

REPORT

The coming of age of galectins as immunomodulatory agents: impact of these carbohydrate binding proteins in T cell physiology and chronic inflammatory disorders

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Immune cell homeostasis is attributed to multiple distinct safety valves that are interconnected and intervene at defined checkpoints of the life cycle of immunocytes to guarantee clonal expansion and functional inactivation of self-reactive potentially autoaggressive lymphocytes. Galectins, animal lectins defined by shared consensus amino acid sequence and affinity for β -galactose containing oligosaccharides, are found on various cells of the immune system, and their expression is associated with the differentiation and activation status of these cells. Over the past few years, galectins have been implicated in the regulation of many aspects of T cell physiology such as cell activation, differentiation, and apoptosis. In addition, a growing body of experimental evidence indicates that galectins may play critical roles in the modulation of chronic inflammatory disorders, autoimmunity, and cancer. Given the increased interest of immunologists in this field, the growing body of information raised during the past few years and the potential use of galectins as novel anti-inflammatory agents or targets for immunosuppressive drugs, we will summarise recent advances on the role of galectins in different aspects of T cell physiology and their impact in the development and/or resolution of chronic inflammatory disorders, autoimmunity, and cancer.

Galectins, a growing family of evolutionarily conserved carbohydrate binding proteins, have recently attracted the attention of immunologists as novel regulators of inflammation and autoimmunity.^{1,2}

GALECTINS AS IMMUNOMODULATORY AGENTS: BIOCHEMISTRY, STRUCTURE, AND SUBCELLULAR LOCALISATION

Members of the galectin family are defined by a conserved carbohydrate-recognition domain (CRD) with a canonical amino acid sequence and affinity for β -galactosides.^{3,4} To date, 15 mammalian galectins have been identified, which can be subdivided into those that have one CRD and those that have two CRDs in tandem. In addition, galectin-3, a one-CRD galectin, is unique in that it contains unusual tandem repeats of short amino acid stretches fused onto the CRD.^{4,5}

Many galectins are either bivalent or multivalent with regard to their carbohydrate binding activities—some one-CRD galectins exist as dimers; two-CRD galectins have two carbohydrate binding sites, and galectin-3 forms oligomers when it binds to multivalent carbohydrates.⁵ In this way, galectins can form ordered arrays of complexes when they bind to multivalent glycoconjugates much like the lattices formed by antibodies and multivalent antigens.⁶ Some galectins are distributed in a wide variety of tissues, whereas

others have a more restricted localisation. The expression of galectins is modulated during the activation and differentiation of immune cells and changes under different physiological or pathological conditions.⁷

Galectins are present both inside and outside cells, and function both intracellularly and extracellularly. Although galectins have features of cytosolic proteins, they are secreted by non-classical (non-ER-Golgi) pathways.^{4,8} Extracellularly, galectins can bind to cell surface glycoconjugates that contain suitable galactose-containing oligosaccharides. As galectins can bind either bivalently or multivalently, they can crosslink cell surface glycoconjugates, which, like many other receptor–ligand systems, can trigger a cascade of transmembrane signalling events. Through this mechanism, galectins can modulate processes that include apoptosis, cytokine secretion, and cell adhesion and migration.⁷ Furthermore, intracellularly galectins are engaged in processes that are essential for basic cellular functions such as pre-mRNA splicing, regulation of cell growth, and cell cycle progression.⁸

GALECTINS IN THE REGULATION OF T CELL PHYSIOLOGY

Galectins as regulators of T cell survival: influence in the maintenance of central and peripheral T cell tolerance

A dynamic balance in the quantity and activation state of the cells participating in immune responses is essential for achieving appropriate proliferative and effector responses to antigens while avoiding autoimmunity.⁹ Immune cells are subject to cell death checkpoints at many stages during their lifespan to ensure proper development, maintain homeostasis and prevent disease. Different families of proteins can regulate immune cell death, including death inducing ligands, death receptors, and intracellular regulators of death pathways.

