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Review article

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The role of galectins in immunity and infection

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Abstract

The galectin family consists of carbohydrate (glycan) binding proteins that are expressed by a wide variety of cells and bind to galactose-containing glycans. Galectins can be located in the nucleus or the cytoplasm, or can be secreted into the extracellular space. They can modulate innate and adaptive immune cells by binding to glycans on the surface of immune cells or intracellularly via carbohydrate-dependent or carbohydrate-independent interactions. Galectins expressed by immune cells can also participate in host responses to infection by directly binding to microorganisms or by modulating antimicrobial functions such as autophagy. Here we explore the diverse ways in which galectins have been shown to impact immunity and discuss the opportunities and challenges in the field.

Sections

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Introduction

Canonical pathways of immune regulation involve ligand–receptor interactions that transmit predicable signalling outcomes[1]. The discrete nature of such ligand–receptor pairs, including the specific signalling pathways they engage, lends well to genetic and pharmacological approaches to define their biological functions. However, another level of essential immune regulation involves interactions of greater complexity. Mammals express various carbohydrate (glycan) binding proteins (GBPs) that recognize glycosylated proteins and glycosylated lipids (glycoconjugates). The targeted modifications on these glycoconjugates are highly variable[2]. Glycan modifications cannot be predicted based on the amino acid sequence of a given glycoprotein but, instead, are dictated by the repertoire of glycosyltransferases and substrates responsible for their synthesis[3]. As a result, the same protein (or lipid) can be decorated by different carbohydrate modifications depending on the type, differentiation and overall activation state of a given cell[3]. In this way, the nature and abundance of glycans of a given glycoconjugate, not protein or lipid levels alone, can dictate how well a given GBP binds. As different glycoconjugates may express the same glycan structure, different proteins or lipids on a cell surface may be engaged by a given GBP, allowing GBPs to potentially modulate or activate various distinct receptors. Thus, the role of GBPs in immune signalling and regulation fundamentally differs from other immune regulators, such as cytokine-receptor pathways.

Galectins were the first family of GBPs for which immune regulatory activity was described and they represent the most ancient arm of mammalian GBP evolution[4,5]. In vertebrates, more than 16 galectins have been characterized (galectin-1–galectin-16), and are often classified according to their overall structure into prototype galectins, chimaera-type galectins and tandem-repeat galectins[6] (Fig. 1). Galectin-1 and -3 are expressed in nearly every tissue and by most cell types examined[7,8], whereas other galectins appear to have a more restricted expression profile: galectin-7 is mainly expressed by stratified epithelial cells; galectin-9 is mainly expressed by gastrointestinal epithelial cells, the thymus and endothelial cells; and galectin-12 is mainly expressed by adipocytes[9–12].

Galectins can be found in the extracellular space where they are able to bind a broad range of glycoconjugates[13,14], including those located on the cell surface and extracellular matrix. In addition, galectins are unique amongst GBPs in that they can regulate diverse processes intracellularly, which can occur through glycan-independent or glycan-dependent interactions[14]. Given that galectins are expressed in many tissues, coupled with their ability to regulate

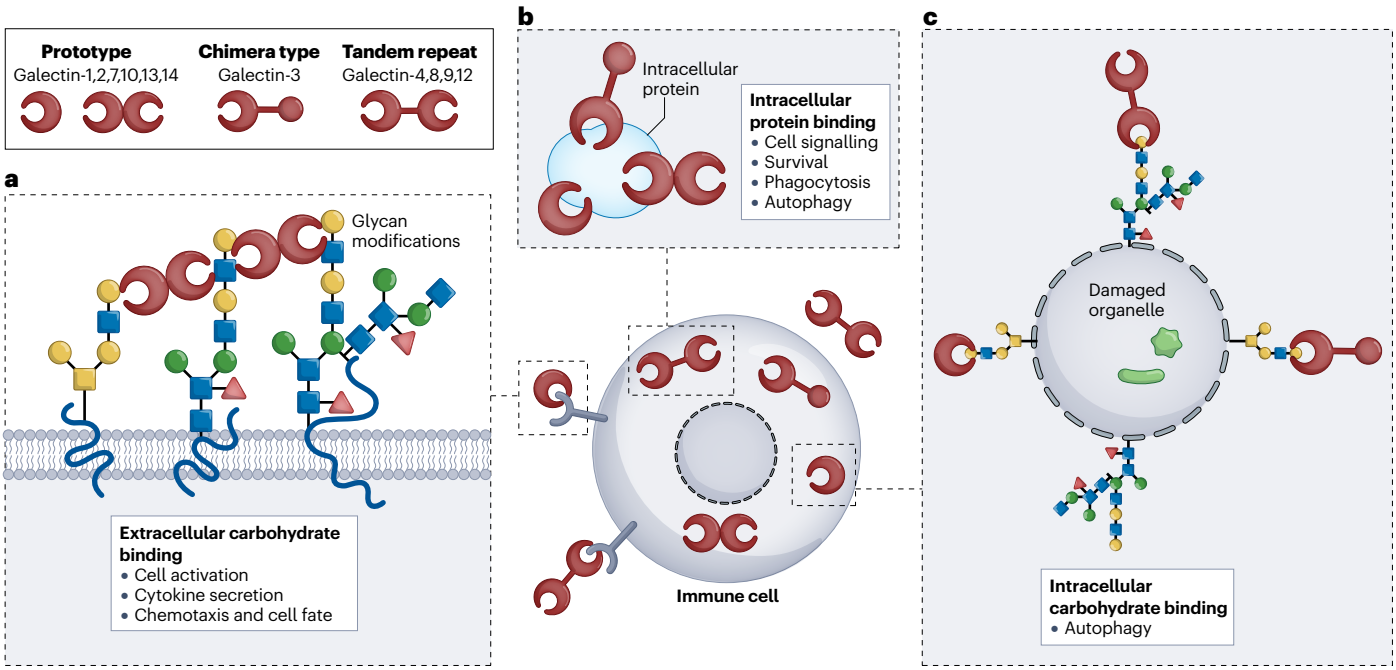

Fig. 1 | Galectins are unique regulators of host immunity. Galectins can be classified into prototypical galectins, chimaera-type galectins and tandem-repeat galectins according to their overall structural features. Prototypical galectins exist in a monomer–dimer equilibrium with the extent of dimerization at steady state differing for each individual galectin. **a**, Extracellular galectins can engage common glycan motifs that can be present on many different glycoproteins and glycolipids, which are expressed on the surface of most cells. As such, galectins can bind a wide variety of glycoprotein and glycolipid receptors, which can result in distinct signalling outcomes depending on the different types of receptors engaged. Importantly, the glycan modifications of cell surface proteins can change following cellular activation and differentiation. This can result in galectin engagement of completely different glycoprotein or glycolipid targets and, therefore, different signalling outcomes. In immune cells (both innate immune cells such as macrophages and dendritic cells, and adaptive immune cells such as T cells and B cells), such interactions can affect cell activation, cytokine secretion, chemotaxis and cell fate. **b**, Galectins are synthesized and are either secreted or reside in the cytosol, due to their lack of a classical signal sequence, but can also shuttle to the nucleus. Cytosolic galectins can impact cellular signalling by directly binding intracellular targets such as BCL-2 and ALG2-interacting protein X (ALIX) (Supplementary Table 3) through carbohydrate-independent interactions. This can affect cell signalling, survival, phagocytosis and autophagy. **c**, Intracellular galectins can also bind exposed glycans following organelle damage, inducing autophagy (shown in detail in Fig. 4).

immune activities through diverse intracellular and extracellular processes, galectin-mediated immune regulation shows a high level of complexity.

Here, we highlight the diverse mechanisms by which galectins can regulate adaptive and innate immune responses. We first describe the general characteristics of galectins and explore their roles in regulating adaptive immunity, in particular T cell biology, and in regulating innate immunity, including macrophage activation, immune cell recruitment and phagocytosis. We also discuss the diverse ways in which galectins may influence the consequence of pathogen exposure and highlight the recent discovery that cytosolic galectins can function as sensors of organelle injury. It should be noted that galectins have also been implicated in modulating the tumour microenvironment through altering vascular biology and by engaging the immune system, which has been discussed in detail elsewhere[15–19].

General characteristics of galectins

Nearly all mammalian members of the galectin family bind predominantly to a common glycan modification, galactose β1, 4-*N*-acetylglucosamine (LacNAc), which is present on different glycoproteins on the surface of nearly every cell[20–22]. Similar to other GBPs, galectins are present in the extracellular space, where they can bind to glycosylated cell surface receptors and, thereby, modulate the behaviour of many different types of cell. However, many galectins have been shown to bind to a large number of different cell surface glycoproteins (Supplementary Table 1), which makes it very challenging to study these proteins using approaches commonly employed to study the classical one protein–one receptor paradigm. In addition, LacNAc modifications, which can change depending on the differentiation and activation state of a given cell, can modulate different galectin–glycan interactions, potentially altering the intracellular signalling pathways that are activated following galectin exposure (Supplementary Table 2). In this way, the impact of extracellular galectins on a given receptor can change depending on the glycan signature of the receptor.

