. Furthermore, the function of endogenous galectin-3 in dendritic cells, has also been revealed by studying *Lgals3*$^{-/-}$ cells. In particular, galectin-3 suppresses the production of IL-12 in this cell type [92].

Thus, through extracellular modes of actions, galectin-3 can affect cell growth and survival, activate or inhibit cellular responses, modulate cell adhesion, and induce cell migration. These functions are mostly demonstrated with recombinant galectin-3 *in vitro* and whether endogenous galectin-3 exerts all these activities remains to be clarified. In addition, whether these activities are operative *in vivo* is largely unknown. Endogenous galectin-3 can exert many of the same functions through intracellular actions. These functions are not expected for a protein with lectin properties, but are consistent with the protein's intracellular localization. Importantly, a number of these were revealed by using gene transfection or antisense approaches to influence expression of the protein in the cell. Because of the intrinsic potential problems associated with these types of approaches, they should be confirmed by employing other experimental strategies, such as the use of cells from *Lgals3*$^{-/-}$ mice and by knocking down the galectin-3 gene expression by siRNA.

It is important to note that the intracellular functions demonstrated for endogenous galectin-3 may not be the same as those exerted by exogenous added galectin-3. This is best exemplified by the opposite activities noted for the protein in the regulation of apoptosis. The endogenous protein is antiapoptotic presumably through its intracellular action, while exogenously added recombinant protein induces apoptosis, through an extracellular mechanism. Thus, a comprehensive understanding of the physiological and pathological functions of galectin-3 *in vivo* will require the use of specific inhibitors that can differentially target intracellular and extracellular galectin-3 (e.g. using those unable to penetrate the cell and affect only extracellular galectin-3 and those can get inside the cell and inhibit the intracellular action of the protein).

Functions of galectin-3 in inflammatory responses *in vivo*

Galectin-3 in inflammation and allergy

Some of the functions described above have been confirmed by *in vivo* studies of mouse models using *Lgals3*$^{-/-}$ mice or by injecting exogenous galectin-3. For example, injection of recombinant galectin-3 into airpouches created in mice can induce migration of monocytes/macrophages to the injected sites [88]. In addition, the function of galectin-3 as a positive regulator of the mast cell response has been established by showing that *Lgals3*$^{-/-}$ mice exhibit reduced IgE-mediated responses of mast cells compared with wild-type mice, as evidenced by diminished passive cutaneous anaphylactic reactions [91]. The function of galectin-3 in phagocytosis has also been established by showing that *Lgals3*$^{-/-}$ macrophages are defective in phagocytosis of opsonized erythrocytes and apoptotic thymocytes *in vivo* [90].

The above described *in vitro* and *in vivo* studies suggest that galectin-3 may modulate inflammatory responses through its functions on cell activation, cell migration, or inhibition of apoptosis (thus prolonging the survival of inflammatory cells). The emerging data from studies of *Lgals3*$^{-/-}$ mice support the role of galectin-3 in promotion of the inflammatory response. For example, *Lgals3*$^{-/-}$ mice exhibited attenuated peritoneal inflammation induced by peritoneal injection of thioglycollate broth [70, 93], compared with wild-type mice, suggesting a role of galectin-3 in this model of inflammation. Similarly, *Lgals3*$^{-/-}$ mice exhibited reduced airway allergic inflammation compared with wild-type littermates, when they were first sensitized with ovalbumin systemically and then challenged with the same antigen through the airways [94].

It should be mentioned that other investigators observed reduced airway eosinophil infiltration in response to airway antigen challenge in rats and mice

after intranasal delivery of cDNA encoding galectin-3 [95, 96]. These contrasting results may be explained by a potentiating role for endogenous galectin-3 in the airway inflammatory response, but a suppressive effect of pharmacological concentrations of galectin-3 applied to the airways. This contrasting role of endogenous galectin-3 and exogenously added galectin-3 might reflect what has been observed *in vitro*. For example, endogenous galectin-3 is antiapoptotic in T cells, whereas exogenously added galectin-3 induces death in the same cell type, as mentioned above.

The studies of mouse infectious disease models have also revealed the proinflammatory function of galectin-3. *Lgals3*$^{-/-}$ mice infected with *Toxoplasma gondii* exhibited lower inflammatory scores in gut, liver and brain compared with similarly infected wild-type mice [92]. It is to be noted, however, that cellular inflammation was more pronounced in the lungs of *Lgals3*$^{-/-}$ mice compared with wild-type littermates in late infection. The reason for this variable responses in different organs is unknown.

The studies of the functional role of galectin-3 using *Lgals3*$^{-/-}$ mice have also revealed the protein's ability to regulate Th1/Th2 polarization. Thus, by using a mouse model of allergic airway inflammation, compared with wild type mice, *Lgals3*$^{-/-}$ mice showed: (i) decreased IL-4 and IgE levels in bronchoalveolar fluid and serum and (ii) elevated IFN-γ levels and IgG2a/IgG1 ratios in bronchoalveolar fluid and serum [94]. Similarly, compared with wild type mice, *Lgals3*$^{-/-}$ mice exhibited a significantly higher Th1 response after infection with *Toxoplasma gondii* [92]. The mechanism by which galectin-3 exerts such effects remains to be determined, but it could be related to its function in suppressing the production of IL-12 in dendritic cells, as mentioned above, which is the major cytokine that drives the Th1 response.

Finally, increased galetin-3 expression in inflammatory tissues has been noted in some inflammatory diseases in humans. For example, galectin-3 was detected in tears from patients with inflammatory ocular diseases [97]. It is upregulated in synovial tissues in patients with RA and detectable in synovial fluid from these patients [98]. It is also detectable in atherosclerotic lesions [99]. Additional studies will be required to establish the contributory role of galectin-3 in these inflammatory conditions, although *in vitro* studies and *in vivo* studies in experimental animals described above do support such causal relationship.

Galectin-3 in autoimmunity

Galectins can also participate in immune and inflammatory responses by functioning as autoantigens. A number of reports demonstrated the presence of autoantibodies to different galectins in normal individuals and selected patient populations. A high frequency of autoantibodies to galectin-3 was found in patients with systemic lupus erythematosus and polymyositis/dermatomyositis compared with healthy individuals [100]. In addition, a substantial higher percentage of sera from Crohn's disease patients contain anti-galectin-3 autoantibodies [101].

