Turning 'sweet' on immunity: galectin–glycan interactions in immune tolerance and inflammation

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Abstract | The function of deciphering the biological information encoded by the glycome, which is the entire repertoire of complex sugar structures expressed by cells and tissues, is assigned in part to endogenous glycan-binding proteins or lectins. Galectins, a family of animal lectins that bind *N*-acetyllactosamine-containing glycans, have many roles in diverse immune cell processes, including those relevant to pathogen recognition, shaping the course of adaptive immune responses and fine-tuning the inflammatory response. How do galectins translate glycan-encoded information into tolerogenic or inflammatory cell programmes? An improved understanding of the mechanisms underlying these functions will provide further opportunities for developing new therapies based on the immunoregulatory properties of this multifaceted protein family.

Lattice
Spatial array of glycans and endogenous multivalent lectins on the cell surface that controls signalling and cellular responses.

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The regulated expression of cell surface glycans through the coordinated action of glycan-modifying enzymes (glycosyltransferases and glycosidases) is a hallmark of immune cell activation, differentiation and homeostasis[1]. Decoding the biological information encrypted by these glycosylation 'signatures' is a role assigned to endogenous glycan-binding proteins or lectins, the expression patterns of which are regulated during the course of an immune response[2].

Galectins are evolutionarily conserved glycan-binding proteins with pleiotropic roles in innate and adaptive immune responses[3–5]. To date, 15 galectins have been identified in mammals, most with wide tissue distribution, although some galectins are expressed with restricted tissue specificity[5]. Within the immune system, galectins are expressed by virtually all immune cells, either constitutively or in an inducible fashion, and are significantly upregulated by activated B and T cells, inflammatory macrophages, decidual natural killer (NK) cells and $CD4^+CD25^+$ regulatory T (T_{Reg}) cells[2–5]. Galectins share a common structural fold and at least one conserved carbohydrate recognition domain (CRD) of approximately 130 amino acids that mediates carbohydrate binding. A traditional classification based on structural similarities includes: prototype galectins (galectin 1, galectin 2, galectin 5, galectin 7, galectin 10, galectin 11, galectin 13, galectin 14 and galectin 15), which have one CRD and exist as monomers or dimers; tandem repeat-type galectins (galectin 4, galectin 6, galectin 8, galectin 9 and galectin 12), which contain two different CRDs separated by a linker of up to 70 amino acids; and the chimera-type galectin 3, which contains a CRD connected to a non-lectin amino-terminal region[3,5] (FIG. 1). Most galectins are either bivalent or multivalent with regard to their carbohydrate-binding activities, which enable the recognition of multiple binding partners and the activation of distinct signalling pathways. Prototype galectins can dimerize, tandem repeat-type galectins are at least bivalent and galectin 3 can form oligomers following binding to multivalent glycoproteins[3–5].

Although galectins do not contain a classical secretory signal, some members are found in the extracellular compartment and are released through an unusual route that requires intact carbohydrate-binding activity of the secreted protein[5]. Once outside the cells, galectins can bind multiple glycosylated binding partners and translate glycan-encoded information into immune cell activation, differentiation and homeostatic programmes. Remarkably, these proteins can function by forming ordered galectin–glycan structures — often termed lattices — on the cell surface (FIG. 1) or through direct engagement of specific cell surface glycoconjugates by traditional ligand–receptor interactions[3–7]. However, such interactions are limited to galectins that are secreted; some members of the galectin family

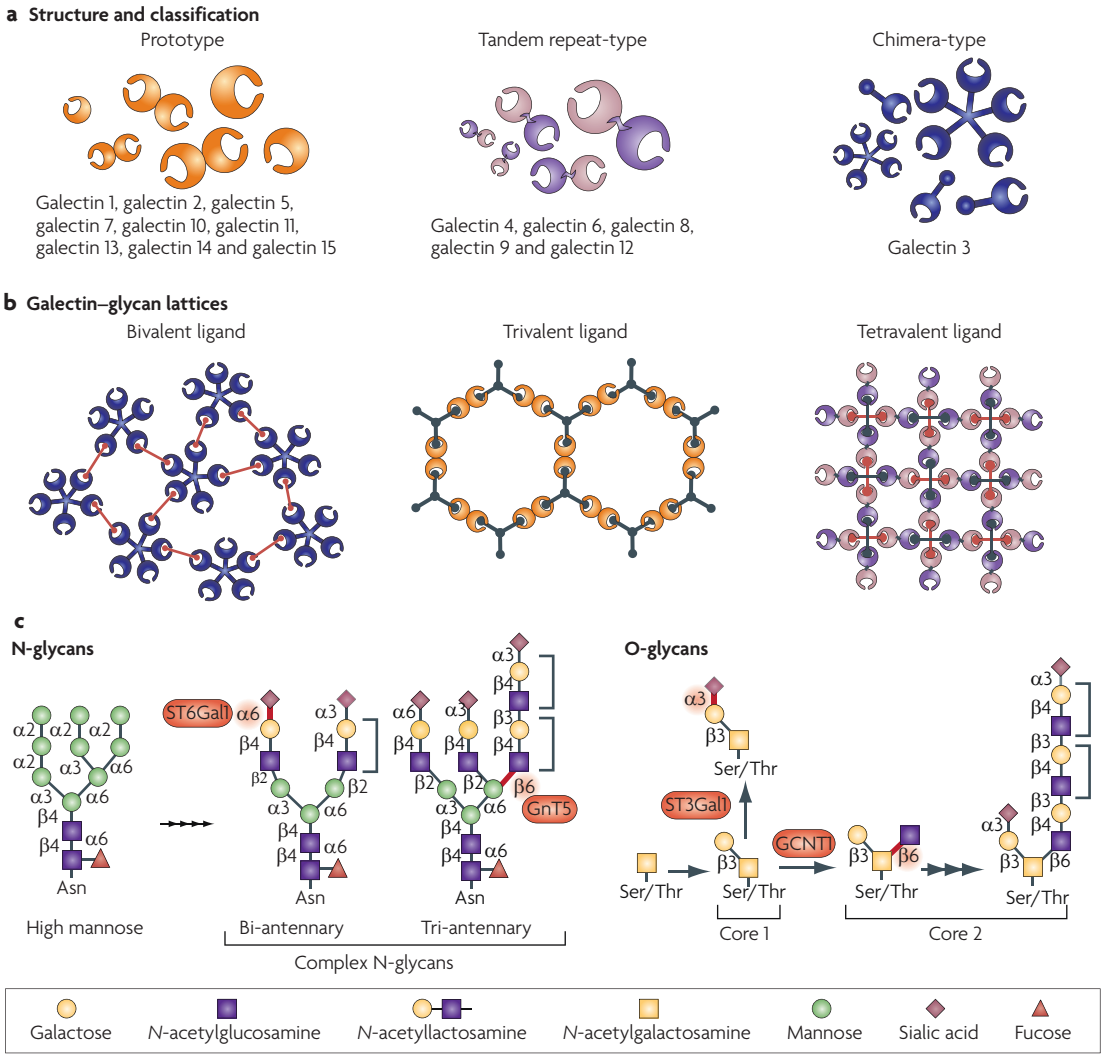

Figure 1 | **Galectin–glycan interactions: structural and biochemical features. a** | Schematic representation of the structure of different members of the galectin family. Galectins can be subdivided into three groups: prototype galectins, which contain one carbohydrate recognition domain (CRD) and can form homodimers; tandem repeat-type galectins, which contain two distinct CRDs in tandem connected by a linker of up to 70 amino acids and are inherently bivalent; and the unique chimera-type galectin 3, which consists of unusual tandem repeats of proline- and glycine-rich short stretches fused onto the CRD. Following ligand binding, galectin 3 undergoes conformational changes, which enable its oligomerization into pentamers. **b** | Schematic representation of distinct types of lattice that could be formed hypothetically between multivalent galectins and multivalent glycans. Examples of bivalent, trivalent and tetravalent ligands are illustrated. **c** | Schematic representation of N- and O-glycan biosynthesis, including relevant glycosyltransferases, such as core 2 *N*-acetylglucosaminyltransferase 1 (GCNT1), *N*-acetylglucosaminyltransferase 5 (GnT5), α2,3 sialyltransferase 1 (ST3Gal1) and α2,6 sialyltransferase 1 (ST6Gal1), the coordinated actions of which lead to the generation or masking of common glycosylated ligands for galectins (such as *N*-acetyllactosamine (LacNAc) or poly-LacNAc residues in complex N-glycans or core 2 O-glycans). Fine specificities of individual galectins to particular glycan structures are illustrated in Supplementary information S1 (table).

might remain associated with cell membranes or might function within the intracellular compartment[3–5]. These include galectin 3 and galectin 10, which function intracellularly to either modulate cell survival and pre-mRNA splicing or to control the suppressive activity of T_{Reg} cells[5,8].