Galectin-1

A growing body of experimental evidence indicates that galectins may play critical roles in the regulation of immune cell death (reviewed in references 1 and 7) (fig 1). Galectin-1 induces cell growth inhibition and cell cycle control and promotes apoptosis of activated T cells.^{10–20} Galectin-1 has shown specific growth inhibitory properties towards concanavalin A (Con A) stimulated rat and mouse T cells,^{19,20} phytohaemagglutinin (PHA) activated human T cells^{10,17} and

Abbreviations: APC, antigen presenting cell; CIA, collagen-induced arthritis; CRD, carbohydrate recognition domain; EAE, experimental autoimmune encephalomyelitis; EAMG, experimental autoimmune myasthenia gravis; ECM, extracellular matrix; ER, endoplasmic reticulum; GVHD, graft versus host disease; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; NSN, nephrotoxic serum nephritis; Th, T helper; TNF, tumour necrosis factor

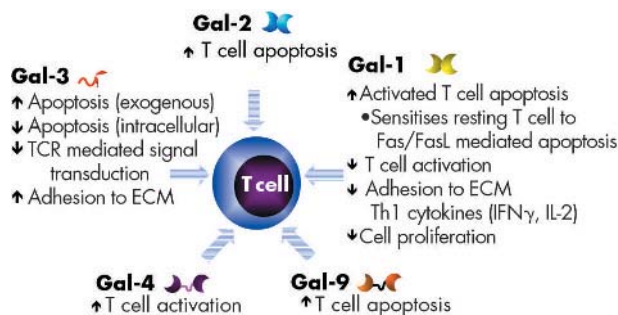


Figure 1 Influence of galectins (Gal) in the regulation of T cell physiology. This scheme illustrates the influence of different members of the galectin family on different T cell functions including T cell apoptosis, activation, adhesion, and cytokine secretion. ECM, extracellular matrix.

human alloreactive T cells.¹⁵ Furthermore, galectin-1 induces apoptosis of developing thymocytes and peripheral T lymphocytes.^{11–20}

One concern regarding the proapoptotic activity of galectin-1 is that this effect has been demonstrated in most cases at relatively high concentrations (micromolar range) and it is uncertain whether high levels of soluble protein can be produced in vivo. Interestingly, recent evidence indicates that the amount of galectin-1 secreted by different cell types is sufficient to kill T cells, when galectin-1 is presented in the context of extracellular matrix (ECM) glycoproteins.²¹ Moreover, the presence of galectin-1 in activated but not resting T cells^{10, 22} suggests a potential autocrine suicide mechanism to achieve homeostasis during the termination of an immune response. Interestingly, this regulated expression involves signalling through MEK1/extracellular regulating kinase (ERK), p38 mitogen activated protein kinase (MAPK), and p70^{S6} kinase.²²

Different glycoconjugates on the surface of activated T cells appear to be primary receptors for galectins, including CD45, CD43, CD7, and CD2.^{23–25} In particular, CD7 has been identified as a critical receptor for galectin-1 induced cell death,²⁵ and it has been recently demonstrated that CD7 negative T cells from patients with mycosis fungoides/Sézary syndrome are protected from galectin-1 triggered death.^{26, 27} Interestingly, galectin-1 binding to T cells results in a marked redistribution of receptors into segregated membrane microdomains.²³

The expression of *N*-acetylglucosamine ligands by T cells, which may determine susceptibility to galectin-1 induced cell death, is tightly controlled by specific glycosyltransferases, which are regulated during T cell development, activation, and differentiation.^{28, 29} Galvan and colleagues²⁸ demonstrated that CD45 positive T cells lacking the core-2- β -1,6-*N*-acetylglucosaminyltransferase (C2GnT) are resistant to galectin-1 induced cell death. This enzyme is responsible for creating branched structures on *O*-glycans of T cell surface glycoproteins, such as CD45. In addition, other glycosyltransferases can also act to reduce galectin binding to indirectly or directly masking galectin saccharide ligands. In this sense, addition of α 2,6-linked sialic acids to lactosamine units by the ST6Gal-I sialyltransferase has been shown to block galectin-1 binding by interfering with lactosamine insertion into the binding pocket of galectin-1.²⁹ Interestingly, recent findings suggest that altered glycosylation of T cells during human immunodeficiency virus (HIV)-1 infection increases their susceptibility to galectin-1 induced cell death,³⁰ suggesting that this apoptotic pathway may contribute to HIV-1 induced immunosuppression.