Autoantibodies to galectins have also been detected in association with neoplasm. A subject with newly diagnosed adenocarcinoma of the colon was found to have a significantly elevated level of IgG anti-galectin-3 [102]. Similarly, the occurrence of IgG antibodies to galectin-3 was noted in a high percentage of sera from patients with pharynx/larynx squamous cell carcinoma [103]. However, in the latter studies, such autoantibodies were also found in healthy donors and there was no statistically significant difference between the two populations. Hence, the pathophysiological roles of these antibodies still remain to be elucidated.

Mgat5-deficient mice develop kidney autoimmune disease and increased susceptibility to EAE [83]. As these authors also showed that galectin-3 binds to Mgat5-modified glycans, they attributed this to the lack of galectin-3 binding to these glycans, including TCR, that normally would result in suppression of the T-cell response (as described above in *Functions of galectin-3 demonstrated in vitro*). Thus, similarly to galectin-1, galectin-3 may play a critical role in suppressing autoimmune responses.

Galectin-9

Functions of galectin-9 *in vitro*

Identification of galectin-9 as a ligand for Tim-3

Interferon-γ-producing Th1 cells are a central component of cell mediated immunity against intracellular pathogens and are deleterious for autoimmunity. Consequently, considerable effort has been spent on identifying cell surface proteins that would not only facilitate the identification of Th1 cells, but might also play a role in their regulation. In 2002, Monney *et al.* [104] identified Tim-3 as a protein expressed selectively on terminally differentiated Th1 but not Th2 cells. *In vivo* modulation of Tim-3/Tim-3 ligand interactions resulted in exacerbated Th1 responses and central nervous system (CNS) autoimmunity [104, 105], suggesting that Tim-3 plays an important role in the regulation of Th1 immune responses. Thus, a search for the endogenous Tim-3 ligand ensued. Immunoprecipitation studies coupled with mass spectrometry led to the identification of galectin-9 as a Tim-3 ligand [106]. Both deglycosylation of Tim-3 and mutation of the galectin-9 CRD domains abrogated Tim-3 binding, confirming that the interaction of galectin-9 with Tim-3 involves galectin-9 binding to carbohydrate residues on Tim-3.

Role of galectin-9 in regulating survival of thymocytes and Th1 cells

Galectin-9 has been shown to induce apoptosis of thymocytes but not hepatocytes [107]. Further studies using several different cells lines and both activated $CD4^+$ and $CD8^+$ T cells have shown that galectin-9 induces cell death via the Ca^{2+}-calpain-caspase-1 pathway [108]. Not surprisingly, galectin-9 has now been shown to specifically induce rapid cell death in Th1 cells in a Tim-3-dependent manner [106]. Thus, galectin-9 triggering of Tim-3 on Th1 cells can serve to negatively regulate Th1 immunity. In this regard, it is important to note that galectin-9 expression is itself upregulated by interferon-γ [109]. Thus, a negative feedback loop may exist whereby Tim-3^+ Th1 cells produce IFN-γ which in turn upregulates galectin-9 which then selectively eliminates Th1 cells.

Recently, a new subset of $CD4^+$ Th cells characterized by the production of IL-17 (Th-17) has been the subject of intense research. Interestingly, Th-17 cells, which are highly pathogenic, have recently been found to express lower levels of Tim-3 relative to Th1 cells [110]. While the effects of galectin-9 on Th-17 cells have not been examined, the lower level of Tim-3 expression on Th-17 cells raises the possibility that Th-17 cells may be less susceptible to galectin-9/TIM-3 regulation and that this may underlie their pathogenicity. Current studies are being conducted to explore the differential susceptibility of distinct effector T-cell populations, including Th1, Th2 and Th17 cells, to different members of the galectin family.

Role of galectin-9 *in vivo*

Galectin-9 in CNS inflammation

Experimental autoimmune encephalomyelitis is a model of CNS autoimmunity in which both Th1 and, more recently, Th-17 cells have been implicated. EAE can be induced in susceptible strains of mice by immunization with components of CNS myelin. Treatment of immunized mice with galectin-9 during the induction phase of EAE resulted in a specific decrease in myelin specific IFN-γ producing cells [106]. Furthermore, *in vivo* knockdown of galectin-9 using siRNA during the induction of EAE resulted in blunting of disease [106]. Collectively these data support the concept that galectin-9 functions *in vivo* to eliminate Tim-3^+ Th1 cells and thereby terminate Th1 immunity. As galectin-9 is widely expressed, it remains to be seen which cell types mediate the elimination of Tim-3^+ Th1 cells *in vivo*.

Galectin-9 in rheumatoid arthritis

We recently found that both galectin-9 mRNA and protein are more highly expressed in the synovial tissues of patients with RA than those with osteoarthritis. Lining and sublining cells, macrophages, T cells, B cells and endothelial cells were positive for galectin-9. Not surprisingly, galectin-9 preferentially induces apoptosis of RA synovial cells compared with synovial cells from osteoarthritis (M. Seki, K. Sakata, S. Oomizu, T. Arikawa, A. Sakata, M. Ueno, A. Nobumoto, T. Niki, N. Saita, K. Ito, S. Dai, S. Katoh, N. Nishi, M. Tsukano, K. Ishikawa, A. Yamauchi, V. Kuchroo, M. Hirashima, submitted manuscript). Interestingly, galectin-9, but not galectin-1 or -3, induces apoptosis and suppresses proliferation of RA synovial cells. We have found that galectin-9 modified by truncating its linker peptide, stable Gal-9 (sGal-9), exhibits more potent activity than wild type galectin-9 [111]. Thus, we used sGal-9 for *in vivo* experiments to clarify whether sGal-9 plays beneficial effects on mouse CIA, and found that sGal-9 reduced severity and incidence of the inflammatory osteoarticular disease Therefore, galectin-9 has therapeutic effects on RA by at least two different mechanisms. The first mechanism involves the induction of apoptosis of pathogenic Th cells (similarly to galectin-1), while the second involves the induction of apoptosis of synovial cells that play a crucial role in pannus formation (M. Seki, K. Sakata, S. Oomizu, T. Arikawa, A. Sakata, M. Ueno, A. Nobumoto, T. Niki, N. Saita, K. Ito, S. Dai, S. Katoh, N. Nishi, M. Tsukano, K. Ishikawa, A. Yamauchi, V. Kuchroo, M. Hirashima, submitted manuscript).