The picture that has emerged is that secreted galectins, in contrast to cytokines or chemokines, do not have specific receptors, but can mediate communication between immune cells through the recognition of a preferred set of cell surface glycoconjugates[5] (TABLE 1). In this context, the minimal structure recognized by galectins is the disaccharide *N*-acetyllactosamine (LacNAc), which is found in N-glycans and O-glycans and can be presented as multiple units (poly-LacNAc) on cell surface glycoproteins[9] (FIG. 1). However, substantial differences exist in the glycan-binding preferences of individual members of the galectin family[9,10], which might be the basis of functional differences in their biological activity. These variations in glycan recognition

Table 1 | **Glycoprotein and glycolipid receptors for galectins on immune cells**

Receptor	Galectin	Cell	Associated processes	Refs
CD45	Galectin 1	T cells and thymocytes	Apoptosis	28,31,39
	Galectin 3	T cells and thymocytes	Apoptosis	31
CD43	Galectin 1	T cells and thymocytes	Apoptosis and transendothelial migration	31,39,42,104
CD7	Galectin 1	T cells and thymocytes	Apoptosis	39
	Galectin 3	T cells and thymocytes	Apoptosis	36
CD71	Galectin 3	T cells	Apoptosis	31
CD29	Galectin 2	T cells	Apoptosis	32
	Galectin 3	T cells	Apoptosis	36
TCR	Galectin 1	T cells	Signalling and activation	24
	Galectin 3	T cells	Signalling and activation	20–23
TIM3	Galectin 9	T_H1 cells	Apoptosis	7
		Dendritic cells and monocytes	Maturation and cytokine secretion	90
CD98	Galectin 3	Macrophages	Alternative activation	96
CD44	Galectin 8	Synovial fluid cells	Apoptosis	109
	Galectin 9	T cells	Adhesion	117
Neuropilin 1	Galectin 1	Endothelial cells	Migration	141
Pre-BCR	Galectin 1	Pre-B cells	Signalling and maturation	78,79
α4 integrins	Galectin 1	Pre-B cells	Signalling and maturation	78,79
	Galectin 8	T cells	Adhesion	142
CD2	Galectin 1	T cells	Apoptosis	143
CD3	Galectin 1	T cells	Apoptosis	143
	Galectin 4	T cells	Apoptosis	34
CTLA4	Galectin 3	T cells	Cell growth arrest	25
GM1	Galectin 1	T cells	T_{Reg} cell-mediated immunosuppression	77

BCR, B cell receptor; CTLA4, cytotoxic T lymphocyte antigen 4; TCR, T cell receptor; TIM3, T cell immunoglobulin domain and mucin domain protein 3; T_H1, T helper 1; T_{Reg}, regulatory T.

are mainly associated with the extent of N-glycan branching, the multiplicity of LacNAc residues and/or the modification of terminal saccharides (for example, sialylation or fucosylation)[9–11] (see Supplementary information S1 (table)). Surprisingly, differences in carbohydrate recognition of individual galectins can be even more pronounced, as the specific binding of galectin 10 to mannose is of higher affinity than its binding to LacNAc[5]. In addition, selective binding of galectins to different glycoprotein partners can result from the particular spatial orientation of individual CRDs and the unique glycoprotein topologies determined by the number of attached N-glycans[6,11].

In addition to binding glycan structures that are expressed by host cells, galectins can also recognize saccharide ligands that are present on microorganisms, suggesting an evolutionarily conserved function of these endogenous lectins as soluble pattern recognition receptors[2,12,13] (BOX 1). In fact, some galectins can act as 'alarmins' by converting damage-associated signals into innate immune responses, and others have been associated with the resolution of acute inflammation[2,5,14,15]. As other review articles have covered the contribution of galectins to pathogen recognition and innate immunity in greater depth[2,5,12,13], in this Review we highlight recent insights into the mechanisms by which galectins and their specific saccharide ligands contribute to immune tolerance and homeostasis during the course of an adaptive immune response. Furthermore, we discuss the relevance of these interactions to chronic inflammation and cancer.

Galectins in immune tolerance and homeostasis

Studies carried out over the past decade have been immensely fruitful in terms of advances in our understanding of the cellular and molecular mechanisms of immune tolerance and homeostasis. Important developments include the identification of gene products that are responsible for central and peripheral T cell deletion, anergy and cytokine deviation, as well as the dissection of the molecular pathways that lead to the differentiation of T_{Reg} cells[16]. These landmarks have paved the way for successful exploitation of these mechanisms for the treatment of autoimmune disorders and the prevention of transplant rejection.

In addition to their established role in pathogen recognition, emerging evidence indicates that endogenous lectins, including galectins, have important functions in deciphering glycan-encoded information on host immune cells and modulating their effector functions[2].

Anergy
A state of non-responsiveness to antigen. Anergic B or T cells cannot responds to their cognate antigens under optimal conditions of stimulation.

Box 1 | **Galectins in host–pathogen interactions**

The recognition of glycans from different organisms is one mechanism used by the host to identify pathogens. Galectins can influence immune responses by interacting with β-galactoside-containing glycans in a range of invading microorganisms. Although these interactions are not as well-characterized as those involving pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) or C-type lectin receptors, galectin oligomerization and/or lattice formation probably has an important role in pathogen recognition[12,13,134,135].

Parasites

Galectin 3 and galectin 9 specifically bind to lipophosphoglycans of *Leishmania major*, thus enabling glycan-dependent interactions between the parasite and host macrophages. However, recognition of parasite glycans by galectin 3 leads to proteolytic removal of the amino-terminal domain of this protein, thereby preventing oligomerization and lattice formation[134]. In addition, galectin 3 acts as a PRR for LacdiNAc (GalNAc β1–4GlcNAc)-containing glycans that are present in helminths such as *Schistosoma mansoni*[135]. This specific recognition seems to be evolutionarily conserved, as a galectin with four carbohydrate recognition domains, which was identified in the eastern oyster *Crassostrea virginica*, mediates the recognition of the pathogenic parasite *Perkinsus marinus* in this species[13].

Viruses

Galectin 1 inhibits envelope-mediated cell–cell fusion of the zoonotic Nipah virus through binding to specific N-glycans on viral glycoproteins[136]. However, galectin 1 but not galectin 3 increases HIV-1 infectivity by facilitating virus adsorption to human macrophages[137].

Bacteria

Many bacteria, including *Neisseria meningitides*, *Neisseria gonorrhoeae* and *Helicobacter pylori*, carry various different saccharides, including lipooligosaccharides or capsular oligosaccharides, which are specifically recognized by host galectins[2,12,138]. Although the biological relevance of these interactions remains poorly understood, recent studies indicated that macrophages constitutively express galectin 3, which binds bacterial lipopolysaccharide (LPS) and acts as a negative regulator of LPS functions[130].

Fungi

Macrophages differentially sense the fungi *Candida albicans* and *Saccharomyces cerevisiae* through mechanisms involving TLR2 and galectin 3 (REF. 139). Interestingly, galectin 3 specifically binds and kills *C. albicans* species that bear β1,2-linked oligomannans on the cell surface, providing evidence of a direct antifungal activity of this lectin[140].

How do endogenous lectins influence T cell homeostasis? Most lectins, including C-type lectins and sialic acid-binding immunoglobulin-like lectins (Siglecs), are associated with the cell surface of antigen-presenting cells (APCs), where they can modulate T cell signalling and survival through glycan-dependent cell–cell interactions[2,17]. By contrast, secreted galectins can bind LacNAc-containing cell surface glycoconjugates and influence immune tolerance by controlling T cell signalling and activation, modulating T cell survival, altering cytokine balance and shaping the B cell compartment[4] (see below). In addition, research over the past few years has shed light on a previously unappreciated role for endogenous galectins as crucial mediators of the suppressive function of T_{Reg} cells[8,18].