Unlike other GBPs, galectins also reside intracellularly and, indeed, represent the only protein family with known intracellular carbohydrate-binding activity. The presence of galectins in the cytosol was initially intriguing, considering that their ligands are carbohydrates that are synthesized and exocytosed through the endoplasmic reticulum–Golgi pathway and are primarily found in the extracellular environment (Box 1). However, it has since been shown that galectins can also interact with intracellular proteins through glycan-independent mechanisms[2] (Fig. 1). Different galectins appear to engage distinct sets of intracellular partners, which is consistent with the fact that galectins have only a moderate degree of homology (approximately 30–40% sequence identity among most members). Galectin-3, in particular, has a unique amino-terminal non-lectin domain that can interact with intracellular proteins. Moreover, it was recently discovered that intracellular galectins can interact with glycans that are exposed to the cytosol after endolysosomal damage. Here, the binding of galectins allows for the formation of 'signalling platforms' that can modulate numerous cellular processes, including autophagy.

In contrast to most other immune regulators, many galectins are constitutively expressed in a wide range of tissues. However, each galectin can exhibit unique tissue expression patterns, and tissue differentiation, injury and inflammation can enhance galectin expression. The mechanism by which galectins are secreted from cells include an as yet incompletely defined, endoplasmic reticulum–Golgi-independent pathway following cell activation, differentiation or injury[23–26]. As galectins bind to glycan ligands that are widely found on most cells, key regulatory mechanisms have evolved that limit galectin activity (both spatially and temporally) following their release into the extracellular space. For example, some galectin family members are sensitive to oxidative inactivation or proteolytic degradation following exposure to the extracellular environment[6,27–31].

It is important to note that these properties have significant implications for the design and interpretation of experiments that interrogate the activity of galectins. This is especially true for in vivo models where galectins are systemically administered, as well as in vitro experiments where cells are incubated with galectins (Box 2). Thus, although galectins have been implicated in a wide variety of immune pathways, investigations to define exactly how they influence immune activity within an organism can be challenging.

Here, we primarily focus our discussion on studies that addressed the function of endogenous galectins in experiments in which specific galectins were either inhibited or genetically deleted. We have also included select studies in which the function of galectins were demonstrated in vitro using galectins administered to the cell culture, but describe the limitations of these studies in Box 2.

Box 1

Compartmentalization of cellular glycoproteins and glycolipids

Glycoproteins are initially generated by co-translational insertion of a growing polypeptide through the translocon of the endoplasmic reticulum, where the transplanted protein is secreted into the endoplasmic reticulum lumen or becomes inserted into the membrane. During this process, N-glycosylation (attachment of glycans to asparagine residues) is initiated by the addition of a preformed glycan. This glycan assists in protein folding within the endoplasmic reticulum lumen and is further modified before glycoprotein transport to the Golgi apparatus. Once in the Golgi apparatus, O-glycosylation (attachment of glycans to serine or threonine resides) is initiated in a stepwise fashion. Both O-glycans and N-glycans on the protein surface can be extended and further modified by additional glycosyltransferases. The types of modifications are dictated by the repertoire of glycosyltransferases and available donor nucleotide sugars. In each of these pathways, glycoproteins are not normally accessible to intracellular galectins. As protein glycosylation matures through the Golgi apparatus, glycoproteins are finally released from the cell or take up residence on the cell surface. Selected populations of glycoproteins can serve as glycan ligands for extracellular galectins. Glycolipids can also serve as glycan ligands for galectins[1,2]. Through distinct oligomeric quaternary organization, galectins — in particular galectin-3 — when bound to cell surface glycoconjugates, can regulate endocytosis, receptor activation, lattice formation and the secretion of glycoproteins[145,154–158].

Box 2

Special considerations when examining galectin function

A common approach to study the functions of galectins has been through the use of recombinant galectins that are added to cell cultures in vitro, followed by the evaluation of various cellular outcomes. Beyond the general concerns regarding the possibility of super-physiological concentrations of added agents for any in vitro assay, discerning whether effects observed in vitro actually occur in vivo can be particularly challenging with galectins. This is especially important when considering that galectins can engage many different cells by virtue of common galactose β1,4-*N*-acetylglucosamine (LacNAc)-containing glycans on cell surfaces (Supplementary Table 1). As a result, cognate receptor levels alone rarely dictate a given cell's sensitivity to a particular galectin. Instead, key cellular programmes that govern the extracellular bioavailability of a given galectin appear to regulate cell exposure to galectins. Thus, interactions of particular galectins with specific cell types that are observed in vitro need to be verified in vivo. This can be especially challenging when considering that the actual concentration and activity of individual galectins inside and outside cells, within distinct tissues and in the context of different disease states, is rarely known.

Similar caveats need to be considered in studies where recombinant galectins are injected into experimental animals. High concentrations of galectins following injection may impact immune cells at the injection site that do not normally encounter galectins, and these affected cells may, ultimately, be responsible for driving an observed outcome. Thus, although galectin injection into a galectin knockout animal may appear to rescue a phenotype, whether the results obtained using this approach truly reflect the restoration of endogenous galectin activity in a knockout recipient often remains difficult to define. In addition, although endogenous galectins can be released into the extracellular space, these proteins are also involved in many intracellular processes. As a result, galectins can regulate immune cell activity through the engagement of dynamic extracellular glycan ligands, as well as interacting with a multitude of intracellular targets. Consequently, the regulation of immune cells by galectins is likely significantly more complex when compared with immune regulators that activate discrete signalling networks. Unfortunately, the ability to distinguish clearly whether a phenotype observed in a galectin-deficient animal is entirely a reflection of an intracellular activity, an extracellular pathway or both is not currently possible. These challenges have made it difficult to pinpoint the exact mechanisms responsible for the activity of a given galectin, despite compelling phenotypes that continue to extend from numerous experimental approaches.

Regulation of adaptive immunity

The earliest and most extensive studies demonstrating that galectins regulate immune activity primarily focused on T cells. Key findings include the ability of galectins to modulate processes in the development and contraction of an adaptive immune response such as T cell activation and apoptosis (Fig. 2). These studies have implicated an anti-inflammatory role for many galectins in nearly every aspect of adaptive immune responses, from activation to resolution, and thereby provide a possible negative feedback mechanism in the setting of autoimmunity through various distinct extracellular and intracellular mechanisms.

T cell activation

Recombinant galectins have been shown to engage a range of glycoproteins that are involved in orchestrating T cell activation, including CD2, CD7, CD8, CD43, CD45 and the T cell receptor (TCR)[32]. This can result in their reorganization and alter the half-life of surface receptors, as well as changes in membrane structure, which may impact signalling microdomains and influence T cell activation[33,34].

Early studies of the role of galectins in T cell function examined the impact of β1,6-*N*-acetylglucosaminyltransferase V (MGAT5), which is responsible for generating branched N-glycans bearing polylactosamine structures commonly recognized by galectins[21,35,36]. In the mouse experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, *Mgat5* knockout mice showed higher levels of autoimmunity and enhanced TCR signalling[36]. In vitro incubation of wild type T cells with lactose, which removes galectin-3 from the cell surface, resulted in the relocalization of key signalling constituents to the immunological synapse and in enhanced signalling outcomes[36]. This suggests that altered galectin binding on the surface of T cells in *Mgat5* knockout mice may account for the observed T cell phenotype.

More recently, the anti-inflammatory cytokine IL-10 was shown to upregulate the expression of *Mgat5* in T cells. The authors demonstrated that MGAT5 enhances the glycosylation of the TCR, and that increased binding of extracellular galectin-3 to the TCR affects the co-localization of the TCR and CD8, thereby increasing the threshold for T cell activation[37]. Importantly, unlike in wild-type mice, IL-10-mediated upregulation of MGAT5 expression in galectin-3 knockout mice did not lower the activation threshold of $CD8^+$ T cells[37], supporting a role for galectin-3 in elevating the threshold for TCR signalling.

Intracellular galectin-3 has been shown to translocate to the cytosolic side of the immunological synapse in $CD4^+$ T cells upon activation, where it downregulates the surface expression of key components of the TCR, thereby limiting T cell activation[38]. Although additional studies are needed to define the mechanisms responsible for this outcome, intracellular galectin-3 may accomplish this through non-carbohydrate-dependent intracellular interactions with the multifunctional adapter protein ALG2-interacting protein X (ALIX)[38,39]. Intracellular galectin-3 was also found to be localized in the immunological synapse in both naive and memory $CD8^+$ T cells when activated by γ-herpesvirus (MHV68)[40]. Galectin-3 knockout mice mount a stronger MHV68-specific $CD8^+$ T cell response compared with wild-type mice[40], indicating that intracellular galectin-3 can upregulate the threshold for T cell activation. Overall, these studies suggest that both extracellular and intracellular galectin-3 can dampen T cell activation by altering the distribution and half-life of distinct cell surface glycoproteins.