Galectin-9 in allergic inflammation and infection

We first identified galectin-9 as a T cell-derived eosinophil chemoattractant mediating the delayed phase of allergic tissue eosinophilia [112]. Not surprisingly, galectin-9 was also discovered in patients with Hodgkin's disease, because eosinophil accumulation is one of characteristics of certain types of Hodgkin's disease [113]. Galectin-9 indeed exhibits chemoattractant activity *in vitro* and *in vivo* and we have shown that divalent galactoside binding activity is required for its chemotactic activity [114]. Galectin-9 is not only chemotactic, but also behaves as an eosinophil activating factor capable of inducing eosinophil aggregation, suppressing superoxide production, and promoting eosinophil survival [115]. However, the effect of galectin-9 may differ depending on the origin of eosinophils. For example, galectin-9 suppresses apoptosis of eosinophils from eosinophilic patients, but it enhances apoptosis of eosinophils from healthy donors [116].

In a guinea pig model of allergic airway hypersensitivity, galectin-9 levels correlated with eosinophil peroxidase, but not with airway resistance, suggesting little or no involvement of galectin-9 in airway resistance [117]. However, administration of galectin-9 in a mouse model of allergic airway hypersensitivity reduced airway

hyperresponsiveness as well as Th2-associated airway inflammation. Galectin-9 inhibited the infiltration of peripheral blood Th2 cells into the airway by inhibiting the binding of the adhesion molecule CD44 to hyaluronan, indicating that galectin-9 inhibits allergic inflammation of the airway and airway hyperresponsiveness by modulating CD44 dependent leucocyte recognition of the ECM [118].

As described above, the source of galectin-9 was first thought to be antigen-stimulated T cells [112], and its release was associated with some unidentified matrix metalloprotease [119]. More recently, we found that macrophages, dendritic cells, mast cells and NKT cells are also source of galectin-9, and galectin-9 is released by stimulation with LPS [120]. It was also found that *Lgals9* transgenic mice were resistant to LPS, whereas *Lgals9* deficient mice became susceptible to LPS, confirming the important role of galectin-9 in LPS-induced inflammation. We also found that pro-inflammatory cytokines, e.g. IFN-γ, also induced galectin-9 expression on the surface of fibroblasts and vascular endothelial cells though little galectin-9 was released from those cells [121].

Lipopolysaccharide from *Porphylomonas gingivalis* upregulated *Lgals9* mRNA and protein in human periodontal ligament-derived cells suggesting that galectin-9 is associated with inflammatory reactions in the periodontal ligament [122]. We recently found that galectin-9 regulates LPS-induced inflammation and protects mice from Shwartzman reaction by attracting prostaglandin-E2 (PGE_2)-producing neutrophils, indicating the crucial role of galectin-9 in bacterial infection [120]. These results imply that galectin-9 has suppressive effects on bacterial infection-induced inflammation.

Galectin-9 probably exhibits its suppressive role not only in bacterial, but also in other infections, such as parasitic and viral infections. We showed that both galectin-3 and -9 recognize *Leishmania major* by binding to the *Leishmania major*-specific polygalactosyl epitope [123]. Although both galectins exhibit comparable affinities toward the epitopes, only galectin-9 enhances the interaction between *Leishmania major* and macrophages, indicating distinctive roles for the galectins in the *Leishmania major*-specific development of leishmaniasis in the host.

Warke *et al.* [124] found that galectin-9 expression is enhanced in human umbilical vein endothelial cells (HUVEC) infected with dengue virus. We also found that galectin-9 mRNA and protein expression is enhanced in HUVEC when they are treated with double-stranded RNA [125], and further studies revealed that the TLR3, PI3K and IRF3 pathways are involved in double-stranded RNA-induced galectin-9 expression [126]. These results suggest that galectin-9 is also associated with viral infections.

Galectin-9 in tumour immunity

Like other galectins, galectin-9 is also expressed by various different tumours. Lahm *et al.* [127, 128] described different levels of regulation of galectin production in colon cancer cells in the cases of the tandem-repeat-type galectin-8 and -9. Galectin-9 was found to be correlated with oral squamous cell cancer cell-matrix interactions and may therefore play an important role in the metastasis of squamous cell carcinoma [129].

Indeed, we also found that a melanoma cell line that proliferated strongly and promoted colony formation expressed more galectin-9 than another melanoma cell line which proliferated less and did not induce colony formation. Therefore, we examined the association between galectin-9 expression in melanoma cells and the prognosis of patients bearing melanocytic tumours, and found that high galectin-9 expression was inversely correlated with the progression of disease [130]. Immunohistochemical analysis in patients with breast cancer revealed a similar inverse relationship between galectin-9 expression in cancer cells and distant metastasis in breast cancer. The cumulative distant metastasis-free survival ratio for galectin-9-positive patients was significantly better than for the galectin-9-negative group. Multivariate analysis revealed that galectin-9 status influenced distant metastasis independent of and much more than lymph node metastasis. We also found that galectin-9 expression on the surface of cancer cells also reduced cell adhesion to ECM [131, 132].

In addition, galectin-9 seems to be a novel nasopharyngeal carcinoma (NPC)-specific raft partner of the Epstein–Barr virus latent membrane protein 1 (LMP1). LMP1 was further shown to bind galectin 9 in a TRAF3-independent manner [133]. More recently, it was found that human leucocyte antigen-class II (HLA-class II)-positive exosomes purified from NPC contained galectin 9 [134].

Adult T-cell leukaemia (ATL) is a fatal malignancy of T lymphocytes caused by human T-lymphotropic virus-I (HTLV-I) infection. We have found that sGal-9 prevents cell growth of HTLV-I-infected T-cell lines and primary ATL cells. sGal-9 induced cell cycle arrest by reducing the expression of cyclin D1, cyclin D2, cyclin B1, Cdk1, Cdk4, Cdk6, Cdc25C and c-Myc, and apoptosis by reducing the expression of XIAP, c-IAP2 and survivin. Furthermore, sGal-9 suppressed IκBα phosphorylation, resulting in suppression of the transcriptional activity of NF-κB [135].