Fine-tuning T cell signalling and activation. T cell activation requires stable contacts with APCs to assemble the immunological synapse, an effect that involves extensive interdigitation of the cell surface glycocalyx and lateral compartmentalization of signalling proteins into membrane microdomains[19]. Several molecular contacts, including those elicited by lectins and glycans, contribute to defining the nature and magnitude of APC–T cell interactions as well as the balance between immune cell responsiveness and tolerance[19]. In this regard, the T cell receptor (TCR) is 'decorated' by β1,6 N-glycan branch structures that are generated by the glycosyltransferase *N*-acetylglucosaminyltransferase 5 (GnT5) (FIG. 1). Elegant work has shown that multivalent galectin 3–N-glycan complexes can limit TCR clustering by restricting lateral TCR movement within the plane of the membrane, thereby increasing agonist threshold for TCR signalling[20] (FIG. 2). Accordingly, deficiency in GnT5 lowers the threshold for T cell activation by enabling TCR clustering and signalling, which results in augmented T helper 1 (T_H1) cell responses and enhanced susceptibility to autoimmune disease[20,21]. These observations were recently extended to show that supplementation of T cells with metabolites of the hexosamine pathway, which leads to increased β1,6 *N*-acetylglucosamine (GlcNAc)-branching of N-glycans, prevents sustained TCR signalling, T_H1 cell polarization and the development of autoimmunity[22]. Additional studies aimed at dissecting the mechanistic basis of this effect revealed that galectin–glycan lattices and actin microfilaments act on opposing sides of the plasma membrane to regulate receptor distribution and signalling[23]. In the absence of TCR engagement, galectin binding to N-glycans prevents filamentous actin-dependent targeting of the TCR, CD4 and the protein tyrosine kinase LCK to GM1-enriched membrane microdomains[23]. Moreover, galectin–glycan lattices contribute to the inactivation of LCK by specifically retaining the CD45 phosphatase at these membrane areas, thereby preventing spontaneous TCR activation in the absence of specific ligands[23]. In addition, galectin 1 modulates the early steps of T cell activation by limiting the coalescence of lipid rafts and by promoting incomplete phosphorylation of the TCR ζ-chain, thereby allowing T cell responses that only require partial TCR signals to occur[24].

C-type lectin
A receptor protein that binds carbohydrates in a calcium-dependent manner. The binding activity of C-type lectins is based on the structure of the carbohydrate recognition domain.

Immunological synapse
A large junctional structure that is formed at the cell surface between a T cell and an antigen-presenting cell (APC); it consists of molecules required for adhesion and signalling. This structure is important in establishing T cell adhesion and polarity, is influenced by the cytoskeleton and transduces highly controlled secretory signals, thereby allowing the directed release of cytokines or lytic granules towards the APC or target cell.

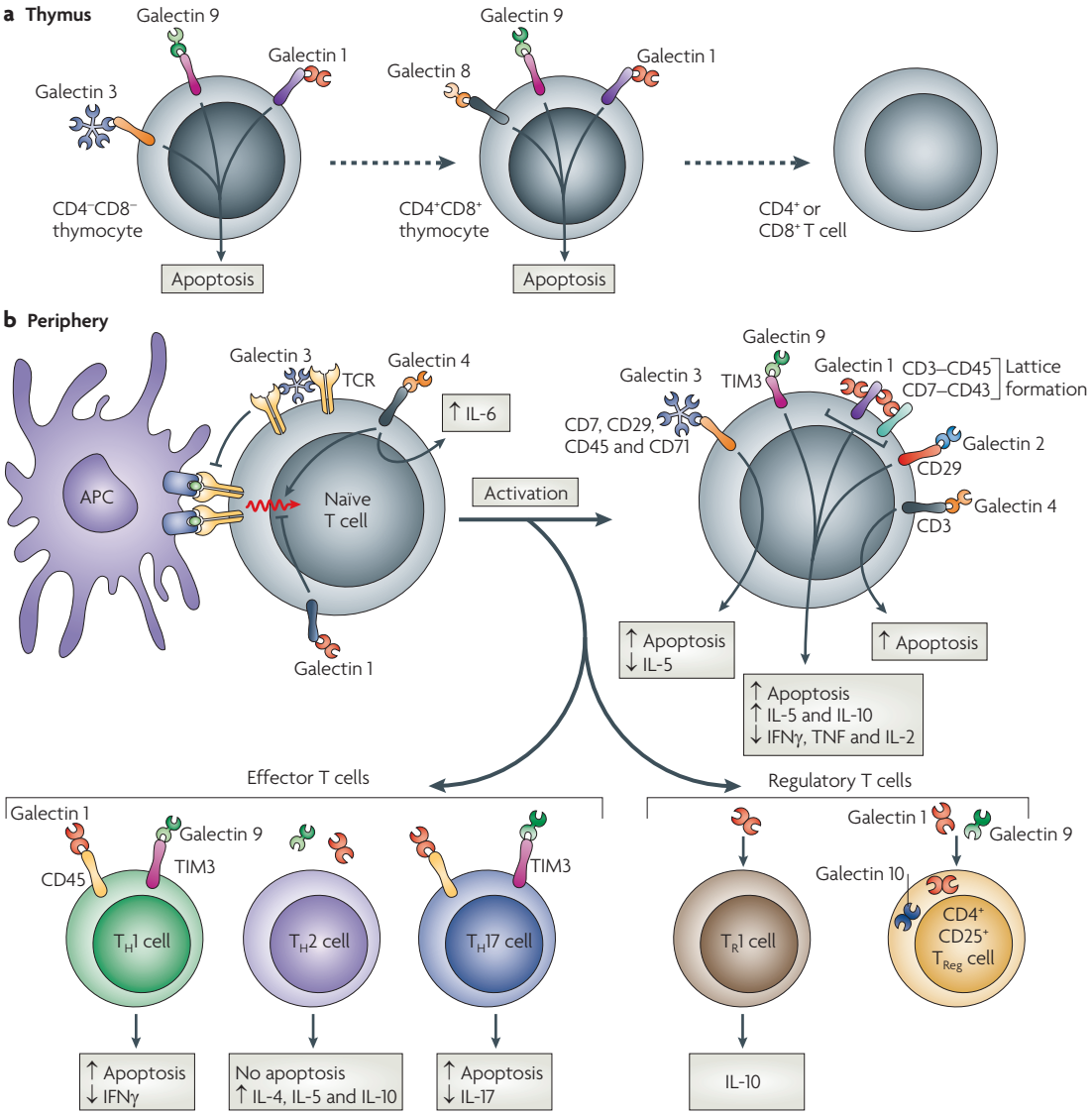

Figure 2 | **Galectins in the control of T cell homeostasis.** Galectins regulate a range of T cell processes including T cell signalling, activation, apoptosis, cytokine secretion and regulatory T (T_{Reg}) cell expansion. **a** | In the thymic microenvironment, galectin 1, galectin 3, galectin 8 and galectin 9 induce apoptosis in double-negative ($CD4^-CD8^-$) or double-positive ($CD4^+CD8^+$) thymocytes, suggesting a possible role for these galectins in regulating central tolerance. **b** | Once in the periphery, galectin 1 blocks early T cell receptor (TCR)-mediated activation signals and prolongs the survival of naive T cells, and galectin 4 triggers T cell activation and interleukin-6 (IL-6) production. Galectin 3 forms lattices with complex N-glycans to limit TCR clustering, thus increasing agonist threshold for TCR signalling. In the absence of TCR engagement, binding of galectins to N-glycans prevents filamentous actin-dependent targeting of the TCR, CD4 and the protein tyrosine kinase LCK to GM1-enriched membrane microdomains, thus preventing spontaneous TCR activation in the absence of specific ligands. Following T cell activation, galectin 1, galectin 2, galectin3, galectin 4 and galectin 9 bind to particular glycosylated receptors, including CD3, CD7, CD29, CD43, CD45, CD71 and T cell immunoglobulin domain and mucin domain protein 3 (TIM3) and trigger distinct intracellular events to induce T cell death. The repertoire of N- and O-glycan structures that 'decorate' cell surface glycoproteins or the expression of specific glycoproteins determines the differential susceptibility of T helper 1 (T_H1), T_H2 and T_H17 cells to galectin 1- and galectin 9-mediated apoptosis. Galectins can also regulate the secretion of pro- or anti-inflammatory cytokines and promote the expansion of IL-10-producing regulatory T (T_R1) cells. In addition, galectin 1 and galectin 10 contribute to the suppressive activity of $CD4^+CD25^+$ T_{Reg} cells. APC, antigen-presenting cell; IFNγ, interferon-γ; TNF, tumour necrosis factor.

Interestingly, the number of N-glycans available for the formation of galectin–glycan lattices is different for stimulatory receptors, such as epidermal growth factor receptor, compared with inhibitory receptors, such as transforming growth factor-β receptor (TGFβR) and cytotoxic T lymphocyte antigen 4 (CTLA4). This variable number of N-glycans can selectively regulate receptor turnover and endocytosis[25], thus highlighting a new mechanism of transition between cell growth and arrest based on the number and branching of N-glycans.

In this regard, at late stages of T cell activation, galectin–glycan lattices can contribute to T cell homeostasis by promoting cell surface retention of the inhibitory molecule CTLA4, thus favouring T cell growth arrest[25]. In addition, a recent study highlighted a crucial role for galectin 3–N-glycan interactions in mediating anergy of tumour-specific cytotoxic T lymphocytes (CTLs) by favouring the segregation of CD8 from the TCR[26]. Therefore, galectin–glycan lattices may have evolved as an endogenous homeostatic mechanism to prevent spontaneous T cell activation, to regulate early TCR signals and to turn off T cell effector functions after the completion of an immune response.