T cell differentiation

Similar to galectin-3, intracellular galectin-9 is recruited to the cytosolic side of the immune synapse upon T cell activation[41]. Enhanced expression of galectin-9 is associated with an increased production of T cell cytokines, including IL-17, following T cell activation[41]. Galectin-9 knockout mice exhibit reduced IgA production following oral antigen exposure, which correlated with a reduced number of T helper 17 cells (T_H17 cells) and lower levels of IL-17 production[42]. As exposure to an IL-17 blocking antibody can reduce IgA production, these results suggest that galectin-9 may increase IgA production by promoting the differentiation of $CD4^+$ T cells into T_H17 cells[42]. Moreover, galectins can affect T cell activation and differentiation through the modulation of antigen-presenting cells (APCs), as described in the discussion of innate immune cells.

T cell contraction

Following the initial activation and expansion of T cells in response to pathogen challenge, a contraction of the T cell population occurs[43]. The earliest and most informative studies that examined the direct interactions of galectins with T cells implicated galectin-1 in the process of T cell contraction. Here, the incubation of activated T cells with

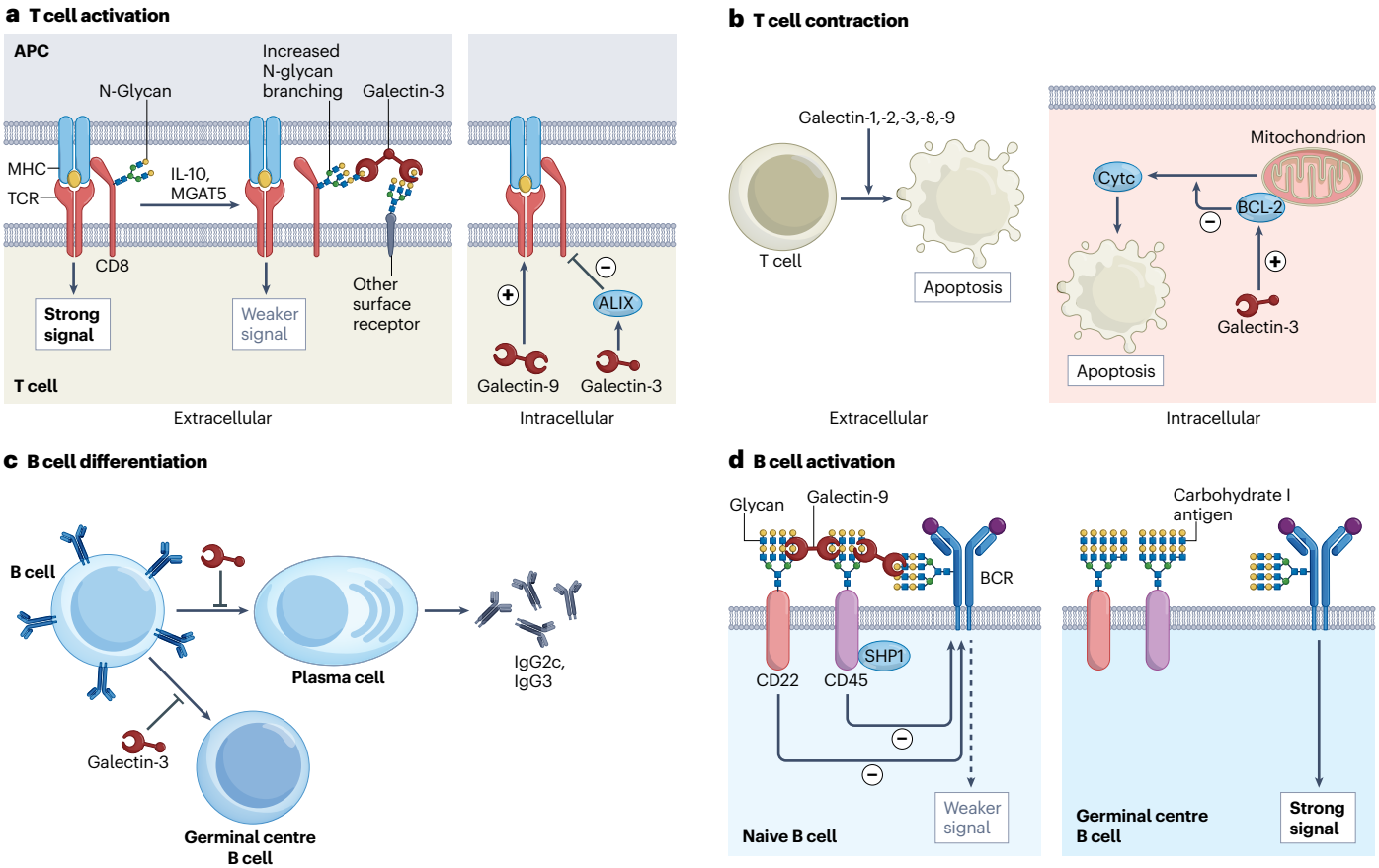

Fig. 2 | Regulation of adaptive immunity by galectins. Galectins can modulate adaptive immune cells through both intracellular and extracellular mechanisms and via their effects on antigen-presenting cells (APCs) and other innate immune cells. **a**, Extracellular galectin-3 can modulate T cell activation through engagement of extracellular glycans on co-receptors such as CD8 and other cell surface glycoconjugates, which restricts CD8 interactions with the T cell receptor (TCR) and, ultimately, MHC class I at the immmunological synapse. This, in turn, weakens TCR signalling. Galectin-3-mediated restriction of CD8 in this manner is mediated by β1,6-*N*-acetylglucosaminyltransferase V (MGAT5), an enzyme that is induced by IL-10 and catalyses the formation of branched N-glycans bearing polylactosamine structures that are common galectin ligands[37]. Intracellular galectin-3 can upregulate the threshold for TCR activation by binding the adaptor protein ALG2-interacting protein X (ALIX)[38], whereas galectin-9 can have the opposite effect on the TCR through engagement of intracellular partners that remain to be described[41]. **b**, Galectin-1, -8 or -9 knockout mice appear to have a defect in T cell contraction after challenge, and extracellular administration of galectin-1, -2, -3, -8 and -9 has been shown to induce apoptosis in T cells in vitro[45–48]. However, whether and how these galectins regulate T cell fate in vivo remains to be elucidated, especially in view of the issues described in Box 2. In addition, intracellular galectin-3 can inhibit T cell apoptosis through interactions with the anti-apoptotic protein BCL-2 (refs. [49,50]). **c**, Galectins have also been shown to regulate B cell differentiation through extracellular and possibly intracellular processes. For example, galectin-3 knockout B cells display an enhanced proclivity to differentiate into germinal centre B cells and plasma cells that produce IgG2c or IgG3, all of which may enhance the probability of antibody-mediated autoimmunity[59]. Galectin-3 expressed by B cells can suppress B cell differentiation, probably via intracellular mechanisms[59]. **d**, On naive B cells, galectin-9 can recruit glycosylated forms of the transmembrane proteins CD22 and CD45 to the B cell receptor (BCR). CD45 is bound by the phosphatase SHP1, which downregulates BCR signalling[55,56]. In contrast, germinal centre B cells express the carbohydrate I antigen which reduces the ability of galectin-9 to facilitate CD22 and CD45-mediated inhibition of B cell signalling and enhances B cell responses following antigen engagement.

galectin-1 in vitro resulted in apoptosis[44]. This activity does not appear to be limited to galectin-1, as similar in vitro studies suggested that extracellular galectin-2, -3, -8 and -9 may exert an immunosuppressive function by directly inducing apoptosis in T cells[45–48]. Additional studies indicate that intracellular galectin-3 can also inhibit T cell apoptosis in a cell-intrinsic manner[49,50] and galectin-3 was shown to have sequence homology with and bind to the anti-apoptosis protein BCL-2 (ref. [50]).

In the EAE mouse model of multiple sclerosis, systemic administration of galectin-1 and -9 inhibits autoimmunity, which likely reflects the ability of these galectins to induce apoptosis of autoreactive T cells[44]. However, as outlined in Box 2, there are caveats to interpreting galectin activity in animal studies where galectins are systemically administered. Nevertheless, these findings were strengthened in experiments with knockout mice where the deletion of specific galectins exacerbated disease in models of autoimmune conditions[45–47,51]. For example, galectin-1, -8 or -9 knockouts have an exaggerated clinical score and increased numbers of T_H1 cells and T_H17 cells in the EAE model[45]. Of note, increases in the number of $CD8^+$ T cells have also been observed in galectin-9 knockout mice following challenge with herpes simplex virus (HSV)[52], suggesting that the regulation of T cell fate is not limited to $CD4^+$ T cells or autoimmunity. Together, these studies suggest that several different types of galectins have a role in the contraction of T cell populations and raises the possibility that systemic administration of galectins may be of therapeutic value in autoimmune diseases.