The signal transduction events that lead to galectin-1 induced cell death include the induction of specific

transcription factors (that is adaptor protein (AP)-1 and nuclear factor of activated T cells (NFAT)),^{16, 31} the modulation of Bcl-2 protein production,¹⁶ activation of caspases,¹⁷ modulation of the ceramide pathway,^{17, 32} and release of cytochrome *c*.¹⁷ However, a recent study shows that apoptosis induced by galectin-1 in a T cell line is not dependent on the activation of caspase-3 or on cytochrome *c* release.¹⁸ Therefore, it seems evident that galectin-1 might trigger different death pathways or different apoptosis endpoints in different cell types.

In addition, however, using a human allogeneic T cell model we have recently demonstrated that alternative mechanisms may operate to achieve T cell immunosuppression including inhibition of proinflammatory cytokines in the non-apoptotic cell population.¹⁵ Moreover, there is recent evidence that exposure of activated leukocytes to dimeric galectin-1 may contribute to phagocytic recognition of these cells by inducing surface exposure of phosphatidylserine with no apparent signs of apoptosis.³³ Most recently Endharti and colleagues³⁴ demonstrated that, in contrast to the proapoptotic role of galectin-1 on activated T cells, secretion of this protein by stromal cells is capable of supporting survival of naive T cells without promoting proliferation.³⁴

Although these findings suggest a pivotal role for galectin-1 in the establishment and maintenance of T cell tolerance and homeostasis, targeted disruption of the galectin-1 gene in knockout mice resulted in the absence of major spontaneous abnormalities, suggesting potential redundancy between different members of the galectin family.^{2, 4} However, in contrast to these assumptions, recent work clearly indicates that galectin family members are not redundant and that there are subtle but functionally relevant differences in the specificity and function of individual members of the galectin family to regulate inflammatory responses (reviewed in reference 5).

Galectin-2

Galectin-2, a member of the galectin family structurally related to galectin-1 has been also demonstrated to act as a proapoptotic protein.³⁵ Investigation of the molecular mechanisms and intracellular pathways involved in the proapoptotic activity of this protein revealed binding to cell surface β -integrins but not to CD3 or CD7 receptors, involvement of caspase-3 and caspase-9, enhanced cytochrome *c* release, disruption of the mitochondrial membrane potential and an increase of the Bax/Bcl-2 ratio.³⁵ Studies of cytokine secretion by activated T cells incubated with galectin-2 revealed a significantly shift towards a T helper (Th) 2 profile.³⁵ These observations revealed substantial differences between galectin-2 and galectin-1 in terms of cell surface ligands and intracellular signalling pathways leading to cell death.

Galectin-3

Recent evidence indicates that galectin-3 could act in a dual manner either protecting cells from apoptosis or stimulating cell death depending on whether the protein is found in the intracellular compartment^{36, 37} or it acts extracellularly.³⁸ Yang and colleagues³⁶ demonstrated that T cell transfectants overexpressing galectin-3 display higher growth rates than control transfectants and are protected from apoptosis induced by a variety of agents including Fas ligation and staurosporine.³⁶ Furthermore, the authors demonstrated that galectin-3 interacts with Bcl-2 in a lactose dependent manner.³⁶ Interestingly, Fukumori and colleagues³⁸ demonstrated that secreted extracellular galectin-3 can signal apoptosis of human T cells. after binding to cell surface glycoconjugate receptors through carbohydrate dependent interactions. Analysis of the routes of signalling revealed that

galectin-3 binds mainly to CD7 and CD29 (β 1-integrins) resulting in activation of the mitochondrial pathway including cytochrome *c* release and caspase-3, but not caspase-10 activation.³⁸ Furthermore, recent evidence suggested a functional cross-talk between intracellular and extracellular galectins in the regulation of T cell death.¹⁸ Hahn *et al*¹⁸ demonstrated that galectin-1-induced cell death is inhibited by intracellular expression of galectin-3. In addition, it has been recently demonstrated that galectin-3 can act as a novel Fas-binding partner involved in the selection of different apoptotic signalling pathways.³⁹ Finally, the antiapoptotic activity of galectin-3 has been directly or indirectly demonstrated in pathological situations including lymphomas,⁴⁰ other types of cancer,⁴¹ and rheumatoid arthritis.^{42, 43}

Galectin-9

Similarly to the proapoptotic role of galectin-1 in the thymic microenvironment, mouse galectin-9 also induces apoptosis of thymocytes in a lactose-inhibitable fashion.⁴⁴ Furthermore, galectin-9 induces apoptosis of peripheral CD4+ and CD8+ T cells through a Ca^{2+} -calpain-caspase-1 signalling pathway.⁴⁵