Galectin–glycan lattices have also been shown to modulate signalling thresholds in the thymic microenvironment, as galectin 1 imprints a negative selection signalling signature in developing thymocytes by inducing rapid and transient activation of extracellular signal-regulated kinase (ERK), while antagonizing sustained ERK activation in thymocytes that are undergoing positive selection[27]. Hence, the synchronized expression of galectins and/or the control of glycan branching can positively or negatively influence priming versus tolerance decisions by differentially regulating TCR signalling during T cell development, activation and homeostasis.

Controlling T cell survival. A promising strategy for achieving immune tolerance involves the targeted deletion of pathogenic T cells, which mimics the natural mechanisms of central tolerance and peripheral tolerance[16]. Several members of the galectin family bind to surface glycoprotein receptors on immature or mature T cells and trigger various intracellular signal pathways that lead to impaired cell growth and induction of T cell apoptosis[7,28–38] (FIG. 2, TABLE 1). Clustering and segregation of CD45 with CD3 and CD43 (also known as leukosialin) with CD7 seem to be crucial for galectin 1 signalling and subsequent cell death[6,39]. However, these glycoproteins can either positively or negatively regulate cell susceptibility to galectin 1-induced apoptosis depending on their glycosylation status[40–43]. Expression of the glycosyltransferase α2,6 sialyltransferase 1 (ST6Gal1), which incorporates α2,6 sialic acid to terminal galactose, selectively modifies the N-glycans on CD45 to inhibit galectin 1 binding[40]. Furthermore, $CD45^+$ T cells that lack expression of the core 2 *N*-acetylglucosaminyltransferase 1 (GCNT1), which is a glycosyltransferase that generates core 2 O-glycans, were resistant to galectin 1 binding[41]. This effect seems to be specific for CD45, as CD43 glycoforms that bear either core 1 or core 2 O-glycans can both mediate galectin 1-induced cell death independently of the activities of GCNT1 (REF. 42). Consistent with the ability of galectin 1 to bind CD7, $CD4^+CD7^-$ leukaemic T cells[44] are resistant to galectin 1-triggered cell death. Moreover, haploinsufficiency of GCNT1 in lymphoma T cells results in altered glycosylation and decreased binding to galectin 1 (REF. 45). These findings may represent a new escape mechanism of distinct leukaemia and lymphoma cell types that allows their survival in galectin 1-enriched microenvironments.

Remarkably, glycosylation can change dramatically during activation and differentiation of immune cells, resulting in the creation or masking of specific carbohydrate ligands for endogenous lectins[1,2]. A clear example illustrating this concept is the differential glycosylation of cell surface glycoproteins that can selectively control the survival of T_H cells by modulating their susceptibility to galectin 1 (REF. 43). Although differentiated T_H1 and T_H17 cells express the repertoire of cell surface glycans that is required for galectin 1 binding and the subsequent induction of cell death, T_H2 cells are protected from galectin 1-mediated cell death through differential α2,6 sialylation of cell surface glycoproteins[43]. In keeping with this finding, galectin 1-deficient (*Lgals1*$^{-/-}$) mice have an increased frequency of T_H1 and T_H17 cells and enhanced susceptibility to autoimmune neuroinflammation[43]. These observations unveil a molecular link between differential glycosylation of T_H cells, susceptibility to cell death and termination of the inflammatory response. Accordingly, recent studies showed that T_H2 cells promote T_H1 cell apoptosis through the secretion of galectin 1 (REF. 46), suggesting a galectin 1-dependent mechanism of counter-regulation between distinct T_H cell subsets.

Although further work is needed to fully dissect a hierarchy of upstream and downstream signalling events that are triggered by galectin 1 in effector T cells, it has been shown that this glycan-binding protein induces the activation of the transcription factor activator protein 1 (AP1), modulation of B cell lymphoma 2 (BCL-2) expression and sphingomyelinase-mediated release of ceramide[47–49]. Notably, although some studies found that apoptosis induced by galectin 1 proceeds through CD95-, cytochrome c- and caspase-independent mechanisms[50], other observation have shown that galectin 1 sensitizes T cells to CD95- and caspase 8-mediated apoptosis[38,49].

In spite of these findings, the notion that galectin 1 is a general pro-apoptotic factor has been challenged by observations that the formation of galectin 1–glycan lattices does not always result in T cell death, as exposure of naive T cells to galectin 1 results in heightened survival[51]. In addition, another study showed that galectin 1 does not alter T cell viability when included in cell cultures in the absence of dithiothreitol (a reducing agent commonly used to avoid CRD inactivation), although it still favoured the synthesis of interleukin-10 (IL-10) and the inhibition of interferon-γ (IFNγ) secretion by T cells[52] (see later). Therefore, the pro-apoptotic effects of galectin 1, but not other immunoregulatory actions, may be affected by the prevalence of reducing or oxidative microenvironments. As the thiol redox state of lymphoid organs and peripheral tissues is markedly altered during ongoing T cell responses[53], the possibility that galectin 1 might display different immunoregulatory effects *in vivo* in different tissues awaits further consideration. These apparently divergent outcomes may also be associated with the different activation and/or differentiation states of target cells, the biochemical properties of galectin 1 (that is, dimeric or monomeric forms of the protein) or the contextual regulation of different tissues

Negative selection
One step in the process of T cell differentiation in the thymus. Cells that express T cell receptors with high affinity for self antigens are eliminated from the repertoire by apoptosis after recognition of their target antigen presented by thymic dendritic cells.

Central tolerance
Tolerance created at the level of the central lymphoid organs. For T cells, positive and negative selection occurs in the thymus.

Peripheral tolerance
The lack of self-responsiveness of mature lymphocytes to specific antigens in the periphery. These mechanisms control potentially self-reactive lymphocytes that have escaped central tolerance mechanisms and prevent exuberant inflammatory reactions.

T_H cells
Subsets of activated $CD4^+$ T cells that are specialized in different functions. T helper 1 (T_H1) cells produce interleukin-2 (IL-2), interferon-γ and lymphotoxin and support cell-mediated immune responses. T_H2 cells produce IL-4, IL-5, IL-10 and IL-13, support antibody-mediated immune responses and downregulate T_H1 and T_H17 cell responses. T_H17 cells produce IL-17A, IL-17F, IL-21 and IL-22 and are important in inflammatory and autoimmune diseases.

(inflammatory or tolerogenic microenvironments). In this regard, a recent report has shown that binding of galectin 1 to specific ligands enhances dimer formation and reduces its sensitivity to oxidative inactivation, thus facilitating its biological functions[54]. Further studies are essential to bridge *in vitro* observations to characterized *in vivo* functions.

Interestingly, the chimera-type galectin 3 acts in a dual manner, either protecting T cells from apoptosis or promoting cell death, depending on whether it functions intracellularly or is added exogenously to T cell cultures[36,55]. T cells that overexpress galectin 3 are protected from apoptosis that is induced by various stimuli[55]. By contrast, extracellular galectin 3 induces apoptosis in human T cells by binding to CD7 and CD29 (also known as β1 integrin)[36] or to CD45 and CD71 (REF. 31) and mobilizes intracellular Ca^{2+} to promote the exposure of phosphatidylserine on the cell surface, thus preparing the cells for phagocytic removal[52]. Nevertheless, it is still not clear whether galectin 3 has anti- or pro-apoptotic effects during ongoing T cell responses *in vivo*. Given that galectin 3-deficient (*Lgals3*$^{-/-}$) mice frequently display attenuated T cell responses[5,56–58], it seems that dominant anti-apoptotic and pro-inflammatory activities of endogenous galectin 3 may override its pro-apoptotic potential.

Moreover, accumulating evidence indicates an important role of the tandem repeat-type galectin 9 in regulating the fate of effector T cells. This lectin acts as a binding partner for T cell immunoglobulin domain and mucin domain protein 3 (TIM3) to induce apoptosis of T_H1 cells, an effect that is associated with attenuation of autoimmune disorders and prolongation of allograft survival[7,59]. Following binding to T cells, galectin 9 induces intracellular Ca^{2+} flux and favours the activation of caspase 1 or the release of apoptosis-inducing factor depending on the nature of the target T cells[7,35,37]. The physiological relevance of this effect was recently shown in galectin 9-deficient (*Lgals9*$^{-/-}$) mice, which had increased numbers of $CD4^+TIM3^+$ T cells and showed enhanced susceptibility to the induction of autoimmune arthritis compared with control mice[60].