B cells

The various mechanisms by which galectins can modulate T cells would also be expected to impact T cell-dependent B cell responses. As described above, galectin-9 appears to enhance the production of IgA by plasma cells by promoting the differentiation of T_H17 cells[42]. In contrast, intracellular galectin-3 appears to impair the differentiation of B cells into IgA-producing plasma cells, as it was shown that peritoneal B1 cells from galectin-3 knockout mice exhibited enhanced differentiation to IgA-producing plasma cells when treated with IL-5 plus TGFβ1 in vitro[53]. Moreover, there are indications that extracellular galectins may also directly engage B cell surface glycans and affect B cell signalling, activation and differentiation into antibody secreting cells[54]. For example, endogenous galectin-9 can be found on the surface of primary naive B cells, where it appears to regulate microdomain clustering, particularly of CD22 and CD45. This can suppress B cell receptor (BCR) signalling through CD45-mediated recruitment of SHP1 (refs. [55,56]), resulting in an inhibition of B cell activation and their subsequent differentiation into plasma cells[57]. Likewise, changes in the glycosylation of surface proteins of B cells can impact the binding of extracellular galectin-1 and galectin-8 and affect B cell differentiation. For example, it was shown that functional impairment of both galectin-1 and galectin-8 through knockout, knockdown or the use of inhibitors can reduce B cell differentiation into plasma cells in vitro[58]. Galectin-3 can also attenuate germinal centre B cell development through the suppression of IFNγ production and T follicular helper cell differentiation, as shown in a mouse model of lupus[59]. Galectin-3 knockout mice exhibited a higher number of germinal centre B cells, increased levels of autoantibodies and a propensity to develop lupus-like autoimmune disease. This is likely mediated through a B cell intrinsic processs[59], as demonstrated by adoptive transfer experiments.

Regulation of innate immunity

As galectins are expressed and potentially released by a wide range of cells, innate immune cells may encounter them at various stages of an immune response, and various innate immune cells are known to express galectins. Accordingly, a large number of studies have shown that galectins can regulate various aspects of innate immunity (reviewed elsewhere[31,60–64]) and contribute to diverse immune and inflammatory responses (Fig. 3). Some galectins promote these responses, whereas others have suppressive roles; for a given galectin, this may depend on the experimental conditions or models used. Additionally, numerous studies have shown that galectins can also function as intracellular regulators in innate immune cells and that many intracellular galectin activities are independent of carbohydrate binding (Supplementary Table 3).

Overall, these studies point to the differential regulation of common intracellular pathways in macrophages and other immune cells by different galectins. The potential role of intracellular versus extracellular galectins in this context continues to be studied. Galectin-3, which is released following tissue injury, has been proposed to act as an extracellular damage-associated molecular pattern that induces innate immune cell activation[65]. However, most studies in this context have the caveat of relying on recombinant (rather than endogenous) galectin-3 for the assessment of its activity.

Regulation of APCs

In addition to directly affecting T cell signalling, galectins can inhibit or augment the activation of APCs and their cytokine secretion, which, in turn, can impact T cell activation and differentiation. This was first observed following allogeneic mating of galectin-1 knockout mice, which led to an increased rate of spontaneous abortions, consistent with impaired fetal–maternal tolerance[66]. Galectin-1 was also shown to enhance the ability of tolerogenic dendritic cells to suppress autoimmunity in the EAE mouse model of multiple sclerosis, where the adoptive transfer of dendritic cells isolated from wild-type mice, but not galectin-1 knockout mice, reduced EAE progression. This occurred even when transferred after the initial disease onset, suggesting that galectin-1 can facilitate tolerance through a dendritic cell-mediated process in this setting[67]. Similar observations were made in a mouse model of the autoimmune condition Sjögren syndrome[68].

Two studies using bone marrow-derived dendritic cells from galectin-3 knockout mice concluded that galectin-3 suppresses the production of various cytokines, including IL-10 and IL-23, in dendritic cells[69,70]. This activity may explain how galectin-3 can suppress T_H2-type and T_H17-type polarization in $CD4^+$ T cells in experiments in which these were co-cultured with dendritic cells[40,70]. Another study using galectin-3 knockout mice indicated that galectin-3 suppresses Notch signalling upon stimulation with the Notch ligand Jagged1 (JAG1), which attenuates bone marrow-derived dendritic cell IL12p40 production[71]. In vitro experiments showed that knockdown of galectin-1 or galectin-3 in dendritic cells increases allogenic T cell responses[72], implying that these galectins dampen such responses. However, galectin-3 has also been shown to enhance APC-mediated T cell activation in some settings. For example, in a model of ConA-induced hepatitis, galectin-3 knockout mice had attenuated T cell responses, which was linked to a reduced production of IL-12 and enhanced production of IL-10 by dendritic cells and macrophages[73]. Such apparent discrepancies in the role of galectin-3 during APC-mediated T cell activation or suppression have not yet been resolved.

Macrophage activation

Various stimuli can trigger the activation of macrophages via extracellular and intracellular mediators. Among the intracellular mediators, the inflammasome is critical for the secretion of the pro-inflammatory cytokines IL-1β and IL-18. Studies indicate that galectin-3 can affect

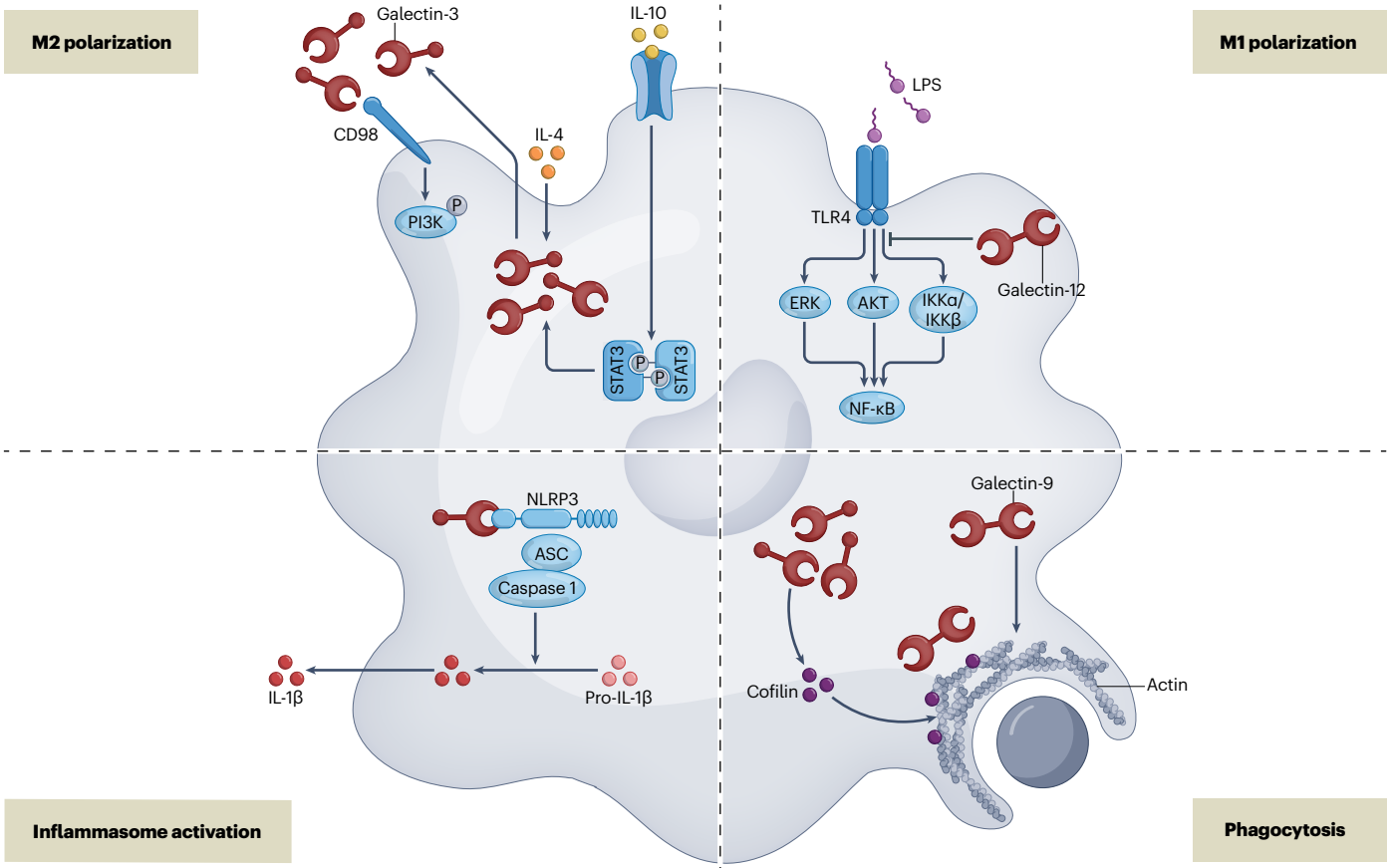

Fig. 3 | Regulation of macrophages by extracellular and intracellular galectins. Several different galectins have been shown to affect macrophage polarization, phagocytosis and inflammasome activation. For example, galectin-3 was shown to be induced in cardiac macrophages in models of myocardial infarction where it promoted their polarization into M2-like macrophages, which contribute to tissue repair by promoting fibrosis and clearance of apoptotic cells after myocardial infarction[79]. Here, galectin-3 expression was induced via IL-10-mediated activation of the transcription factor STAT3. In vitro experiments have also shown that IL-4 induces galectin-3 expression and galectin-3 facilitates M2-like macrophage polarization by binding CD98, which induces PI3K (ref. [78]). By contrast, galectin-12 (which is primarily located intracellularly and expressed by adipocytes, where it has a key role in adipogenesis[12]) is also expressed by myeloid cells[80], where it was shown to be a positive regulator of M1 macrophage polarization in response to treatment with lipopolysaccharide (LPS)[81]. This was related to decreased activation of IKKα/β, AKT and ERK downstream of Toll-like receptor 4 (TLR4) which results in decreased activation of NF-κB, resulting in M1 polarization. Intracellular galectin-3 is also a positive regulator of the NLRP3 inflammasome[75,76] and was shown to physically associate with NLRP3 in isolated hepatic macrophages, promoting IL-1β production[75]. Moreover, intracellular galectin-3 is a positive regulator of macrophage phagocytosis. In vitro experiments showed that it is localized at the cytosolic side of phagosomes where it is involved in actin rearrangement[82]. Similar observations were made in cultured primary microglia[83]. Additional studies suggest that galectin-3 controls the activation state of cofilin, which affects actin filament organization and stability[83]. Intracellular galectin-9 has also been shown to enhance phagocytosis through the regulation of actin filament formation[84].