Taken together, these data support that sGal-9 can inhibit invasion and metastasis of tumour cells, and induce apoptosis or cell arrest. When immature dendritic cells were cultured with galectin-1, -3, -8 or -9, only galectin-9 dramatically upregulated the expression of CD40, CD54, CD80, CD83, CD86 and HLA-DR on dendritic cells. Galectin-9 stimulates dendritic cells to

secrete IL-12, but not IL-10. Thus, galectin-9 induces dendritic cell maturation and galectin-9-matured dendritic cells stimulate IFN-γ, but not IL-4 and IL-5 production from allogenic $CD4^+$ T cells. As it is well known that activated dendritic cells play a crucial role in tumour immunity, galectin-9-mediated dendritic cell maturation may also contribute to tumour-immune surveillance [136]. We recently found that galectin-9 can serve as an anti-tumour and anti-metastatic agent. For example, treatment with sGal-9 reduced tumour formation of an HTLV-I-infected T-cell line when these cells were subcutaneously inoculated into severe combined immunodeficient (SCID) mice [135]. Moreover, our preliminary experiments have revealed that sGal-9 administration protects MethA-bearing mice from death, and suppresses metastasis of melanoma cells in an intravenous B16/F10-injection model, indicating the usefulness of galectin-9 in the therapy against malignant tumours.

Conclusions and future perspectives

As illustrated in this review, different members of the galectin family may provide inhibitory or stimulatory signals to control immune cell response and regulate inflammation following an antigenic challenge. However, like many other cytokines and growth factors (e.g. TGF-β), galectins may exhibit a 'double-edge sword' effect depending on many different intrinsic factors, such as the physicochemical properties of the protein (monomer/dimer equilibrium), stability in tissues, concentrations and oxidation state, and other extrinsic factors, such as the target cell type and the general context or microenvironment. In fact, some galectins clearly share most of the characteristics of cytokines including an upregulated expression in activated inflammatory cells. Hence, it is attractive to speculate that the body responds to an exacerbated inflammatory response by increasing the peripheral production of endogenous anti-inflammatory galectins in an attempt to restore immune cell homeostasis. In fact, *in vivo* studies, including those using galectin-deficient mice, are starting to provide relevant information on the selective functions of several members of the galectin family in the inflammatory response.

Given the broad spectrum of immunoregulatory effects in autoimmune diseases, inflammatory processes and cancer, galectins have been postulated as candidates for the design of novel anti-inflammatory drugs, and as targets for anti-cancer therapies. Challenges for the future will be to employ recombinant galectins or inhibitors of galectins for the treatment of different pathological conditions. In fact, molecules with such properties have already been developed including: (i) glycoamine analogues [137, 138]; (ii) modified citrus polysaccharides (pectin) [139]; (iii) wedgelike glycodendrimers consisting of 2, 4 and 8 lactose moieties linked using 3,5 di-(2-aminoethoxy) benzoic acid as branching unit [140] and (iv) a collection of small specific inhibitors based on derivatization of N-acetyllactosamine [141–143].

However, before galectin-based therapeutic agents can be fully realized, a more thorough understanding of the lesser studied galectins and the mechanisms involved in their different immunoregulatory functions is required. To what extent is there functional redundancy and specificity of action within the galectin family? What is the precise explanation of the different functions exerted by the same galectins in different environmental contexts? What are the precise mechanisms involved in the anti-inflammatory and immunoregulatory effects of different members of the galectin family? What are the levels of galectins attained *in vivo* during an inflammatory reaction, infectious process or tumour dissemination? Increased understanding of the role of galectins in immunoregulation, inflammation and cancer should provide more insights into how the regulation of galectin expression and activity can be exploited for therapeutic purposes.

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References

1 Daniels MA, Hogquist KA, Jameson SC. Sweet 'n' sour: the impact of differential glycosylation on T cell responses. *Nat Immunol* 2002;3:903–10.
2 Leffler H, Carlsson S, Hedlund M, Qian Y, Poirier F. Introduction to galectins. *Glycoconj J* 2004;19:422–40.
3 Camby I, Mercier ML, Lefranc F, Kiss R. Galectin-1: A small protein with major functions. *Glycobiology* 2006;16:137–57.
4 Cooper DN, Barondes SH. God must love galectins; he made so many of them. *Glycobiology* 1999;9:979–84.
5 Brewer CF. Binding and cross-linking properties of galectins. *Biochim Biophys Acta* 2002;1572:255–62.
6 Liu FT, Rabinovich GA. Galectins as modulators of tumour progression. *Nat Rev Cancer* 2005;5:29–41.
7 Rabinovich GA, Baum LG, Tinari N *et al.* Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* 2002a;23:313–20.

8 Blaser C, Kaufmann M, Muller C *et al.* β-galactoside-binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. *Eur J Immunol* 1998;28:2311–9.

9 Rabinovich GA, Modesti NM, Castagna LF *et al.* Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells: biochemical and functional characterization. *J Immunol* 1998;160:4831–40.

10 Zuniga EI, Rabinovich GA, Iglesias MM, Gruppi A. Regulated expression of galectin-1 during B-cell activation and implications for T-cell apoptosis. *J Leukoc Biol* 2001;70:73–9.

11 Sugimoto N, Oida T, Hirota K *et al.* Foxp3-dependent and -independent molecules specific for $CD25^+CD4^+$ natural regulatory T cells revealed by DNA microarray analysis. *Int Immunol* 2006;18:1197–209.

12 Garin MI, Chu CC, Golshayan D, Cernuda-Morollon E, Wait R, Lechler RI. Galectin-1: a key effector of regulation mediated by $CD4^+CD25^+$ T cells. *Blood* 2007;109:2058–65.

13 Ocklenburg F, Moharregh-Khiabani D, Geffers R *et al.* UBD, a downstream element of FOXP3, allows the identification of LGALS3, a new marker of human regulatory T cells. *Lab Invest* 2006;86:724–37.

14 Kubach J, Lutter P, Bopp T *et al.* Human $CD4^+CD25^+$ regulatory T cells: proteome analysis identifies galectin-10 as a novel marker essential for their anergy and suppressive function. *Blood* 2007;[Epub ahead of print].

15 Toscano MA, Ilarregui JM, Bianco GA *et al.* Dissecting the pathophysiologic role of endogenous lectins: glycan-binding proteins with cytokine-like activity? *Cytokine Growth Factor Rev* 2007;18:57–71.

16 Perillo NL, Uittehbogaart CH, Nguyen JT, Baum LG. Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J Exp Med* 1997;185:1851–8.