Other galectins: apoptosis on other cell types

Although there is still no evidence regarding the role of other members of the galectin family in T cell death, it is becoming increasingly apparent that other galectins can regulate survival of many different cell types. For example, transfection of galectin-7 (p53-induced gene 1) in epithelial tumour cell lines revealed its intracellular proapoptotic activity.⁴⁶ On the other hand, Arbel-Goren and colleagues⁴⁷ revealed a major role for galectin-8 in the regulation of cell growth and apoptosis of tumour cell lines. Interestingly the authors found that cyclin dependent kinase inhibitors and jun kinase (c-Jun-N-terminal kinase (JNK)) can regulate the choice between growth arrest and apoptosis induced by galectin-8.⁴⁷ In addition, previous studies demonstrated that galectin-12 expression regulates cell cycle progression⁴⁸ and apoptosis of adipocytes.⁴⁹ Thus, different members of the galectin family, can positively or negatively regulate survival of different cell types.

Galectins as regulators of T cell activation and differentiation

Immune cell activation and differentiation are key processes which determine the successful resolution of an inflammatory response following an antigenic challenge. In this context galectins have also been shown to influence different events associated with T cell activation and differentiation (see fig 1).

Galectin-1

In addition to its role in the modulation of T cell survival, Vespa and colleagues⁵⁰ demonstrated that galectin-1 acts as a potent modulator of T cell receptor (TCR) signals and antagonises TCR induced interleukin (IL)-2 production in a murine T cell hybridoma clone. Interestingly, the same group further demonstrated that galectin-1 induces partial TCR- ζ chain phosphorylation and is able to antagonise TCR responses known to require costimulation and processive protein tyrosine phosphorylation, such as IL-2 production, whereas it is permissive for TCR responses that only require partial TCR signals, such as CD69 upregulation and apoptosis.⁵¹ Thus, by acting at early events of TCR signalling, galectin-1 can also control T cell activation.

Galectin-3

Galectin-3 has been shown to positively contribute to cell interactions between dendritic cells and naive T cells in T cell dependent areas of lymph nodes.⁵² In contrast, Demetriou

and colleagues⁵³ reported that galectin-3 might play a role in restricting signal transduction initiated by TCR complexes. The authors hypothesised that galectin-3 might form multi-valent complexes with *N*-glycans on the TCR, thereby restraining the lateral mobility of TCR complexes.⁵³ This effect was abrogated in mice deficient in β 1,6 *N*-acetylglucosaminyltransferase (Mgat5), a crucial enzyme in the *N*-glycosylation pathway. These mice showed enhanced delayed-type hypersensitivity (DTH) responses and increased susceptibility to autoimmunity.⁵³ Thus, galectin-3 may directly or indirectly influence T cell interactions with antigen presenting cells (APCs) and control T cell activation by regulating the immunological synapse.⁵³

Galectin-4

Using a combined antigen-screening approach, Hokama and colleagues⁵⁴ found that galectin-4 plays a key role in CD4+ T cell activation under intestinal inflammatory conditions.⁵⁴ Galectin-4-mediated stimulation of T cells was reflected by increase IL-6 production and was shown to exacerbate chronic colitis and delay recovery from acute intestinal injury.⁵⁴

Galectins as regulators of cytokine secretion

The Th 1/Th 2 cytokine balance is crucial for determining the outcome of chronic inflammatory disorders and autoimmunity. It has been demonstrated that galectins can differentially regulate the cytokine balance in different inflammatory conditions (see fig 1).

Galectin-1

Galectin-1 has been shown to block secretion of proinflammatory cytokines in vitro, including IL-2, interferon γ (IFN γ), and tumour necrosis factor α (TNF α).^{51, 55} In addition in vivo studies in experimental models of chronic inflammation and autoimmunity showed the ability of galectin-1 to skew the balance towards a Th 2-type cytokine response, with decreased levels of IFN γ and increased secretion of IL-5 or IL-10 by pathogenic T cells.⁵⁶⁻⁵⁹ In the context of infection, we have demonstrated in an experimental model of *Trypanosoma cruzi* infection, that very low concentrations of galectin-1 (nanomolar range) are sufficient to downregulate the production of IL-12 and this effect correlated with an inhibitory effect on parasite replication without affecting cell viability.⁶⁰ Furthermore, recent evidence indicates that, upon viral infection, galectin-1 can induce dendritic cell secretion of IL-6, a critical proinflammatory cytokine.⁶¹ In addition, van der Leij and colleagues⁶² recently reported a marked increase in IL-10 mRNA and protein levels in non-activated and activated CD4+ and CD8+ T cells following treatment with recombinant galectin-1.⁶² Since IL-10 is known to suppress Th 1-type responses, upregulation of IL-10 may thus contribute to the immunoregulatory activity of galectin-1.