inflammasome activation. For example, in a model of dextran sulfate sodium (DSS)-induced colitis, galectin-3 knockout mice developed less severe colitis than wild-type mice, and the authors demonstrated that endogenous galectin-3 can promote NLRP3 inflammasome activation within macrophages[74]. In another study using isolated hepatic macrophages, a physical association between galectin-3 and NLRP3 was demonstrated[75]. Also, in a model of biliary disease, galectin-3 knockout mice exhibited reduced disease severity along with attenuated T_H17-type immune responses in the liver[76]. Here, the deficiency in galectin-3 resulted in lower NLRP3 inflammasome expression and a lower amount of IL-1β in macrophages[76]. These studies indicate that galectin-3 can induce both NLRP3 inflammasome expression and activation in macrophages, and thereby enhance their inflammatory activity.

Similar to galectin-3, galectin-9 also interacts intracellularly with NLRP3, but with opposite effects. In primary peritoneal macrophages and HEK293T cells, galectin-9 was shown to promote the formation of a complex between NLRP3 and the autophagy receptor p62, thereby facilitating the autophagic degradation of NLRP3 (ref. [77]). Galectin-9 knockout mice exhibited enhanced NLRP3 inflammasome activation and NLRP3-dependent inflammation[77].

Macrophage polarization

Under the influence of various extracellular stimuli, macrophages can be differentiated in vitro into pro-inflammatory M1-like macrophages (when 'classically' activated with IFNγ and lipopolysaccharide (LPS)) or anti-inflammatory M2-like macrophages (when 'alternatively' activated

with IL-4 or IL-10). Experiments with bone marrow-derived macrophages from galectin-3 knockout, siRNA-mediated galectin-3 knockdown and pharmacologic inhibition of galectin-3 revealed that galectin-3 facilitates the IL-4-induced activation of M2-like macrophages[78]. Moreover, galectin-3 was shown to be expressed by cardiac macrophages in animal models of myocardial infarction, where it promoted their polarization into M2-like macrophages. These contribute to tissue repair by promoting fibrosis and clearance of apoptotic cells after myocardial infarction[79]. In this system, the expression of galectin-3 was induced by IL-10, which activates the transcription factor STAT3. In contrast, galectin-12, which is primarily expressed by adipocytes and plays a key role in adipogenesis[12], but is also expressed by myeloid cells[80], was shown to be a positive regulator of M1-like macrophage polarization in response to treatment with LPS[81]. This was linked to its enhancement of IKKα/β, AKT and ERK activation, which results in increased activation of NF-κB and AP1, favouring M1-like macrophage polarization[81].

Macrophage phagocytosis

Various cell surface receptors facilitate phagocytosis, and intracellular galectins appear to have a role in this process. For example, it was shown that peritoneal macrophages isolated from galectin-3 knockout mice exhibited a lower capacity to phagocytose IgG-opsonized erythrocytes when compared with wild type cells[82]. Here, intracellular galectin-3 was found to localize to the cytosolic side of the erythrocyte-containing phagosomes. It appeared to be involved in actin rearrangement, given that galectin-3 knockout macrophages exhibited a lower degree of actin rearrangement upon Fcγ receptor cross-linking[82]. Similar observations were made following the knockdown of galectin-3 in cultured primary microglia, where these cells exhibited reduced phagocytosis and altered actin rearrangement[83]. Additional studies suggest that intracellular galectin-3 controls the activation state of cofilin, which affects actin filament organization and stability[83], providing a potential mechanism whereby galectin-3 may upregulate phagocytosis.

Intracellular galectin-9 may likewise enhance phagocytosis. Bone marrow-derived dendritic cells from galectin-9 knockout mice showed a reduced uptake of zymosan particles in vitro[84]. Knockdown of galectin-9 in human monocyte-derived dendritic cells yielded similar findings and also reduced actin filament formation[84]. Additional in vitro studies with the above-stated sources of dendritic cells, with galectin-9 knocked out or knocked down, suggested that galectin-9, similar to galectin-3, has an intracellular function in the control of phagocytosis, possibly by affecting actin dynamics[84].

Innate immune cell recruitment

Galectins also appear to modulate key cellular activities that culminate in leukocyte recruitment, likely by affecting chemoattraction and/or cell adhesion, potentially through both extracellular and intracellular signalling pathways. For example, in psoriasis, a chronic skin condition manifested by epidermal hyperplasia and an infiltration of inflammatory cells into the skin (in particular neutrophils), galectin-3 was found to be downregulated in the lesional epidermis both in humans and in mouse models[85]. In a mouse model of psoriasis induced by topical application of imiquimod, galectin-3 was shown to inhibit neutrophil accumulation in lesional skin by serving as a negative regulator of the expression of mediators associated with neutrophil recruitment, including S100A7, CXCL1 and CXCL8 (ref. [85]). Galectin-3 can also suppress the JNK pathway, which is involved in S100A7 production[85].

Similar to galectin-3, galectin-7 was also downregulated in lesional skin in patients with psoriasis, as well as in a mouse model of psoriasis where disease is induced by intradermal injection of IL-23, known to promote the production of IL-17A, a key cytokine responsible for the development of the disease[86]. Knockdown experiments in a human keratinocyte cell line and in primary cultures of human keratinocytes showed that endogenous galectin-7 suppresses the IL-17A-induced expression of IL-6 and IL-8, two key cytokines responsible for neutrophil accumulation in psoriatic skin lesions. This appeared to be due to the suppression of miR-146 by galectin-7, resulting in a downregulation of the ERK pathway and a subsequent reduction in the production of IL-6 and IL-8 (ref. [86]). These findings were further supported by the observation that galectin-7 knockout mice have enhanced epidermal hyperplasia and skin inflammation in response to intradermal IL-23 injection[86], further indicating that galectin-7 has a suppressive role in psoriasis pathogenesis.

In contrast, in models of airway inflammation induced by various infectious agents, galectin-3 knockout mice displayed impaired neutrophil recruitment[87–89]. Experiments with myeloid cell-specific galectin-3 knockout mice indicated that myeloid cells are a source of secreted galectin-3 that contributes to neutrophil accumulation in the lungs in response to LPS-induced airway inflammation[90]. Moreover, galectin-3 was also implicated in neutrophil recruitment in mice that received a footpad injection of the parasite *Leishmania major*[91]. It is not clear why galectin-3 deficiency in these models results in an attenuated neutrophil response, whereas in the model of skin inflammation it results in an enhanced response[85].

Galectin-3 has also been linked to the development of allergic airway inflammation through the modulation of the eosinophil response. Compared with wild-type mice, galectin-3 knockout mice had lower levels of airway inflammation, including eosinophil infiltration, in models of acute and chronic asthma[92,93]. By contrast, in a mouse model of acute asthma, galectin-1 deficiency resulted in greater airway hyperresponsiveness and eosinophil airway infiltration, suggesting that galectin-1 can suppress allergic airway inflammation in this context[94].

Another example of a role of galectin-3 in promoting immune cell recruitment was provided by the study of a model of multiple sclerosis induced by Theiler murine encephalomyelitis virus (TMEV). Galectin-3 knockout mice exhibited lower numbers of $CD45^+$ immune cells infiltrating the subventricular zone in the brain compared with wild-type mice, which correlated with lower levels of chemokines (CCL2, CCL5, CCL and CXCL10)[95].

Thus, the roles of endogenous galectins in inflammatory cell recruitment continue to be clarified. Some galectins, such as galectin-3, appear to promote inflammation via the activation or recruitment of innate immune cells in conditions such as colitis and airway inflammation. In contrast, both galectin-3 and galectin-7 can inhibit leukocyte chemotaxis in the setting of psoriasis, and galectin-1 appears to suppress airway eosinophil accumulation in a mouse model of asthma.