17 Perillo NL, Pace KE, Seihamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. *Nature* 1995;378:736–9.

18 Rabinovich GA, Modesti NM, Castagna LF, Landa CA, Riera CM, Sotomayor CE. Specific inhibition of lymphocyte proliferation and induction of apoptosis by CLL-I, a β-galactoside-binding lectin. *J Biochem* 1997;122:365–73.

19 Rabinovich GA, Ramhorst RE, Rubinstein N *et al.* Induction of allogenic T-cell hyporesponsiveness by galectin-1-mediated apoptotic and non-apoptotic mechanisms. *Cell Death Differ* 2002b;9:661–70.

20 Stillman BN, Hsu DK, Pang M *et al.* Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *J Immunol* 2006;176:778–89.

21 Nguyen JT, Evans DP, Galvan M *et al.* CD45 modulates galectin-1-induced T cell death: regulation by expression of core 2 O-glycans. *J Immunol* 2001;167:5697–707.

22 Pace KE, Lee C, Stewart PL, Baum LG. Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1. *J Immunol* 1999;163:3801–11.

23 Walzel H, Fahmi AA, Eldesouky MA *et al.* Effects of N-glycan processing inhibitors on signaling events and induction of apoptosis in galectin-1-stimulated Jurkat T lymphocytes. *Glycobiology* 2006;16:1261–71.

24 Amano M, Galvan M, He J, Baum LG. The ST6Gal I sialyltransferase selectively modifies N-glycans on CD45 to negatively regulate galectin-1-induced CD45 clustering, phosphatase modulation, and T cell death. *J Biol Chem* 2003;278:7469–75.

25 Rabinovich GA, Alonso CR, Sotomayor CE, Durand S, Bocco JL, Riera CM. Molecular mechanisms implicated in galectin-1-induced apoptosis: activation of the AP-1 transcription factor and downregulation of Bcl-2. *Cell Death Differ* 2000a;7:747–53.

26 Matarrese P, Tinari A, Mormone E *et al.* Galectin-1 sensitizes resting human T lymphocytes to Fas (CD95)-mediated cell death via mitochondrial hyperpolarization, budding, and fission. *J Biol Chem* 2005;280:6969–85.

27 Ion G, Fajka-Boja R, Toth GK, Caron M, Monostori E. Role of p56lck and ZAP70-mediated tyrosine phosphorylation in galectin-1-induced cell death. *Cell Death Differ* 2005;12:1145–7.

28 Ion G, Fajka-Boja R, Kovacs F *et al.* Acid sphingomyelinase mediated release of ceramide is essential to trigger the mitochondrial pathway of apoptosis by galectin-1. *Cell Signal* 2006;18:1887–96.

29 Hahn HP, Pang M, He J *et al.* Galectin-1 induces nuclear translocation of endonuclease G in caspase- and cytochrome *c*-independent T cell death. *Cell Death Differ* 2004;11:1277–86.

30 Endharti AT, Zhou YW, Nakashima I, Suzuki H. Galectin-1 supports survival of naive T cells without promoting cell proliferation. *Eur J Immunol* 2005;35:86–97.

31 Vespa GN, Lewis LA, Kozak KR *et al.* Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibit IL-2 production and proliferation. *J Immunol* 1999;162:799–806.

32 Chung CD, Patel VP, Moran M, Lewis LA, Miceli MC. Galectin-1 induces partial TCR ζ-chain phosphorylation and antagonizes processive TCR signal transduction. *J Immunol* 2000;165:3722–9.

33 Rabinovich GA, Ariel A, Hershkoviz R, Hirabayashi J, Kasai KI, Lider O. Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. *Immunology* 1999a;97:100–6.

34 van der Leij J, van den Berg A, Harms G *et al.* Strongly enhanced IL-10 production using stable galectin-1 homodimers. *Mol Immunol* 2007;44:506–13.

35 Rabinovich GA, Daly G, Dreja H *et al.* Recombinant galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. *J Exp Med* 1999b;190:385–98.

36 Santucci L, Fiorucci S, Cammilleri F, Servillo G, Federici B, Morelli A. Galectin-1 exerts immunomodulatory and protective effects on concanavalin A-induced hepatitis in mice. *Hepatology* 2000;31:399–406.

37 Santucci L, Fiorucci S, Rubinstein N *et al.* Galectin-1 suppresses experimental colitis in mice. *Gastroenterology* 2003;124:1381–94.

38 Toscano MA, Commodaro AG, Ilarregui JM *et al.* Galectin-1 suppresses autoimmune retinal disease by promoting concomitant Th2- and T regulatory-mediated anti-inflammatory responses. *J Immunol* 2006;176:6323–32.

39 Perone MJ, Bertera S, Tawadrous ZS *et al.* Dendritic cells expressing transgenic galectin-1 delay onset of autoimmune diabetes in mice. *J Immunol* 2006a;177:5278–89.

40 Baum LG, Blackall DP, Arias-Magallano S *et al.* Amelioration of graft versus host disease by galectin-1. *Clin Immunol* 2003;109:295–307.

41 Rubinstein N, Alvarez M, Zwirner NW *et al.* Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: a potential mechanism of tumor-immune privilege. *Cancer Cell* 2004;5:241–51.

42 He J, Baum LG. Endothelial cell expression of galectin-1 induced by prostate cancer cells inhibits T-cell transendothelial migration. *Lab Invest* 2006;86:578–90.

43 Gauthier L, Rossi B, Roux F, Termine E, Schiff C. Galectin-1 is a stromal cell ligand of the pre-B cell receptor (BCR) implicated in synapse formation between pre-B and stromal cells and in pre-BCR triggering. *Proc Natl Acad Sci U S A* 2002;99:13014–9.

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

45 Clark AG, Chen S, Zhang H *et al.* Multifunctional regulators of cell growth are differentially expressed in anergic murine B cells. *Mol Immunol* 2007;44:1274–85.

46 Correa SG, Sotomayor CE, Aoki MP, Maldonado CA, Rabinovich GA. Opposite effects of galectin-1 on alternative metabolic pathways of L-arginine in resident, inflammatory, and activated macrophages. *Glycobiology* 2003;13:119–28.

47 Barrionuevo P, Beigier-Bompadre M, Ilarregui JM *et al.* A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. *J Immunol* 2007;178:436–45.