Although other members of the galectin family, including galectin 2, galectin 4 and galectin 8, can also affect T cell viability and exert immunoregulatory effects, their physiological relevance is still uncertain and awaits further examination *in vivo*. *In vitro* studies showed that galectin 2 triggers an apoptotic programme that involves the engagement of CD29 and activation of caspase 3 and caspase 9 (REF. 32), whereas galectin 4 preferentially binds CD3 and promotes a caspase-independent pathway of T cell death[34]. Therapeutic administration of galectin 2 or galectin 4 resulted in enhanced apoptosis of mucosal T cells and amelioration of inflammatory bowel disease[34,61]. These observations apparently contrast with reports showing that epithelial cell-derived galectin 4 activated mucosal T cells[62]. However, galectin 4, as well as other galectins, could have dual functions, exerting stimulatory or inhibitory activities depending on the activation state of the target T cells. Finally, galectin 1, galectin 3, galectin 8 and galectin 9, which are widely expressed in the thymic microenvironment, can differentially regulate the survival of $CD4^-CD8^-$ or $CD4^+CD8^+$ thymocytes[31,33,35,63], suggesting their possible involvement in regulating central tolerance mechanisms (FIG. 2a).

Thus, galectin–glycan lattices seem to be capable of regulating T cell fate, although differences exist between the type of cell death programme that is triggered by individual galectins. These differences are mainly based on variations in receptor engagement, intracellular pathways and target cell specificities. More important, the contribution of T cell apoptosis to immunoregulation *in vivo* still remains to be fully established. This is because the prevailing immunological phenotype of galectin-deficient mice might be due to dysregulation of other galectin-mediated biological processes (such as T cell signalling, cytokine production and modulation of APC function) in addition to the regulation of cell survival.

Regulating T cell cytokine secretion. An imbalance of pro-inflammatory and anti-inflammatory cytokines results in the breakdown of immune cell homeostasis and enhanced inflammation[16]. Several galectins have been shown to restore tolerance in experimental models of chronic inflammation by skewing the immune response towards a T_H2-type cytokine profile[7,34,43,60,64–69]. *In vitro*, galectin 1, galectin 2 and galectin 9 inhibit the secretion of T_H1- and T_H17-type cytokines[30,32,60,70,71] and promote the synthesis of T_H2-type cytokines[46,52,72,73]. Investigation of the mechanisms underlying these effects revealed a link between cytokine deviation and selective induction of apoptosis of T_H cells[43,46]. However, emerging evidence indicates that galectin 1 can also promote IL-10 secretion[52,64,73], inhibit IFNγ synthesis[30,70,71] and bolster TCR-induced T_H2-type cytokine production[46] in a manner that is independent of its pro-apoptotic functions. In light of these observations, it could be assumed that both apoptotic and non-apoptotic mechanisms may account for the T cell inhibitory activities of these glycan-binding proteins *in vivo*. Although the enhanced T_H1 and T_H17 cell responses that have been observed in *Lgals1*$^{-/-}$ and *Lgals9*$^{-/-}$ mice broadly support the *in vitro* effects of the recombinant proteins[43,60], it is more likely that the observed *in vivo* phenotypes involve a combination of different biological effects that are mediated by the endogenous galectins.

As mentioned above, galectin 3 exerts differential effects depending on whether it acts intracellularly or extracellularly. Administration of a galectin 3-encoding plasmid inhibited the synthesis IL-5, a typical T_H2-type cytokine[74], suggesting that galectin 3 downregulates T_H2 cell responses. However, studies using *Lgals3*$^{-/-}$ mice have indicated an essential role for endogenous galectin 3 in downregulating T_H1 cell responses in experimental models of allergic inflammation and parasite infection[58,75,76]. Therefore, genetic delivery of galectin 3 may not exactly reproduce the function of endogenous galectin 3, as it may not be expressed by the same cell types and may differ in its intracellular and extracellular mode of action. However, the phenotype of the *Lgals3*$^{-/-}$ mice in the experimental models

described above was different from that observed in a model of autoimmune neuroinflammation, in which *Lgals3*$^{-/-}$ mice showed decreased T_H1- and T_H17-type cytokine responses[56]. This suggests that endogenous galectin 3 might differentially regulate cytokine production in different pathophysiological settings.

Finally, although the phenotype of galectin 4-deficient mice has yet to be described, antibody-mediated blockade of epithelial cell-derived galectin 4 revealed a role for this two-CRD galectin in driving protein kinase Cθ-dependent IL-6 production by mucosal T cells[62]. Thus, individual members of the galectin family may differentially imprint a pro- or anti-inflammatory cytokine signature, which could dictate the development or resolution of adaptive immune responses.

Boosting regulatory T cell function. T cell homeostasis can also be achieved by active immune suppression mediated by regulatory T cell populations, including $CD4^+CD25^+FOXP3^+$ T_{Reg} cells (either inducible or naturally occurring), IL-10-producing T cells and TGFβ-producing T_H3 cells[16]. Remarkably, exposure to galectin 1 or galectin 9 promoted the differentiation of $CD4^+CD25^+$ T_{Reg} cells *in vitro*[60,72], whereas treatment with galectin 1 *in vivo* resulted in the expansion of IL-10-producing T cells, which successfully suppressed autoimmune inflammation and stress-induced fetal loss[64,69]. By contrast, galectin 3 deficiency resulted in increased frequency of $CD4^+CD25^+FOXP3^+$ T_{Reg} cells, suggesting a negative role for this galectin in T_{Reg} cell expansion[56]. Whether other members of the galectin family have an effect on T_{Reg} cell recruitment, differentiation or proliferation still remains uncertain.

A seminal advance in defining the mechanisms of T_{Reg} cell function emerged from comparative gene and proteomic analyses that revealed an upregulation of galectin 1 and galectin 10 expression within human and mouse $CD4^+CD25^+$ T_{Reg} cells compared with effector T cells[8,18]. Notably, blockade of these endogenous lectins substantially reduced the suppressive function of T_{Reg} cells[8,18] (FIG. 2). The effect seems to be mediated by cross-linking of the ganglioside GM1 by galectin 1 and activation of the TRPC5 (transient receptor potential cation channel, subfamily C, member 5) ion channel on effector T cells[77]. Although the exact mechanisms of this regulatory effect still remain elusive, the essential contribution of endogenous galectins to the suppressive function of T_{Reg} cells further emphasizes the relevance of these proteins in T cell tolerance and homeostasis.

Shaping the B cell compartment. Although compelling evidence supports the function of galectins in the control of T cell fate, limited information is available on how these proteins could shape the B cell compartment. In pioneer studies, Gauthier and colleagues[78,79] showed that the binding of the pre-B cell receptor (pre-BCR) to stromal cells during B cell development depends on the anchoring of galectin 1 to glycosylated receptors, specifically α4β1 integrin, α5β1 integrin and α4β7 integrin. In the peripheral compartment, the expression of galectin 1 is regulated by the transcription factor B lymphocyte-induced maturation protein 1 (BLIMP1) and contributes to B cell differentiation into antibody-secreting plasma cells, probably through the formation of galectin–glycan lattices[80]. In addition, endogenous galectin 3 favours B cell survival and mediates IL-4-induced differentiation towards a memory B cell phenotype[81] (FIG. 3). In addition, recent work has shown that *Lgals1* and *Lgals3* are over-represented in anergic compared with conventional B cells[82], suggesting a role of these lectins in the regulation of B cell tolerance. Although B cells were highly resistant to apoptosis induced by exogenous galectin 1 (REF. 83), recent experiments showed that enforced expression of this protein accelerated the death of memory B cells[84]. Additional studies are required to determine whether this results from an intracellular or an extracellular role of the endogenous galectin 1. So, galectins may differentially shape the B cell compartment by modulating B cell maturation, activation, differentiation and survival; however, the global influence of the biological functions of galectins *in vivo* still needs to be determined in both physiological and pathological settings.

Fine-tuning APC function. APCs, including dendritic cells (DCs) and macrophages, play an integral part in establishing immune cell homeostasis through their dual role in orchestrating protective immune responses and promoting immune tolerance[16,85]. Of note, marked glycan changes have been identified during DC maturation that determine differential binding of galectins to immature or mature DCs[86]. Consistent with a regulatory function of galectin–glycan lattices in the control of APC physiology, Partridge and colleagues[87] showed that the interruption of β1,6 branching of N-glycans by targeted deletion of GnT5 results in altered APC sensitivity to cytokine signalling. Investigation of the underlying mechanisms revealed a crucial role for galectin 3–N-glycan lattices in promoting cell surface retention of cytokine receptors by blocking the endocytic machinery, thus facilitating cytokine signalling[87]. In accordance with this finding, blockade of the synthesis of polylactosamine by deleting β1,3 *N*-acetylglucosaminyltransferase 2 reduces the threshold for macrophage activation[88], suggesting a potential role for galectin–glycan lattices in controlling APC function.