Galectin-2

Sturm and colleagues³⁵ have demonstrated that galectin-2 can modulate T cell derived cytokines in vitro and shift the balance towards a Th 2 profile.³⁵ In addition it has been shown that this protein can regulate lymphotoxin α secretion, which can in turn affect the degree of inflammation during myocardial infarction.⁶³ Therefore, galectin-2, as well as galectin-1, can modulate the balance of proinflammatory and anti-inflammatory cytokines.

Galectin-3

Galectin-3 has been shown to act in most cases as a "proinflammatory cytokine", as has been clearly demonstrated by the attenuated inflammatory response in galectin-3 knockout mice⁶⁴ (and reviewed in reference 65). This has

been also demonstrated by the reciprocal regulation of proinflammatory cytokines to regulate galectin-3 expression.⁴³ In addition, this lectin suppresses T h2-mediated allergic inflammation by blocking IL-5 secretion by antigen-specific T cell lines.⁶⁶ Furthermore, we have recently demonstrated that galectin-3 is a critical intracellular mediator of IL-4 induced survival and differentiation of B cells into a memory phenotype.⁶⁷

Galectin-4

As described above, galectin-4 has been recently shown to induce reactivity of intestinal CD4+ T cells and exacerbate inflammation in intestinal tissue.⁵⁴ Galectin-4 produced by epithelial cells induces IL-6 production by CD4+ T cells, an effect mediated by PKC θ associated pathways through the immunological synapse.⁵⁴ Hence, galectin-4 functions as a T cell activator by favoring secretion of proinflammatory cytokines.

Taken together, these data suggest, that under distinct physiological or pathological conditions, different members of the galectin family provide inhibitory or stimulatory signals to regulate immune cell homeostasis and regulate inflammation following an antigenic challenge. Although the mechanisms of galectin mediated modulation of cytokine production are still obscure, we might hypothesise that different galectin family members trigger different signalling cascades to modulate the expression of different transcription factors involved in the regulation of cytokine synthesis.

Other functions of galectins: regulations of innate immune responses

In addition to their effects on T cell physiology, galectins have also been shown to influence the development of innate immune responses.⁶⁸ We have demonstrated that galectin-1 suppresses the acute inflammatory response and inhibits neutrophil extravasation.⁶⁹ Furthermore, this lectin suppresses arachidonic acid release and nitric oxide production from activated macrophages.^{69, 70} In addition, recent findings indicate that galectin-1 can inhibit neutrophil chemotaxis and transendothelial migration.⁷¹

In contrast to the anti-inflammatory effects of galectin-1 studies of acute peritonitis in mice deficient in galectin-3 provided significant support for the proinflammatory role of this lectin during acute inflammatory responses.^{64, 72} After intraperitoneal injection of thioglycolate, galectin-3 deficient mice had significantly reduced numbers of recoverable granulocytes compared with wild-type animals.^{64, 72} Interestingly, however, Karlsson and colleagues^{73, 74} showed that both galectin-1 and galectin-3 are able to induce activation of the superoxide producing NADPH (nicotinamide adenosine dinucleotide phosphate (reduced form)) oxidase at similar levels in primed neutrophils.^{73, 74} Furthermore, regarding the role of galectins in cell-ECM interactions, galectin-3 has been shown to promote neutrophil adhesion to laminin⁷⁵ and endothelial cells⁷⁶ in vitro following an immunological challenge.