Autophagy in response to intracellular vacuolar damage

As described in Box 1, glycoconjugates reside in the lumen of various organelles; here, they are not accessible to intracellular galectins. However, under certain conditions these vesicles are damaged, thereby allowing glycoconjugates to be exposed to the cytoplasmic milieu where they can be bound by cytosolic galectins. The exposed glycans serve as anchors or adaptors for intracellular galectins, although different galectins appear to engage distinct sets of glycoconjugates. In this context, the intracellular galectins bind both carbohydrates and non-carbohydrates – and thereby form a signalling platform that allows for the modulation of cellular responses including autophagy (Fig. 4),

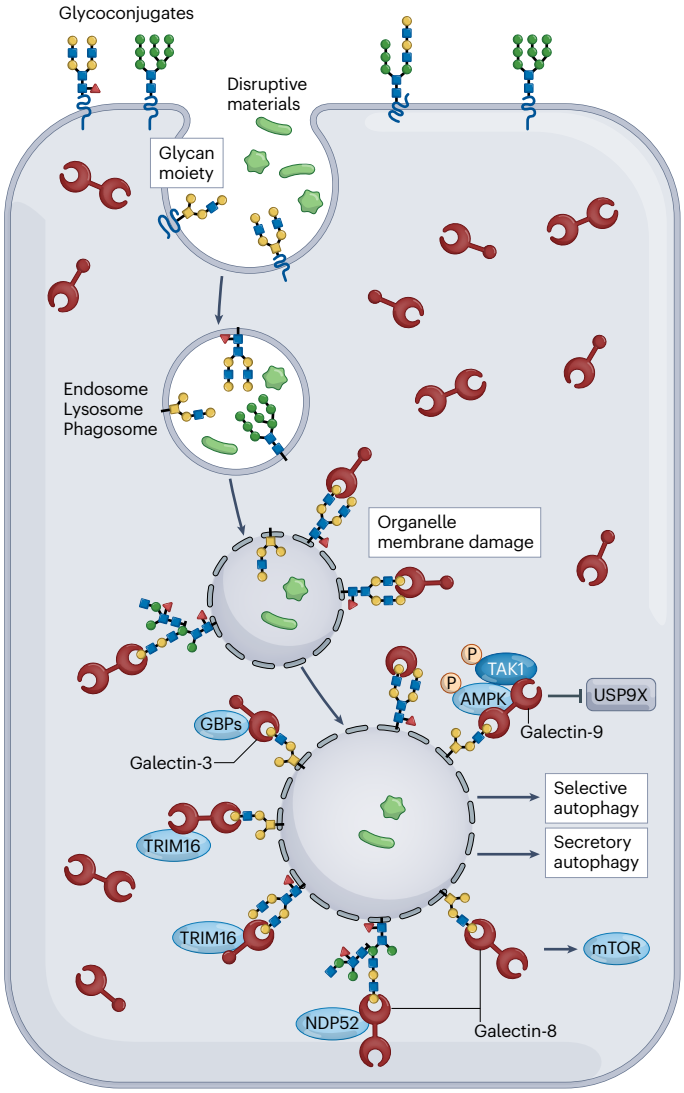

Fig. 4 | Galectins as sensors for endolysosomal damage. Under normal conditions, glycoconjugates reside in the lumen of organelles and thus are not accessible to intracellular galectins, which are located in the cytosol or the nucleus. However, these organelles can become disrupted by intracellular bacteria that cause damage to phagosomes as they escape into the cytosol, toxins secreted into cells by extracellular bacteria, certain viruses and intracellular protein aggregates. Lysosomal damage can also be induced with agents such as Leu-Leu-O-Me (LLOMe) and glycyl-L-phenylalanine 2-naphthylamide (GPN). This can lead to the exposure of glycoconjugates to the cytosolic milieu, where these can be bound by various intracellular galectins. Given that galectins can also bind to various signalling molecules via non-carbohydrate interaction, this can lead to the formation of 'signalling platforms' that can induce autophagic activation and bacterial destruction. For example, galectin-8 was shown to bind to the autophagy adaptor NDP52 and initiate the formation of antibacterial autophagosomes around *Salmonella*-containing vesicles[117]. Galectin-3 was shown to direct the recruitment of the interferon-inducible guanylate binding proteins to intracellular vacuoles damaged by intracellular bacteria[116]. In a model of lysosomal damage, galectin-3 and galectin-8 were found to bind to exposed β-galactosides on damaged membranes, where both galectins can become associated with the autophagy regulator TRIM16 and triggered autophagy[104]. In another model, galectin-8 was shown to associate with the mTOR apparatus, which is normally localized on the surface of lysosomes and serves as an inhibitor of autophagy. This results in inactivation of mTOR and thus autophagic activation[106]. Galectin-9 binding to glycoproteins on damaged lysosomes results in displacement of its cytosolic binding partner, the deubiquitinase USP9X (ref. [107]). This inhibits the enzyme and enhances cellular ubiquitination responses, leading to the activation of a protein kinase TAK1 and, in turn, its downstream AMP-activated protein kinase (AMPK), an inducer of autophagy[107].

the recruitment of antibacterial mediators and, potentially, also inflammasome activation.

Endolysosomal damage can be caused by stressors such as intracellular infections, including those by bacteria (see below), viruses[96] and protozoa[97], as well as oxidative stress[98], free radical formation[99] and endocytosed nanoparticles[100]. Another group of notable insults are membrane disruptive protein aggregates, which are implicated in neurodegenerative diseases (Box 3).

To date, the binding of galectins to cytosolic glycans on damaged organelles has been shown for galectin-1, -3, -7, -8 and -9. Alterations in host cell surface glycosylation by extracellular glycosidases, as can occur during inflammation[99], may result in the differential recruitment of cytosolic galectins to damaged endosomes/lysosomes and in altered autophagic activation[101]. From a practical perspective, the formation of galectin puncta, resulting from accumulation of these proteins around damaged organelles, is now recognized as a sensitive method to detect lysosomal membrane permeabilization[102].

Experimentally, lysosomal damage can be induced with agents such as Leu-Leu-O-Me (LLOMe), and experiments using this agent have uncovered several mechanisms by which galectins may be involved in the activation of autophagy. For example, the clearance of *Mycobacterium tuberculosis* by macrophages was enhanced by the treatment of cells with LLOMe. This appeared to be mediated by the binding of galectin-3 to exposed β-galactosides on damaged membranes, where it then associates with the autophagy regulator TRIM16, which proved critical for autophagy induction and bacterial clearance[103]. Galectin-8 has also been reported to engage TRIM16 following LLOMe-mediated lysosomal damage. This induced secretory autophagy, resulting in the translocation of IL-1β to the extracellular milieu[104]. Interestingly, galectin-3 was also found to mediate the repair of damaged lysosomes following binding to glycoconjugates in damaged organelles in cells treated with LLOM, which appeared to be mediated through its interaction with ALIX[105], an intracellular binding partner of galectin-3 (refs. [38,39]).

Another agent that causes lysosomal damage is glycyl-L-phenylalanine 2-naphthylamide (GPN). In human peripheral blood monocyte-derived macrophages treated with this agent, galectin-8 becomes associated with the mTOR apparatus, which normally localizes on the surface of lysosomes and serves as an autophagy inhibitor. This results in inactivation of mTOR and, thus, autophagic activation[106].

Lysosomal membrane damage-induced autophagy is also associated with ubiquitination responses. Jia et al. reported that binding of cytosolically exposed glycans by galectin-9 is critical for this response[107]. According to their model, galectin-9 binding to lysosomal glycoproteins following lysosomal injury induced by LLOMe results in displacement of the deubiquitinase USP9X, one of its cytosolic binding partners[107]. This inhibits the enzyme and enhances cellular ubiquitination responses. Ultimately, it leads to the activation of AMP-activated protein kinase (AMPK), an inducer of autophagy[107].

Box 3

Galectin involvement in neurodegenerative diseases

Protein aggregates that can cause endolysosomal damage have been implicated in neurodegenerative diseases such as Parkinson and Huntington disease. In a mouse model of Parkinson disease, the endocytosis of exogenous α-synuclein aggregates induced endolysosome rupture and accumulation of galectin-3 aggregates, which mediated the release of intracellular α-synuclein following vesicular damage[159,160]. There is also evidence that galectins bind to damaged lysosomes in a mouse model of Huntington disease, where galectin-3 knockdown reduced microglial activation and inflammation. Here, galectin-3 appeared to enhance inflammation through an NLRP3 inflammasome-dependent pathway[161]. An increased expression of galectin-3 was also found in microglia in patients with Huntington disease and in a mouse model of the disease[161]. However, exactly how the galectin-3 aggregation leads to inflammasome activation awaits clarification.

The role of galectins in infection

Given that galectins can directly engage pathogens, and also regulate fundamental aspects of innate and adaptive immunity, galectins possess the ability to regulate host responses to pathogens through various mechanisms (see also Box 4; see Supplementary Table 4). Although this has been mostly studied in galectin-3 knockout mice, studies demonstrating the involvement of other galectins have started to emerge and demonstrate that different galectins are involved in host defence to bacteria, viruses, fungi and parasites (Table 1). It should be noted that galectin-mediated regulation of immune defences to microbes is not limited to pathogens, as exposure to galectin inhibitors can result in strain-specific outgrowth of distinct commensal bacteria[108], suggesting that galectins may also directly modulate the composition of the microbiota.