Furthermore, galectin 9 favours IL-12 production by DCs and, by ligating TIM3, synergizes with Toll-like receptors (TLRs) to initiate adaptive immune responses[89,90]. As galectin 9 also binds TIM3 to directly eliminate T_H1 cells[7], it is possible that the TIM3–galectin 9 complex may have different functions during the initiation and termination of adaptive immune responses. Concomitant with this, recent findings indicate that galectin 9 potentiates tumour-specific T cell responses through an enhancement of TIM3-dependent DC–$CD8^+$ T cell interactions[91]. Furthermore, galectin 1 promotes the maturation of DCs with an enhanced migratory phenotype[92] and its administration *in vivo* favours the recruitment of a population of immature DCs to the uterine mucosal tissue[64]. However, the exact

Alternative activation
A process triggered by interleukin-4 (IL-4) and IL-13 in macrophages that determines the development of T helper 2 (T_H2)-type responses, in contrast to classical activation, which is typically mediated by interferon-γ and is essential for the microbicidal activity of macrophages and the promotion of T_H1-type responses.

Experimental autoimmune encephalomyelitis
An inflammatory demyelinating disease of the central nervous system, which shows pathological and clinical similarities to multiple sclerosis.

Graft-versus-host disease
An immune response mounted against the recipient of an allograft (generally in the context of allogeneic bone marrow transplantation) by donor T cells that are derived from the graft.

role of endogenous galectin 1 and galectin 9 within the DC compartment has yet to be described. By contrast, DCs from galectin 3-deficient mice had a defective migratory capacity but secreted higher amounts of IL-12 and showed increased T cell stimulatory potential[75,76,93].

Macrophages are also exposed to diverse stimuli that can dictate different states of activation, including classical activation, alternative activation or deactivation[85]. In keeping with its anti-inflammatory functions, galectin 1 inhibits nitric oxide synthesis[94], increases arginase activity[94] and impairs the ability of macrophages to stimulate T cells[95], suggesting that this protein may trigger a state of alternative activation or deactivation. Moreover, galectin 3 mediates IL-4- and IL-13-induced alternative activation of macrophages by interacting with CD98 (REF. 96). Consistent with a role of alternatively activated macrophages in fuelling parasite growth, galectin 1 blocked IL-12 secretion by *Trypanosoma cruzi*-infected macrophages, resulting in enhanced parasite replication[97]. Thus, galectin–glycan interactions may be 'on and off' switches that regulate APC homeostasis and control their activation and signalling.

Galectins at the crossroads of health and disease

The regulatory effects of galectins might be due to evolutionary constraints to fulfil the particular needs of immune cell homeostasis, including the preservation of feto–maternal tolerance, the suppression of autoimmune pathology and the prevention of collateral tissue damage as a result of microbial invasion. However, these 'safeguard programmes' could also be hijacked by tumours to avoid immune recognition[16,85].

Autoimmunity and chronic inflammation. Several indications suggest that galectins have a role in suppressing chronic inflammation and autoimmunity[4,5] (TABLE 2). These anti-inflammatory activities were originally revealed in pioneer studies by Levi and colleagues[98] who identified a protective effect exerted by electrolectin, a galectin 1 homologue from electric eels, in experimental autoimmune myasthenia gravis. Subsequently, Offner and colleagues[99] reported the suppression of clinical signs of experimental autoimmune encephalomyelitis in rats by galectin 1. However, it was not clear from these early reports whether galectins exert immunoregulatory effects by modulating immune tolerance mechanisms. Subsequent studies dissected the potential mechanisms underlying these anti-inflammatory properties of galectin 1 in several rodent models. These include collagen-induced arthritis[67], concanavalin A-induced hepatitis[100], trinitrobenzene sulfonic acid-induced colitis[68], graft-versus-host disease[65], nephrotoxic serum nephritis[101], experimental autoimmune uveitis[69] and autoimmune diabetes[66,102] (TABLE 2). These effects were recently extended to other members of the galectin family, including galectin 2 and galectin 4 (which were shown to limit the severity of inflammatory bowel disease[34,61]) and galectin 9 (which was shown to suppress autoimmune neuroinflammation and collagen-induced arthritis and to prevent allograft rejection[7,59,60]).

Common features of these mouse models include a loss of T_H1 and T_H17 cells, a pronounced skewing towards a T_H2-type cytokine profile and a substantial increase in the frequency of apoptotic T cells in response to galectin treatment[65–69,100]. Still unresolved is the

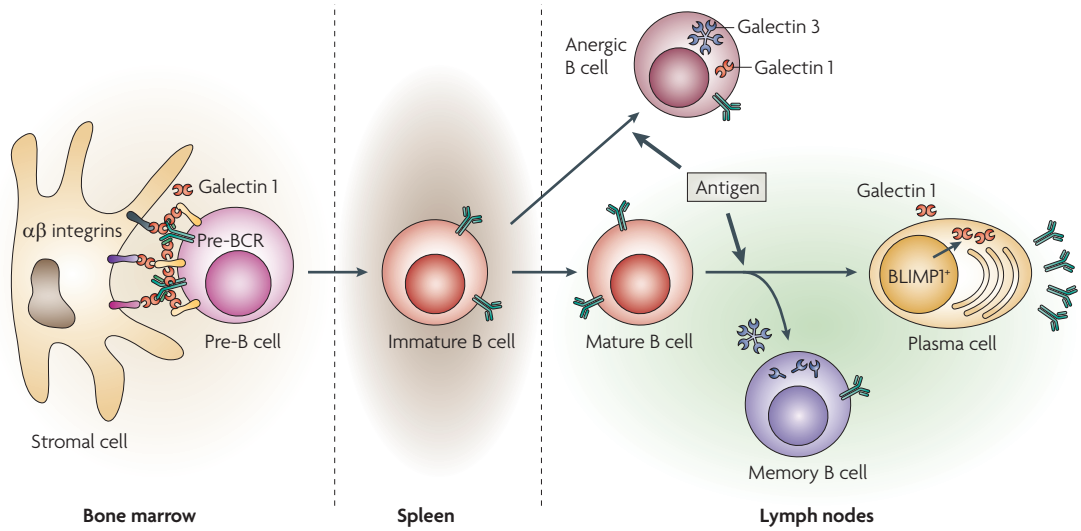

Figure 3 | **Galectins in the regulation of B cell physiology.** Galectins can shape the immature and mature B cell compartment by influencing B cell signalling and differentiation. During B cell development, synapse formation between pre-B cells and stromal cells, which drives pre-B cell receptor (pre-BCR) clustering and signalling, depends on stromal cell-derived galectin 1. This effect is mediated by engagement of α4β1 integrin, α5β1 integrin and α4β7 integrin at the pre-B cell–stromal cell interface. Once in the periphery, galectin 1 expression is upregulated by mature B cells through the activation of the transcription factor B lymphocyte-induced maturation protein 1 (BLIMP1) and promotes plasma cell differentiation and immunoglobulin production. By contrast, intracellular galectin 3 favours B cell survival and mediates interleukin-4-induced differentiation to a memory B cell phenotype. Galectin 1 and galectin 3 are upregulated in anergic and not conventional B cells, suggesting their potential roles in promoting tolerance within the B cell compartment.

question of whether the prevailing mechanisms involve the induction of T cell death and, if so, whether the deletion of pathogenic T cells *in vivo* may offer specific therapeutic advantages. In this regard, antibody-mediated T cell depletion, which showed great promise in animal models, failed to produce long-lasting therapeutic effects in patients with autoimmune disease[103]. The underlying cause of this phenomenon seemed to be that naive T cells were more efficiently depleted than tissue-infiltrating pathogenic T cells. Galectin 1, however, has the potential to eliminate pathogenic T_H1 and T_H17 cells without killing T_H2 cells and naive T cells[43,46,102].