Recently, Liu and colleagues highlighted a critical role for galectin-3 in phagocytosis by macrophages.⁷⁷ Compared with wild-type macrophages, galectin-3 deficient cells exhibited reduced phagocytic capacity.⁷⁷ In addition, the same group showed that galectin-3 promotes chemotaxis of human monocytes through interaction with a G-protein coupled receptor⁷⁸ and we have recently demonstrated that galectin-3 acts in concert with soluble fibrinogen to regulate neutrophil activation, degranulation, and survival through alternative MAPK pathways.⁷⁹

Although not studied in detail as galectin-1 and galectin-3, other galectins also modulate innate immune responses. For example, galectin-8 can modulate neutrophil functions

related to microbial killing.⁸⁰ On the other hand, galectin-9 can act as an eosinophil specific T cell derived chemoattractant.⁸¹ Thus, galectins can act at different levels of the inflammatory cascade to regulate adaptive and innate immune responses.

GALECTINS AS IMMUNOREGULATORS IN T CELL MEDIATED PATHOLOGY

Failure of tolerance mechanisms may lead to autoreactive T cell activation and to induction of autoimmune diseases. To selectively interfere with the activation of pathogenic T cells involved in autoimmune disorders,⁸² immune intervention can be primarily directed to three cellular targets: APCs, autoreactive T cells, and suppressor/regulatory T cells. The common goal of these approaches is to selectively inhibit the activation, migration, and survival of pathogenic effector T cells.⁸³ A growing body of experimental evidence indicates that galectins may play a role in the regulation of T cell mediated inflammatory disorders mainly by targeting pathogenic effector T cells.⁷ In this section we will summarise the clinical and immunological consequences of prophylactic or therapeutic administration of galectins (in particular galectin-1) in chronic inflammatory disorders and autoimmunity (table 1).

Experimental autoimmune myasthenia gravis

Myasthenia gravis is a relatively rare neurological disease that is associated with loss of the acetylcholine receptors that initiate muscle contraction. This results in muscle weakness, which can be life threatening.⁸⁴ As early as 1983, Levi and colleagues⁸⁵ reported the prophylactic and therapeutic effects of electrolectin, an endogenous galectin from the fish *Electrophorus electricus* in experimental autoimmune myasthenia gravis (EAMG) induced by immunisation with purified acetylcholine receptors in rabbits.⁸⁵ The administration of electrolectin to myasthenic rabbits led to clinical recovery; however this effect was not accompanied by any significant change in the level of circulating autoantibodies or modifications at the muscular level.⁸⁵ The authors suggested that electrolectin might play a role in the regulation of immune tolerance to self-antigens. However, at that time, the mechanisms involved in this process had not been investigated.

Experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is considered as a model for multiple sclerosis and in both diseases Th 1-type cells specific for myelin antigen appear to play a critical pathogenic role. Offner and colleagues⁸⁶ demonstrated that galectin-1 prevented the development of clinical and histopathological signs of EAE in Lewis rats.⁸⁶ Although the mechanisms of action of the immunoregulatory activity of galectin-1 were not investigated in this study, the authors proposed that galectin-1 might block activation or sensitisation of encephalitogenic T cells.⁸⁶ These pioneer studies in EAMG and EAE prompted us to investigate in vitro and in vivo the molecular mechanisms involved in the immunoregulatory activity of galectin-1.

Collagen induced arthritis

Rheumatoid arthritis (RA) is an immune mediated disease with a prominent involvement of Th 1 cells, characterised by articular inflammation and subsequent tissue damage leading to severe disability and increased mortality.⁸⁷ Animal models of RA, such as collagen induced arthritis (CIA) have been essential in understanding the possible pathogenic mechanisms of this disease. We have demonstrated, using the DBA/1 model, that a single injection syngeneic DAB/1 fibroblasts engineered to secrete galectin-1 at the day of onset

Table 1 Immunoregulatory effects of administration of galectins (therapeutic and preventive) in experimental models of chronic inflammation, autoimmunity, and cancer