Bacterial infections

Compared with wild-type mice, galectin-3 knockout mice have a higher pathogen burden following infection with various bacteria[87,109–111] and this was associated with various different cellular responses and mechanisms, as summarized in Table 1. These include compromised killing of bacteria by macrophages and impaired neutrophil recruitment. Although galectin-3 can directly bind to and even directly mediate the killing of various bacteria (Box 4), such as *Heliobacter pylori*[109,112], it is unclear whether the increased bacterial loads in galectin-3 knockout mice reflect a deficit in microbial killing. On the other hand, galectin-3 knockout mice were more resistant to infections by some other bacteria, including *Brucella abortus* and *Rhodococcus equi*. This was linked to the ability of galectin-3 to suppress the production of pro-inflammatory cytokines that are critical for clearance of bacteria by the host immune cells[113,114].

Galectins can affect the cellular responses by functioning as a sensor of vacuolar damage induced by intracellular bacteria. *Shigella flexneri*[115], *Yersinia pseudotuberculosis*, *Legionella pneumophila*[116], *Salmonella* Typhimurium[117] and *Listeria monocytogenes*[101] can undergo phagosomal escape and induce endolysosomal damage. In these infections, galectin-3 was found to accumulate near the intracellular pathogens[97,115,116], where it bound to host N-glycans that became exposed to the cytosol due to phagosomal damage. In the context of *Y. pseudotuberculosis* or *L. pneumophila* infection, galectin-3 was shown to facilitate the recruitment of the interferon-inducible guanylate binding proteins GBP1 and GBP2 to damaged intracellular vacuoles containing the microbes[116]. GBP1 and GBP2 are antimicrobial factors previously known to be recruited to pathogen-containing vacuoles.

The antibacterial response most extensively studied in the context of bacterial endolysosomal damage is autophagy. This was first shown for galectin-8, which binds to exposed glycans on damaged vesicles containing *S.* Typhimurium[117], along with galectin-3 and galectin-9. The accumulation of galectin-8, but not the other two galectins, on damaged vesicles was shown to initiate autophagic activation, resulting in the destruction of the bacteria. Multiple lines of experimental evidence indicated that intracellular galectin-8 binds directly to NDP52, a regulator of autophagy[117]. In this complex, the N-terminal domain of galectin-8 binds host N-glycans on damaged phagosomes and the carboxy-terminal domain engages NDP52 to facilitate autophagy[117,118].

In the context of *L. monocytogenes* infection, galectin-3 was shown to attenuate autophagy[101]. In this study, galectin-3 and galectin-8 did not significantly compete with each other in binding to glycans on damaged phagosomes[101], consistent with the distinct glycan-binding preferences of these two galectins[21,22,119]. Additional data suggested that alterations in host cell surface glycosylation by extracellular glycosidases may result in the differential recruitment of cytosolic galectins to damaged endosomes/lysosomes and in altered autophagic activation[101]. Similar findings were also observed in Chinese hamster ovary cells with endosomal damage due to free radical formation, where galectin-3 and galectin-8 were found to reside in different microdomains on the damaged endosomes[120].

There have been far fewer studies on other galectins, especially those containing one carbohydrate-recognition domain. In cells infected with group A *Streptococcus*, galectin-1 and galectin-7 were found to accumulate around bacteria-containing autophagosomes, and this was dependent on Toll-interacting protein (TOLLIP), which is recruited to bacteria-containing endosomal vacuoles prior to the escape of the bacteria[121]. Both galectins were shown to participate in bacterial autophagy[121].

A study of the role of galectins in the host defence against *M. tuberculosis* showed that galectin-3, -8 and -9 are all recruited to the same mycobacterial population in infected macrophage cell lines and primary macrophages, where they co-localized with markers of autophagy[122]. By studying macrophage cell lines with these three galectins knocked out individually and comparing these with macrophages missing all three galectins, the critical role of galectin-8 in autophagic clearance of *M. tuberculosis* was established[122].

Lysosomal damage can also be caused by bacterial toxins. Vacuolating cytotoxin A (VacA), secreted by extracellular *H. pylori*, was shown to form pores in host cells[123]. Galectin-8 aggregates around damaged lysosomes after *H. pylori* infection through binding to the host O-glycans[124]. This was followed by autophagic activation — an outcome that failed to occur in galectin-8 knockdown cells. VacA-deficient *H. pylori* induced less galectin-8 aggregation than its wild type counterpart[124], which is consistent with the ability of VacA to induce lysosomal injury.

Viruses

Studies of the roles of galectins in viral infections are still relatively scarce. However, there is evidence that galectin-3 may have a role in the host response to viral infections through intracellular mechanisms. These include the observation that it can promote inflammasome assembly and activation in macrophages infected by avian influenza A H5N1 virus[125] and suppress TNF production in hepatocytes infected by cytomegalovirus[126].

In SARS-CoV-2 infection, serum levels of galectin-1 (ref. [127]), galectin-3 (ref. [128]) and galectin-9 (ref. [129]) are elevated and correlate positively with markers of inflammation and tissue injury. Here, galectins may be predictors of disease severity and may contribute to cytokine release syndrome[130]. The receptor binding domain of SARS-CoV-2 also possesses some similarities to galectins with regards to its sequence, structure and capacity for carbohydrate binding[131], which may account for the observation that glycans can facilitate SARS-CoV-2 entry into cells[132].

Fungi

Galectin-3 knockout mice were shown to have a lower fungal burden compared with wild-type mice when systemically infected with *Candida albicans* (in the kidneys and brain)[133] or *Histoplasma capsulatum* (in the liver)[134], indicating that galectin-3 suppresses the host response against fungi. This was linked to its ability to suppress the production of reactive oxygen species (ROS) in neutrophils and, consequently, the fungal killing activity of these cells[133], and its ability to suppress IL-23 production in dendritic cells[69,134]. However, another study of galectin-3 in *C. albicans* infection showed that galectin-3 knockout mice were more susceptible to infection[135], and additional investigations are needed to resolve this discrepancy. Galectin-3 has also been shown to have a suppressive effect on pulmonary aspergillosis[88] and cryptococcosis[136], as determined by the reduced survival and higher pulmonary fungal burden in galectin-3 knockout mice compared with wild-type mice following intratracheal infections. This was associated with its function in promoting neutrophil infiltration into the airways[88] and promoting T_H17 cell responses[136].

There has been much less research into the role of other galectins in infection, but galectin-1 has been shown to have a protective effect in mice infected with the yeast *H. capsulatum*[137]. Galectin-1 knockout showed shorter survival and an increased yeast burden after intratracheal infection, which was associated with a role of galectin-1 in limiting pro-inflammatory cytokine responses and neutrophil pulmonary infiltration[137].

Parasites

Several studies have investigated the role of galectin-3 in parasite infections. Remarkably, unlike in some bacterial and fungal infections, galectin-3 deficiency consistently resulted in an increased parasite load in all infection models studied[91,138–143], suggesting that galectin-3 has a protective role. In most of these infections, the increase in parasite load correlated with a decrease in inflammatory responses in galectin-3 knockout mice and with decreased neutrophil recruitment to the infected sites (Table 1). Mechanistically, the role of galectin-3 in anti-parasite immunity has been linked to its ability to suppress T_H1 cell responses[141], regulate Toll-like receptor expression on APCs[142] and modulate the frequency of peripheral regulatory T cells (T_{reg} cells), both at the sites of infection and in draining lymph nodes[139].

There are only a limited number of studies of the roles of other galectins in parasite infections. However, it appears that galectin-1 dampens immunity to parasitic infections[144], given that galectin-1 knockout mice survived longer and had a lower parasite count in muscle tissue compared with wild-type mice when subjected to intraperitoneal infection with *Trypanosoma cruzi*. This is consistent with the often observed opposite effects of galectin-1 and galectin-3 in various innate immune cells, as described above.

As summarized in Table 1, galectins have been shown to either promote or suppress host defences against pathogens. The differential effect of the same galectin in the context of different pathogens is likely because different pathogens affect distinct cell types and tissues, where galectin functions may vary. As for the mechanism of action, many studies listed in Table 1 have suggested that galectins can regulate the recruitment and activation of immune cells that are critically associated with host defence against pathogens. However, other studies highlighted the role of galectins in directly interacting with microbes extracellularly (Supplementary Table 4).

Conclusion

Many studies have demonstrated that galectins can modulate immune cell functions by engaging key cell surface receptors. However, very few strategies have been developed to validate these functions in vivo or investigate whether galectins can be of therapeutic use. This may, in part, reflect challenges associated with delivering galectins locally or systemically, given their ability to agglutinate cells in a nonspecific way and the unlikelihood that systemically delivered galectins would reach desired targets.

Box 4

Direct interactions of galectins with microbes

In addition to regulating immune cells, galectins have also been shown to directly interact with the glycans of various pathogens[162–166]. In vitro studies using a microbial glycan microarray demonstrated that galectin-3, -4, -8 and -9, in particular, exhibit a marked preference for selected glycan structures on bacteria that mimic mammalian glycans[167]. Importantly, microbial engagement by each galectin resulted in rapid microbial death independent of complement or other known innate immune proteins. Inclusion of a galectin inhibitor in vivo can lead to a selective outgrowth of microorganisms targeted by galectins[108], suggesting that endogenous galectins may have a direct role in host defence. Consistent with this, galectins can be secreted from the apical sides of cultured polarized epithelial cells[168–170] and their interactions with microorganisms on the luminal side of epithelia have been demonstrated[112]. Galectins have also been described to interact directly with fungi, viruses and parasites, and lead to cell death in some cases[162,171,172]. However, whether galectins interact with many of these pathogens in vivo remains to be tested[162,171]. Finally, galectins have also been shown to bind microbial components in the host cytosol. For example, galectin-3 can engage cytosolic lipopolysaccharide (LPS), resulting in non-canonical inflammasome activation[173].