It is now clear that additional non-apoptotic mechanisms can be induced to limit tissue damage and restore T cell tolerance following galectin treatment. T cells that are refractory to apoptosis can adopt

Table 2 | **Immunoregulatory effects of galectins in experimental models of autoimmunity and chronic inflammation**

Models	Strategies used	Clinical outcome	Mechanisms	Ref.
Experimental autoimmune myasthenia gravis	Administration of electrolectin to rabbits	Reduced severity and delayed onset	ND	98
EAE	Administration of galectin 1 to Lewis rats	Reduced severity	ND	99
	Administration of galectin 1 to C57BL/6 mice	Reduced severity	Selective elimination of T_H1 and T_H17 cells	43
	EAE induction in galectin 1-deficient mice	Increased severity	Increased T_H1 and T_H17 cell responses	43
	Administration of galectin 9 to C57BL/6 mice	Reduced severity	Apoptosis of $TIM3^+$ T_H1 cells	7
	siRNA-mediated galectin 9 silencing in SJL mice	Increased severity	Apoptosis of $TIM3^+$ T_H1 cells	7
	EAE induction in galectin 3-deficient mice	Reduced severity	Decreased T_H17 cell responses and increased T_{Reg} cell responses	56
CIA	Administration of recombinant galectin 1 or genetic delivery of galectin 1 in DBA/1 mice	Reduced severity	Increased apoptosis of activated T cells and bias towards a T_H2 cell profile	67
	Administration of galectin 9 in DBA/1 mice	Reduced severity	Decreased T_H17 cell responses and increased T_{Reg} cell responses	60
	CIA induction in galectin 9-deficient mice	Increased severity	Accumulation of $TIM3^+$ cells and decreased T_{Reg} cell responses	60
Concanavalin A-induced hepatitis	Administration of galectin 1 in BALB/c mice	Reduced severity	Increased apoptosis of effector T cells and inhibition of pro-inflammatory cytokine production	100
Inflammatory bowel disease	Administration of galectin 1 in BALB/c mice with TNBS-induced colitis	Reduced severity	Increased apoptosis of mucosal effector T cells and suppression of pro-inflammatory cytokine production	68
	Administration of galectin 2 in BALB/c mice with DSS-induced colitis	Reduced severity	Suppression of pro-inflammatory cytokine production and apoptosis of mucosal T cells	61
	Antibody-mediated blockade of galectin 4 in C57BL/6 mice	Reduced severity	Decreased IL-6 production	62
	Administration of galectin 4 in BALB/c mice with DSS-induced colitis	Reduced severity	Increased apoptosis of mucosal effector T cells and suppression of T_H1- and T_H17-type cytokines	34
Nephrotoxic nephritis	Administration of galectin 1, galectin 3 and galectin 9 in Kyoto rats	Reduced severity	Blockade of macrophage recruitment by galectin 1 and galectin 3; induction of $CD8^+$ T cell apoptosis by galectin 9	101
Autoimmune diabetes	Genetic delivery of galectin 1 in NOD–*Rag1*$^{-/-}$ mice	Reduced severity	Increased T cell apoptosis and decreased T_H1-type cytokine production	66
	Administration of galectin 1 in NOD mice	Reduced severity	Increased T cell apoptosis and decreased number of T_H1 and T_H17 cells	102
Graft-versus-host disease	Administration of galectin 1 in AKR (H–2k) mice receiving allogeneic haematopoietic stem cell transplant	Reduced severity and increased host survival	Increased apoptosis of T cells and reduced production of T_H1-type cytokines	65
Experimental autoimmune uveitis	Administration of galectin 1 in B10. RIII mice	Reduced severity	Increased frequency of IL-10-producing T cells and bias toward a T_H2-type cytokine profile	69

CIA, collagen-induced arthritis; DSS, dextran sodium sulfate; EAE, experimental autoimmune encephalomyelitis; IL, interleukin; ND, not determined; NOD, non-obese diabetic; *Rag1*, recombination activating gene 1; siRNA, small interfering RNA; T_H, T helper; TIM3, T cell immunoglobulin domain and mucin domain protein 3; TNBS: trinitrobenzene sulfonic acid; T_{Reg}, regulatory T.

a T_H2 or T_{Reg} cell profile or have a modified migration pattern. Galectin 1 inhibits T cell adhesion *in vitro* and blocks T cell transendothelial migration and trafficking through inflamed tissues *in vivo*[70,104,105]. In addition, galectin 1 has been shown to restore T cell-mediated feto–maternal tolerance in stress-challenged pregnancies by modulating the balance of T_H1- and T_H2-type cytokines, by promoting the expansion of IL-10-producing T cells and by recruiting uterine DCs with an immature phenotype[64]. In keeping with these findings, a recent study[106] found that decidual NK cells can negatively regulate the survival of decidual T cells through a galectin 1-mediated pathway, suggesting that this glycan-binding protein can preserve feto–maternal tolerance through both apoptotic and non-apoptotic mechanisms. In addition, administration of recombinant galectin 1 in autoimmune uveitis[69] or injection of recombinant galectin 9 in collagen-induced arthritis[60] resulted in significant expansion of T_{Reg} cells, which coincided with a decline in the numbers of pathogenic T_H1 and T_H17 cells. However, as galectins can target a wide range of different cell types, the results obtained with recombinant proteins given at pharmacological doses in experimental animal models should only be considered with the caveat that the overall *in vivo* effects may not reflect the individual functions shown *in vitro*.

Until recently, the function of endogenous galectins in the evolution of autoimmune inflammation was unknown. However, an essential function of endogenous galectin 1 in limiting pathogenic T cell responses has been identified through the use of *Lgals1*$^{-/-}$ mice, which have enhanced antigen-specific T_H1 and T_H17 cell responses and are more susceptible to autoimmune inflammation and immune-mediated fetal rejection compared with their wild-type counterparts[43,64]. In addition, *Lgals9*$^{-/-}$ mice have enhanced susceptibility to experimental arthritis and diminished frequency of T_{Reg} cells[60]. By contrast, targeted deletion of galectin 3 results in decreased pathogenic T_H1 and T_H17 cell responses, increased expansion of T_{Reg} cells and reduced disease severity in a model of autoimmune neuroinflammation[56]. Thus, in contrast to previous assumptions based on the idea of evolutionarily conservation and functional redundancy, these studies suggest that individual members of the galectin family have distinct functions in the regulation of inflammatory responses. Furthermore, GnT5-deficient mice, which lack β1,6 N-glycan branch structures, develop a spontaneous disease that resembles progressive multiple sclerosis[107]. As various members of the galectin family can bind β1,6 N-glycan branch structures, it is not surprising that GnT5 deficiency results in a more severe phenotype than that of mice lacking individual galectins.

Although there are still limited clinical data on the role of galectins in autoimmunity and chronic inflammation, it has been reported that high serum levels of galectin 3 correlate with disease severity[108], and galectin 1, galectin 3, galectin 8 and galectin 9 are differentially expressed in the synovial tissue of patients with arthritis compared with healthy subjects[60,109,110].

Galectin 3 has recently been suggested to be a biomarker of human atherosclerotic plaque progression[111], which is consistent with the observation that *Lgals3*$^{-/-}$ mice have reduced atherosclerotic lesions[57]. Ozaki and colleagues[112] showed that macrophages from atherosclerotic lesions express galectin 2, which promotes lymphotoxin-α secretion and amplifies the inflammatory cascade. Interestingly, a single nucleotide polymorphism in the *LGALS2* gene, which affects its transcription, is associated with increased susceptibility to myocardial infarction, at least in the Japanese population[112]. Increased incidence of autoantibody responses to galectins was also found in patients with autoimmune disorders, and this correlated with poor disease outcome[113,114]. Taken together, these observations suggest cell-intrinsic roles for galectins in the homeostatic regulation of effector and T_{Reg} cell populations that might collectively contribute to the control of T cell tolerance and autoimmunity.

Allergy. Because galectin 3 can specifically bind IgE[5], *in vivo* studies have been carried out to investigate the functional relevance of this glycan-binding protein in allergic inflammation. In a mouse model of asthma, *Lgals3*$^{-/-}$ mice showed reduced airway hyperresponsiveness, lower levels of lung eosinophilia and increased bias towards a T_H1-type response compared with similarly treated wild-type mice[58]. This suggests that endogenous galectin 3 has a crucial role in sustaining allergic inflammation and promoting T_H2 cell polarization. However, other studies have shown reduced eosinophil infiltration and downregulated IL-5 synthesis following airway antigen challenge in rats that have been treated intranasally with cDNA encoding galectin 3 (REF. 74). These apparently contrasting results could be explained by the stimulatory effects of physiological levels of galectin 3 *in vivo* during inflammatory reactions compared with the inhibitory effects of pharmacological doses of this protein when delivered to sites of inflammation[5].

Supporting a pro-inflammatory role for galectin 3 in the development of airway inflammation, mast cells from *Lgals3*$^{-/-}$ mice showed defective IgE-mediated responses, including reduced histamine release and IL-4 production[115]. Moreover, antibody-mediated blockade revealed a crucial role for galectin 3 in supporting eosinophil rolling and adhesion through the inflamed endothelium[116]. Surprisingly, administration of galectin 9, which was first identified as an eosinophil chemoattractant[5], resulted in attenuated airway hyperresponsiveness and reduced T_H2-type responses in an experimental asthma model[117]. Thus, galectins can act either as amplifiers or silencers of allergic inflammation by regulating T_H2-type cytokine production, mast cell physiology and eosinophil trafficking. In this context, galectin 10 and galectin 14 were originally identified as eosinophil-specific proteins[118,119], although their biological functions in allergic inflammation are still uncertain.