48 Fulcher JA, Hashimi ST, Levroney EL *et al.* Galectin-1-matured human monocyte-derived dendritic cells have enhanced migration through extracellular matrix. *J Immunol* 2006;177:216–26.

49 Perone MJ, Larregina AT, Shufesky WJ *et al.* Transgenic galectin-1 induces maturation of dendritic cells that elicit contrasting responses in naive and activated T cells. *J Immunol* 2006b;176: 7207–20.

50 Rabinovich GA, Sotomayor CE, Riera CM, Bianco I, Correa SG. Evidence of a role for galectin-1 in acute inflammation. *Eur J Immunol* 2000b;30:1331–9.

51 La M, Cao TV, Cerchiaro G *et al.* A novel biological activity for galectin-1: inhibition of leukocyte-endothelial cell interactions in experimental inflammation. *Am J Pathol* 2003;163:1505–15.

52 Stowell SR, Karmakar S, Stowell CJ, Dias-Baruffi M, McEver RP, Cummings RD. Human galectin-1, -2, and -4 induce surface exposure of phosphatidylserine in activated human neutrophils but not in activated T cells. *Blood* 2007;109:219–27.

53 Levi G, Tarrab-Hazdai R, Teichberg VI. Prevention and therapy with electrolectin of experimental autoimmune myasthenia gravis in rabbits. *Eur J Immunol* 1983;13:500–7.

54 Offner H, Celnik B, Bringman TS, Casentini-Borocz D, Nedwin GE, Vandenbark AA. Recombinant human β-galactoside binding lectin suppresses clinical and histological signs of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 1990;28:177–84.

55 Romero MD, Muino JC, Bianco GA *et al.* Circulating anti-galectin-1 antibodies are associated with the severity of ocular disease in autoimmune and infectious uveitis. *Invest Ophthalmol Vis Sci* 2006;47:1550–6.

56 Le QT, Shi G, Cao H *et al.* Galectin-1: a link between tumor hypoxia and tumor immune privilege. *J Clin Oncol* 2005;23:8932–41.

57 Hughes RC. Secretion of the galectin family of mammalian carbohydrate-binding proteins. *Biochim Biophys Acta* 1999;1473:172–85.

58 Liu FT. Regulatory roles of galectins in the immune response. *Int Arch Allergy Immunol* 2005;136:385–400.

59 Lukyanov P, Furtak V, Ochieng J. Galectin-3 interacts with membrane lipids and penetrates the lipid bilayer. *Biochem Biophys Res Commun* 2005;338:1031–6.

60 Delacour D, Cramm-Behrens CI, Drobecq H *et al.* Requirement for galectin-3 in apical protein sorting. *Curr Biol* 2006;16:408–14.

61 Almkvist J, Karlsson A. Galectins as inflammatory mediators. *Glycoconj J* 2004;19:575–81.

62 Ochieng J, Furtak V, Lukyanov P. Extracellular functions of galectin-3. *Glycoconj J* 2004;19:527–35.

63 Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. *Biochim Biophys Acta* 2002;1572:263–73.

64 Joo HG, Goedegebuure PS, Sadanaga N *et al.* Expression and function of galectin-3, a β-galactoside-binding protein in activated T lymphocytes. *J Leukoc Biol* 2001;69:555–64.

65 Muller S, Schaffer T, Flogerzi B *et al.* Galectin-3 modulates T cell activity and is reduced in the inflamed intestinal epithelium in IBD. *Inflamm Bowel Dis* 2006;12:588–97.

66 Fukumori T, Takenaka Y, Yoshii T *et al.* CD29 and CD7 mediate galectin-3-induced type II T-cell apoptosis. *Cancer Res* 2003;63:8302–11.

67 Fernandez GC, Ilarregui JM, Rubel CJ *et al.* Galectin-3 and soluble fibrinogen act in concert to modulate neutrophil activation and survival: involvement of alternative MAPK pathways. *Glycobiology* 2005;15:519–27.

68 Hoyer KK, Pang M, Gui D *et al.* An anti-apoptotic role for galectin-3 in diffuse large B-cell lymphomas. *Am J Pathol* 2004;164:893–902.

69 Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T cell growth and apoptosis. *Proc Natl Acad Sci U S A* 1996;93: 6737–42.

70 Hsu DK, Yang RY, Yu L *et al.* Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. *Am J Pathol* 2000;156:1073–83.

71 Harjacek M, Diaz-Cano S, De Miguel M *et al.* Expression of galectins-1 and -3 correlates with defective mononuclear cell apoptosis in patients with juvenile idiopathic arthritis. *J Rheumatol* 2001;28: 1914–22.

72 Hsu DK, Yang RY, Liu FT. Galectins in apoptosis. *Methods Enzymol* 2006;417:256–73.

73 Matarrese P, Tinari N, Semeraro ML *et al.* Galectin-3 overexpression protects from cell damage and death by influencing mitochondrial homeostasis. *FEBS Lett* 2000;473:311–5.

74 Hughes RC. Galectins as modulators of cell adhesion. *Biochimie* 2001;83(7):667–76.

75 Silva-Monteiro E, Reis Lorenzato L, Kenji NIhei O *et al.* Altered expression of galectin-3 induces cortical thymocyte depletion and premature exit of immature thymocytes during *Trypanosoma cruzi* infection. *Am J Pathol* 2007;170:546–56.

76 Kuwabara I, Liu FT. Galectin-3 promotes adhesion of human neutrophils to laminin. *J Immunol* 1996;156:3939–44.

77 Sato S, Ouellet N, Pelletier I *et al.* Role of galectin-3 as an adhesion molecule for neutrophil extravasation during streptococcal pneumonia. *J Immunol* 2002;168:1813–22.

78 Swarte VV, Mebius RE, Joziasse DH, Van den Eijnden DH, Kraal G. Lymphocyte triggering via L-selectin leads to enhanced galectin-3-mediated binding to dendritic cells. *Eur J Immunol* 1998;28:2864–71.

79 Mina-Osorio P, Soto-Cruz I, Ortega E. A role for galectin-3 in CD13-mediated homotypic aggregation of monocytes. *Biochem Biophys Res Commun* 2007;353:605–10.

80 Kimata H. Enhancement of IgE production in B cells by neutrophils via galectin-3 in IgE-associated atopic eczema/dermatitis syndrome. *Int Arch Allergy Immunol* 2002;128:168–70.