Table 1 | Galectins and infection[a]

Pathogen	Species	Route of infection	Pathogen load in knockout	Cellular response in knockout	Mechanism of action of respective galectin	Refs.
Bacteria	**Galectin-1 knockout**					
	Yersinia enterocolitica	i.g.	Lower	Reduced intestinal pathology	Galectin-1 suppresses NF-κB activation, TNF production and NO synthesis in macrophages and systemic IL-17 and IFNγ responses	150
	Galectin-3 knockout					
	Brucella abortus	i.p.	Lower	Increased amounts of pro-inflammatory cytokines Increased numbers of macrophages, dendritic cells and neutrophils in spleens	Galectin-3 suppresses the recruitment and activation of immune cells, thus hindering clearance of bacteria by immune cells	113
	Helicobacter pylori	i.g.	Higher	Larger amounts of macrophages in the gastric mucosa Larger amounts of lymphoid clusters in the gastric submucosa	Galectin-3 promotes killing of bacteria by macrophages	109
	Leptospira interrogans serovar Copenhageni	i.p.	Higher (kidneys)	More severe interstitial nephritis (subacute phase) More severe renal fibrosis (chronic phase)	Galectin-3 promotes conversion of fibroblasts to myofibroblasts	110
	Rhodococcus equi	i.v.	Lower	Higher granulomatous inflammation Larger amount of IL-12 in serum, higher IL-1β level in the spleen	Galectin-3 suppresses expression of selected cytokines by macrophages that are responsible for clearance of bacteria	114
	Streptococcus pneumoniae	i.n.	Higher	Reduced neutrophil recruitment	Galectin-3 promotes the haemoattraction of neutrophils	87
Viruses	**Galectin-3 knockout**					
	Avian influenza A H5N1	i.n.	No effect	Lower inflammatory response	Galectin-3 promotes inflammasome activation	125
	Cytomegalo virus	i.p.	Higher (liver)	Enhanced hepatitis	Galectin-3 suppresses production of TNF by hepatocytes	126
Fungi	**Galectin-1 knockout**					
	Histoplasma capsulatum	i.t.	Higher (lungs and spleen)	Higher pro-inflammatory cytokine production and neutrophil infiltration in the lung	Galectin-1 promotes phagocytic response of macrophages	137
	Galectin-3 knockout					
	Aspergillus fumigatus	i.t.	Higher	Lower amount of neutrophil recruitment	Stromal galectin-3 promotes neutrophil migration into the airways	88
	Candida albicans	i.v.	Lower	Reduced monocyte and enhanced dendritic cell infiltrations Reduced nephritis	Galectin-3 suppresses neutrophil killing of fungi by suppressing ROS production	133
	Cryptococcus neoformans	i.t.	Higher (lungs and brain)	Lower amounts of IL-17 and IL-23 (lungs and spleen)	Galectin-3 promotes T_H17-type immune responses; also directly inhibits fungal growth and lyses extracellular vesicles produced by the fungi	136
	H. capsulatum	i.v.	Lower	Higher T_H17 cell response	Galectin-3 suppresses the IL-17A response via dendritic cells	134
Parasites	**Galectin-1 knockout**					
	Leishmania donovani	i.v.	Lower (in the liver)	Higher T_H1 cell response	Galectin-1 suppresses hepatic T_H1 cell development	151
	Trypanosoma cruzi	i.p.	Lower (in the muscle)	Increased $CD8^+$ T cells and higher frequency of IFNγ-producing $CD4^+$ T cells in muscle tissues and draining lymph nodes Impaired induction of T_{reg} cells	Galectin-1 promotes development of tolerogenic dendritic cells and T_{reg} cells, and suppresses effector T cell response	152

Table 1 (continued) | Galectins and infection[a]

Pathogen	Species	Route of infection	Pathogen load in knockout	Cellular response in knockout	Mechanism of action of respective galectin	Refs.
Parasites (Cont.)	**Galectin-3 knockout**					
	Leishmania amazonensis	s.c.	Higher (infected sites and lymph nodes)	Enhanced inflammatory response	Galectin-3 controls intracellular proliferation of parasites	138
	Leishmania major	s.c.	Higher	More pronounced swelling of infected sites Increased frequency of peripheral T_{reg} cells	Galectin-3 promotes the suppressive activity of T_{reg} cells	139
	L. major	s.c.	Higher	Reduced number of neutrophils to the infected sites	Galectin-3 facilitates neutrophil migration[b]	91
	Mesocestoides corti	i.c.	No effect	Increased neutrophil and M2 macrophages in the brain	Galectin-3 promotes neutrophil clearance by M2 macrophages	140
	Toxoplasma gondii	Oral	Higher (brain)	Lower inflammatory response in the liver, higher inflammatory response in the lungs Higher T_H1 cell response	Galectin-3 suppresses IL-12 production by dendritic cells	141
	T. cruzi	i.p.	Higher (blood) No effect (heart)	Lower recruitment of leukocytes into peritoneal cavity Lower infiltrating macrophages and T cells in the heart Higher heart fibrosis	Galectin-3 regulates Toll-like receptor expression in dendritic cells	142,143
	Galectin-8 knockout					
	T. cruzi	i.p.	No effect	Increased inflammatory response Increased fibrosis	Galectin-8 is involved in clearance of neutrophils by macrophages	153

i.c., intracranial; i.g., intragastric; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; ROS, reactive oxygen species; s.c.: subcutaneous, T_H17 cells, T helper 17 cells; T_{reg} cells, regulatory T cells. [a]Phenotypes of mice deficient in given galectins and proposed mechanisms of action of galectins. [b]Demonstrated by using exogenously added galectin.

Studies using genetic targeting of the proposed cell surface counter-receptors that phenocopy key findings observed in galectin knockouts have also been lacking. This is likely related to the fact that various galectins have been shown to bind to a large number of different cell surface receptors (Supplementary Table 1), and thus can potentially signal through multiple receptors. This makes it very challenging to verify the role of these putative glyco-receptors in vivo. Instead of specific receptors, the concept of 'galectin lattices'[145], which emphasizes a mechanism whereby glycan modification is key for regulating cellular responses by galectins, is intriguing, although additional studies are needed to fully define such a pathway. A galectin lattice is a complex macromolecular structure composed of glycoproteins and/or glycolipids bound by galectins at the cell surface that can influence receptor signalling, glycoprotein and glycolipid cell surface half-life and overall interactions with other cell surface glycoproteins and glycolipids. Regardless of whether galectins mediate some of their biological functions through galectin lattices or individual receptors, methods to inhibit galectin–glycan interactions in general, either through blocking antibodies or glycan derivatives, have been explored and hold promise for establishing their functions[146].

As galectins also act intracellularly, modulating immune cell functions by targeting intracellular galectin activities also holds promise. Indeed, the list of intracellular partners continues to expand (Supplementary Table 3). However, validation of these functions in vivo, including the role of the intracellular binding partners, is equally challenging. This is, in part, due to the lack of key studies defining the features of galectins responsible for intracellular carbohydrate-independent interactions, making it difficult to use structural approaches to design druggable targets to modulate intracellular functions.

Given the ability of both extracellular and intracellular galectins to exert immunoregulatory activity, new tools and innovative approaches must be developed to more precisely address the functions of different galectins. These approaches may include studies of tissue-specific conditional knockout mice and methods that allow the examination of the trafficking of endogenous galectins in the extracellular space. They may also include the ex vivo culturing of complex tissues, in conjunction with the use of cell-permeable and impermeable galectin inhibitors[147]. Indeed, small molecule and antibody-based inhibitors will be critical in determining the relative contribution of extracellular versus intracellular galectin activities in vivo. Although some well-defined small molecule inhibitors of galectin-3, such as GB0139 and GB1107, are now available[148], they are known to also inhibit other galectins (for example, galectin-1), albeit with lower efficacy. Similarly, reagents capable of defining the concentration and activity state (given their sensitivity to oxidation and/or proteolytic inactivation) of a given galectin are needed, as current agents are often cross-reactive between different galectin family members and fail to distinguish the distinct activity states of a target galectin.

Despite the challenges in studying galectin activity in vivo, numerous studies continue to implicate galectins in various diseases. The discovery and implementation of tools designed to more accurately define galectin function will not only facilitate their study but may also have translational potential. For example, given the role of galectins in autoimmune diseases and inflammatory diseases, approaches to

induce the expression of immunosuppressive galectins, or inhibit pro-inflammatory activities of certain galectins, may prove valuable as a treatment strategy for these diseases[146]. In contrast, during infection, where augmented immune function may be desirable, inhibiting or enhancing the activity of certain immunosuppressive or immunostimulatory activities of individual galectins may be therapeutically beneficial[149]. As galectins regulate a wide variety of biological processes, the manipulation of galectin function remains an open and promising area of research.

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Competing interests

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