Antitumour immune responses. Recent advances have enabled the association of specific glycosylation signatures with particular disease states, including cancer and metastasis[1,2,120], suggesting the possible development of

Exosome
A small lipid bilayer vesicle that is made up of plasma membrane or membrane derived from intracellular vesicles that are released from different cell types.

new therapeutic and diagnostic approaches based on the underlying glycan structures. In addition, gene and protein expression profiles have frequently shown the upregulation of galectins in several tumours and metastatic lesions, which may be used to delineate a glycosylation signature that indicates poor prognosis[121]. But how do galectin–glycan lattices shape the dynamic interactions between tumours and the host immune system? In addition to their appreciated roles in tumour invasiveness and angiogenesis[121,122], galectins that are secreted by tumour or stromal cells can also have tolerogenic effects by targeting the survival of effector T cells, skewing the cytokine balance, favouring T cell anergy and/or promoting T_{Reg} cell expansion[85,121] (FIG. 4). Blockade of galectin 1 expression in melanoma cells results in heightened T cell-mediated tumour rejection and increased secretion of T_H1-type cytokines[123]. Moreover, Reed–Sternberg cells, the malignant component in Hodgkin's lymphoma, selectively overexpress galectin 1, which favours the secretion of T_H2-type cytokines, induces T_{Reg} cell expansion and inhibits Epstein–Barr virus (EBV)-specific T cell immune responses[71,72]. Furthermore, prostate cancer cells that have low expression of the glycosyltransferase GCNT1 are resistant to galectin 1-mediated apoptosis, but express high levels of this protein to selectively eliminate effector T cells[124].

These T cell inhibitory activities were further confirmed in human cancerous tissues, in which a strong inverse correlation was found between galectin 1 expression and the presence of tumour-infiltrating T cells[125]. In addition, a recent report showed that delivery of galectin 3 induces apoptosis of tumour-reactive $CD8^+$ T cells and facilitates tumour growth in a mouse model of colorectal cancer[126]. Furthermore, galectin 3 expression correlated with apoptosis of tumour-associated lymphocytes in human melanoma biopsies[127]. Moreover, a recent study[128] found that exosomes of EBV-infected nasopharyngeal carcinoma cells contained high levels of galectin 9, which triggered apoptosis of $TIM3^+$ EBV-specific T_H1 cells. Galectin 9 can also modulate tumour-specific immune responses by facilitating TIM3-dependent DC–$CD8^+$ T cell interactions[91].

Interestingly, a recent study[26] showed that galectin 3 can promote T cell dysfunction by increasing the distance between the TCR and its co-receptor CD8 in anergic tumour-infiltrating human CTLs. The cytotoxic function of these cells could be restored when T cells were exposed to galectin-specific disaccharide ligands[26], thus providing a rational explanation for the anergic state that is frequently observed in human CTLs. Hence, targeting galectin–glycan interactions may counteract tumour-induced T cell tolerance by preventing T cell apoptosis, blocking T_{Reg} cell expansion and reverting CTL anergy.

Conclusions and future directions

Two decades of intensive research since the cloning of the first galectins have yielded a great deal of useful information regarding the role of these proteins and their glycan partners in a wide range of biological processes. As discussed in this article, galectins are important regulators of immune tolerance and homeostasis, making them attractive therapeutic targets for limiting autoimmune inflammation, preventing allograft rejection and potentiating antitumour responses. However, before galectin-based therapies can be fully embraced, a greater understanding of the mechanisms involved in the functions of these proteins is necessary. This includes *in vivo* studies of the

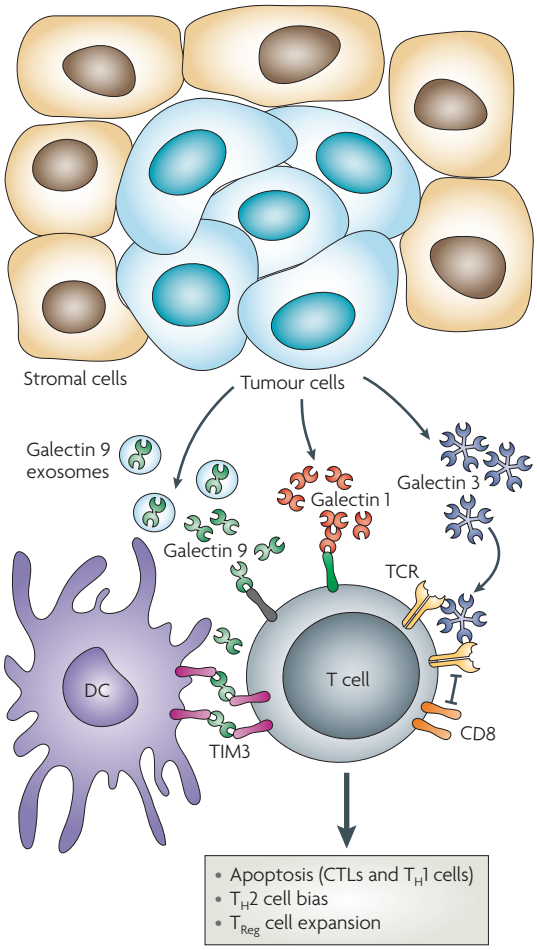

Figure 4 | **Galectin–glycan interactions in the tumour microenvironment.** Malignant transformation and metastasis is often associated with altered glycosylation of tumour and tumour-associated inflammatory cells. Galectins (including galectin 1, galectin 3 and galectin 9) that are secreted by tumour cells or contained in tumour exosomes modulate immune escape of the tumour by targeting the survival of effector cytotoxic T lymphocytes (CTLs) and T helper 1 (T_H1) cells, skewing the balance towards a T_H2-type cytokine profile and/or inducing the differentiation and/or expansion of regulatory T (T_{Reg}) cells. Alternatively, galectin 3 induces T cell dysfunction by distancing the T cell receptor (TCR) from its co-receptor CD8 in anergic infiltrating CTLs, thus contributing to T cell tolerance in the tumour microenvironment. By contrast, galectin 9 potentiates tumour immune responses by modulating T cell immunoglobulin domain and mucin domain protein 3 (TIM3)-dependent interactions between dendritic cells (DCs) and CD8 T cells.

compensatory, unique or synergistic regulatory effects of individual galectins in inflammatory and autoimmune processes, as well as a comparative analysis of the antitumour effects of galectin-specific inhibitors, such as blocking antibodies, synthetic glycomimetics or natural polysaccharides[129].

Similarly to many cytokines and growth factors, galectins can have a differential effect on immune responses. This depends on many different intrinsic factors, including their dimerization or oligomerization status and their concentrations and stability in oxidative microenvironments, as well as extrinsic factors, such as the activation and differentiation status of target cells and the regulated activity of glycan-modifying enzymes in distinct pathophysiological settings. Although original assumptions based on similarities in carbohydrate specificity and evolutionarily conservation suggested extensive redundancy within the galectin family, recent observations highlighted substantial differences in glycan recognition by individual galectins[9,10] and the occurrence of divergent immunological phenotypes in galectin-deficient mice[43,60,64,115,130].

Altogether, the evidence presented here suggests that galectin–glycan lattices might have evolved as an endogenous homeostatic mechanism to turn off T cell effector functions. In support of this concept, the expression of galectin 1 and galectin 3 is upregulated in inflammatory macrophages and activated T cells[29,130–133], and this inhibits clonal expansion in an autocrine fashion[29], thus resembling other negative regulatory molecules such as CTLA4 and programmed death ligand 1 (REFS 16,85). Under this complex but fascinating scenario, future work is warranted to address the cell-specific roles of individual galectins *in vivo* and their coordinated and/or synergistic effects as on and off switches that control tolerogenic or inflammatory responses. In addition, further studies should establish whether the activities observed *in vitro* also operate *in vivo* and whether the demonstrated functions are a result of intracellular or extracellular effects in the *in vivo* setting. Thus, the potential therapeutic manipulation of galectin–glycan interactions to limit autoimmune inflammation, prevent allograft rejection or potentiate antitumour responses prompts further investigation of the mechanisms that underlie the broad immunoregulatory activities of this protein family.

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DATABASES

UniProtKB: http://www.uniprot.org
CD7 | CD43 | CD45 | galectin 1 | galectin 2 | galectin 3 | galectin 4 | galectin 7 | galectin 8 | galectin 12 | TIM3

FURTHER INFORMATION

Consortium for Functional Glycomics: http://www.functionalglycomics.org
Glycoforum Japan: http://www.glycoforum.gr.jp
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