81 Hsu DK, Hammes SR, Kuwabara I, Greene WC, Liu FT. Human T lymphotropic virus-1 infection of human T lymphocytes induces expression of the β-galactose-binding lectin, galectin-3. *Am J Pathol* 1996;148:1661–70.

82 Dong S, Hughes RC. Galectin-3 stimulates uptake of extracellular $Ca2^{+}$ in human Jurkat T-cells. *FEBS Lett* 1996;395:165–9.

83 Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. *Nature* 2001;409:733–79.

84 Frigeri LG, Zuberi RI, Liu FT. ϵBP, a β-galactoside-binding animal lectin, recognizes IgE receptor (FcϵRI) and activates mast cells. *Biochemistry* 1993;32:7644–9.

85 Liu FT, Hsu DK, Zuberi RI *et al.* Expression and function of galectin-3, a β-galactoside-binding lectin, in human monocytes and macrophages. *Am J Pathol* 1995;147:1016–29.

86 Jeng KCG, Frigeri LG, Liu FT. An endogenous lectin, galectin-3 (ϵBP/Mac-2), potentiates IL-1 production by human monocytes. *Immunol Lett* 1994;42:113–6.

87 Nieminen J, St-Pierre C, Sato S. Galectin-3 interacts with naive and primed neutrophils, inducing innate immune responses. *J Leukoc Biol* 2005;78:1127–35.

88 Sano H, Hsu DK, Yu L *et al.* Human galectin-3 is a novel chemoattractant for monocytes and macrophages. *J Immunol* 2000;165:2156–64.

89 Cortegano I, del Pozo V, Cardaba B *et al.* Galectin-3 down-regulates IL-5 gene expression on different cell types. *J Immunol* 1998;161:385–9.
90 Sano H, Hsu DK, Apgar JR *et al.* Critical role of galectin-3 in phagocytosis by macrophages. *J Clin Invest* 2003;112:389–97.
91 Chen HY, Sharma BB, Yu L *et al.* Role of galectin-3 in mast cell functions: galectin-3-deficient mast cells exhibit impaired mediator release and defective JNK expression. *J Immunol* 2006a;177:4991–7.
92 Bernardes ES, Silva NM, Ruas LP *et al. Toxoplasma gondii* infection reveals a novel regulatory role for galectin-3 in the interface of innate and adaptive immunity. *Am J Pathol* 2006; 168:1910–20.
93 Colnot C, Ripoche MA, Milon G *et al.* Maintenance of granulocyte numbers during acute peritonitis is defective in galectin-3-null mutant mice. *Immunology* 1998;94:290–6.
94 Zuberi RI, Hsu DK, Kalayci O *et al.* Critical role for galectin-3 in airway inflammation and bronchial hyperresponsiveness in a murine model of asthma. *Am J Pathol* 2004;165:2045–53.
95 del Pozo V, Rojo M, Rubio ML *et al.* Gene therapy with galectin-3 inhibits bronchial obstruction and inflammation in antigen-challenged rats through interleukin-5 gene downregulation. *Am J Respir Crit Care Med* 2002;166:732–7.
96 Lopez E, Del Pozo V, Miguel T *et al.* Inhibition of chronic airway inflammation and remodeling by galectin-3 gene therapy in a murine model. *J Immunol* 2006;176:1943–50.
97 Hrdlickova-Cela E, Plzak J, Smetana K *et al.* Detection of galectin-3 in tear fluid at disease states and immunohistochemical and lectin histochemical analysis in human corneal and conjunctival epithelium. *Br J Ophthalmol* 2001;85:1336–40.
98 Ohshima S, Kuchen S, Seemayer CA *et al.* Galectin 3 and its binding protein in rheumatoid arthritis. *Arthritis Rheum* 2003;48:2788–95.
99 Nachtigal M, Al-Assaad Z, Mayer EP, Kim K, Monsigny M. Galectin-3 expression in human atherosclerotic lesions. *Am J Pathol* 1998;152:1199–208.
100 Lim Y, Lee DY, Lee S *et al.* Identification of autoantibodies associated with systemic lupus erythematosus. *Biochem Biophys Res Commun* 2002;295:119–24.
101 Jensen-Jarolim E, Neumann C, Oberhuber G *et al.* Anti-galectin-3 IgG autoantibodies in patients with Crohn's disease characterized by means of phage display peptide libraries. *J Clin Immunol* 2001;21:348–56.
102 Mathews KP, Konstantinov KN, Kuwabara I *et al.* Evidence for IgG autoantibodies to galectin-3, a β-galactoside-binding lectin (Mac-2-binding protein, or carbohydrate binding protein 35) in human serum. *J Clin Immunol* 1995;15:329–37.
103 Suarez-Alvarez B, Garcia Suarez MM, Arguelles ME *et al.* Circulating IgG response to stromelysin-3, collagenase-3, galectin-3 and mesothelin in patients with pharynx/larynx squamous cell carcinoma. *Anticancer Res* 2001;21:3677–84.
104 Monney L, Sabatos CA, Gaglia JL *et al.* Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 2002;415:536–41.
105 Sabatos CA, Chakravarti S, Cha E *et al.* Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat Immunol* 2003;4:1102–10.
106 Zhu C, Anderson AC, Schubart A *et al.* The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* 2005;6:1245–52.
107 Wada J, Ota K, Kumar A, Wallner EI, Kanwar YS. Developmental regulation, expression, and apoptotic potential of galectin-9, a β-galactoside binding lectin. *J Clin Invest* 1997;99:2452–61.
108 Kashio Y, Nakamura K, Abedin MJ *et al.* Galectin-9 induces apoptosis through the calcium-calpain-caspase-1 pathway. *J Immunol* 2003;170:3631–6.
109 Chawla-Sarkar M, Leaman DW, Jacobs BS, Borden EC. Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis. *Apoptosis* 2003;8:237–49.
110 Chen Y, Langrish CL, McKenzie B *et al.* Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest* 2006b;116:1317–26.
111 Nishi N, Itoh A, Fujiyama A *et al.* Development of highly stable galectins: truncation of the linker peptide confers protease-resistance on tandem-repeat type galectins. *FEBS Lett* 2005;79:2058–64.
112 Matsumoto R, Matsumoto H, Seki M *et al.* Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes. *J Biol Chem* 1998;273:16976–84.
113 Tureci O, Schmitt H, Fadle N, Pfreundschuh M, Sahin U. Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease. *J Biol Chem* 1997;272:6416–22.
114 Matsushita N, Nishi N, Seki M *et al.* Requirement of divalent galactoside-binding activity of ecalectin/galectin-9 for eosinophil chemoattraction. *J Biol Chem* 2000;275:8355–60.
115 Matsumoto R, Hirashima M, Kita H, Gleich GJ. Biological activities of ecalectin: a novel eosinophil-activating factor. *J Immunol* 2002;168:1961–7.
116 Saita N, Goto E, Yamamoto T *et al.* Association of galectin-9 with eosinophil apoptosis. *Int Arch Allergy Immunol* 2002;128:42–50.
117 Yamamoto H, Kashio Y, Shoji H *et al.* Involvement of galectin-9 in guinea-pig allergic airway inflammation. *Int Arch Allergy Immunol* 2007 (in press).
118 Katoh S, Nobumoto A, Ishii N *et al.* Galectin-9 inhibits CD44-hyaluronan interaction and suppresses a murine model of allergic asthma. *Am J Respir Crit Care Med* 2007 (in press).
119 Chabot S, Kashio Y, Seki M *et al.* Regulation of galectin-9 expression and release in Jurkat T cell line cells. *Glycobiology* 2002;12:111–8.
120 Tsuboi Y, Abe H, Nakagawa R *et al.* Galectin-9 protects mice from Shwartzman reaction by attracting prostaglandin E_2-producing polymorphonuclear leukocytes. *Clin Immunol* 2007 (in press).
121 Asakura H, Kashio Y, Nakamura K *et al.* Selective eosinophil adhesion to fibroblast via IFN-γ-induced galectin-9. *J Immunol* 2002;169:5912–8.
122 Kasamatsu A, Uzawa K, Shimada K *et al.* Elevation of galectin-9 as an inflammatory response in the periodontal ligament cells exposed to *Porphylomonas gingivalis* lipopolysaccharide *in vitro* and *in vivo*. *Int J Biochem Cell Biol* 2005a;37:397–408.
123 Pelletier I, Hashidate T, Urashima T *et al.* Specific recognition of *Leishmania major* poly-beta-galactosyl epitopes by galectin-9: possible implication of galectin-9 in interaction between *L. major* and host cells. *J Biol Chem* 2003;278:22223–30.
124 Warke RV, Xhaja K, Martin KJ *et al.* Dengue virus induces novel changes in gene expression of human umbilical vein endothelial cells. *J Virol* 2004;78:4947–8.
125 Ishikawa A, Imaizumi T, Yoshida H *et al.* Double-stranded RNA enhances the expression of galectin-9 in vascular endothelial cells. *Immunol Cell Biol* 2004;82:410–4.
126 Imaizumi T, Yoshida H, Nishi N *et al.* Double-stranded RNA induces galectin-9 in vascular endothelial cells: involvement of TLR3, PI3 K and IRF3 pathway. *Glycobiology* 2007 (in press).
127 Lahm H, Andre S, Hoeflich A *et al.* Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. *J Cancer Res Clin Oncol* 2001;127:375–86.
128 Lahm H, Andre S, Hoeflich A *et al.* Tumor galectinology: insights into the complex network of a family of endogenous lectins. *Glycoconj J* 2004;20:227–38.

129 Kasamatsu A, Uzawa K, Nakashima D *et al.* Galectin-9 as a regulator of cellular adhesion in human oral squamous cell carcinoma cell lines. *Int J Mol Med* 2005b;16:269–73.
130 Kageshita T, Kashio Y, Yamauchi A *et al.* Possible role of galectin-9 in cell aggregation and apoptosis of human melanoma cell lines and its clinical significance. *Int J Cancer* 2002;99:809–16.
131 Irie A, Yamauchi A, Kontani K *et al.* Galectin-9 as a prognostic factor with antimetastatic potential in breast cancer. *Clin Cancer Res* 2005;11:2962–8.
132 Yamauchi A, Kontani K, Kihara M *et al.* Galectin-9, a novel prognostic factor with antimetastatic potential in breast cancer. *Breast J* 2006;12:S196–200.
133 Pioche-Durieu C, Keryer C, Souquere S *et al.* In nasopharyngeal carcinoma cells, Epstein–Barr virus LMP1 interacts with galectin 9 in membrane raft elements resistant to simvastatin. *J Virol* 2005;79:13326–37.
134 Keryer-Bibens C, Pioche-Durieu C, Villemant C *et al.* Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galectin 9. *BMC Cancer* 2006;6:283.
135 Okudaira T, Hirashima M, Ishikawa C *et al.* A modified version of galectin-9 suppresses cell growth and induces apoptosis of human T-cell leukemia virus type I-infected T-cell lines. *Int J Cancer* 2007;120:2251–61.
136 Dai SY, Nakagawa R, Itoh A *et al.* Galectin-9 induces maturation of human monocyte-derived dendritic cells. *J Immunol* 2005;75:2974–81.
137 Glinsky GV, Price JE, Glinsky VV *et al.* Inhibition of human breast cancer metastasis in nude mice by synthetic glycoamines. *Cancer Res* 1996;56:5319–24.
138 Rabinovich GA, Cumashi A, Bianco GA *et al.* Synthetic lactulose amines: novel class of anticancer agents that induce tumor-cell apoptosis and inhibit galectin-mediated homotypic cell aggregation and endothelial cell morphogenesis. *Glycobiology* 2006;16:210–20.
139 Nangia-Makker P, Hogan V, Honjo Y *et al.* Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *J Natl Cancer Inst* 2002;94:854–62.
140 Andre S, Pieters RJ, Vrasidas I *et al.* Wedgelike glycodendrimers as inhibitors of binding of mammalian galectins to glycoproteins, lactose, maxiclusters, and cell surface glycoconjugates. *Chembiochem* 2001;2:822–30.
141 Sorme P, Kahl-Knutsson B, Wellmar U *et al.* Design and synthesis of galectin inhibitors. *Methods Enzymol* 2003;363:157–69.
142 Tejler J, Leffler H, Nilsson UJ. Synthesis of O-galactosyl aldoximes as potent LacNAc-mimetic galectin-3 inhibitors. *Bioorg Med Chem Lett* 2005;15:2343–5.
143 Ingrassia L, Camby I, Lefranc F *et al.* Anti-galectin compounds as potential anti-cancer drugs. *Curr Med Chem* 2006;13:3513–27.