Experimental models	Strategies used	Clinical outcome	Immunological outcome
<i>Exogenous galectins</i> Experimental autoimmune myasthenia gravis	Electrolectin administration	Clinical recovery	No changes in circulating autoantibodies or modifications at the muscular level
Experimental autoimmune encephalomyelitis (multiple sclerosis)	Prophylactic administration of galectin-1	Prevention of clinical and histopathological manifestations	Blockade of sensitisation of pathogenic T cells
Collagen induced arthritis (rheumatoid arthritis)	Galectin-1 gene therapy and protein administration	Suppression of clinical and histopathological manifestations	Increased IL-5 and decreased IFN γ production Increased T cell susceptibility to activation induced cell death
Concanavalin A induced hepatitis (autoimmune hepatitis)	Prophylactic administration of galectin-1	Prevention of liver injury and T helper cell liver infiltration	Suppressed TNF α and IFN γ production Increased apoptosis of activated T cells
Inflammatory bowel disease (experimental colitis)	Prophylactic and therapeutic administration of galectin-1	Suppression of clinical and histopathological manifestations	Reduced ability of mucosal T cells to produce IFN γ Reduced proliferation of antigen specific T cells Increased number of apoptotic T cells within mucosal tissue
Nephrotoxic nephritis	Galectin-1, galectin-3, and galectin-9 administration	Clinical recovery	Galectin-9 induces apoptosis of activated CD8+ cells Galectin-1 and galectin-3 block the accumulation of macrophages in the renal glomeruli
Graft v host disease	Galectin-1 administration	Increased host survival following allogeneic haematopoietic stem cell transplantation	Reduced production of IFN γ and IL-2 Reduced alloreactivity
<i>Endogenous galectins</i> B16 melanoma	Blockade of galectin-1 expression in the tumour microenvironment (galectin-1 knockdown clones)	Increased T cell mediated tumour rejection	Increased tumour specific IFN γ and IL-2 production Decreased number of infiltrating apoptotic T cells

IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.

of disease was able to abrogate clinical and histopathological manifestations of arthritis.⁵⁶ Furthermore, investigation of the mechanisms involved in this process revealed a shift from a Th 1 to a Th 2-polarised immune response: this effect was manifested by reduced levels of IFN γ and increased levels of IL-5 in draining lymph nodes from mice treated by gene or protein therapy with galectin-1.⁵⁶ In addition, sera from treated mice showed reduced levels of anticollagen type II IgG2a antibodies and increased levels of anticollagen type II IgG1 antibodies. Finally, the molecular bases of the therapeutic effect of galectin-1 were examined in vivo showing that lymph node cells from mice engaged in the galectin-1 gene therapy protocol had increased susceptibility to antigen induced apoptosis. Moreover, galectin-1 expressing fibroblasts revealed an inhibitory effect in antigen-dependent IL-2 production to a collagen type II specific T cell hybridoma clone.⁵⁶ This study provided a strong correlation between the apoptotic properties of galectin-1 in vitro and its therapeutic potential in vivo. In addition, a strong correlation was found between the levels of galectin-1 and galectin-3 and the regulation of apoptosis in synovial tissue from patients with juvenile rheumatoid arthritis.⁴² On the other hand, Ohshima and colleagues⁴³ found that galectin-3 is elevated in sera and synovial fluid from patients with RA. Interestingly, the authors found that RA synovial fibroblasts secrete high levels of galectin-3 into culture medium and that proinflammatory cytokines, such as TNF α markedly upregulate the intracellular content of galectin-3 in these cells.⁴³ A brief outline of the potential role of galectin-1 and galectin-3 in the context of inflamed synovia is shown in fig 2.

Concanavalin A induced hepatitis

Con A induced hepatitis represents a T cell dependent model of liver injury. Con A administration results in liver injury, leucocyte infiltration, increased transaminase plasma levels and hepatocyte apoptosis.⁸⁸ Furthermore, Con A treatment leads to a marked increase in plasma TNF α and IFN γ levels. Santucci and colleagues⁵⁸ found that galectin-1 pretreatment prevented liver injury and Th cell liver infiltration induced by Con A.⁵⁸ Using in vitro and in vivo experiments the authors demonstrated the protective effects of galectin-1 in this model and confirmed that galectin-1 acts in vivo by promoting selective elimination of antigen activated T cells.⁵⁸ In addition, galectin-1 inhibited proinflammatory cytokine secretion from T cells and macrophages in a manner independent of its proapoptotic properties.⁵⁸

Inflammatory bowel disease

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis, are idiopathic chronic inflammatory conditions occurring with increasing frequency in Western populations.⁸⁹ Although the etiology of IBD remains unknown, there is consistent evidence to link this condition to a failure of the mucosal immune system to attenuate the immune response to endogenous antigens. Support for this view has come from animal models of colitis, including the hapten model of colonic inflammation induced by intrarectal delivery of 2,4,6-trinitrobenzene sulphonic acid (TNBS), which consistently exhibits an excessive production of Th 1 derived cytokines.⁸⁹ It has been demonstrated that prophylactic and therapeutic administration of galectin-1

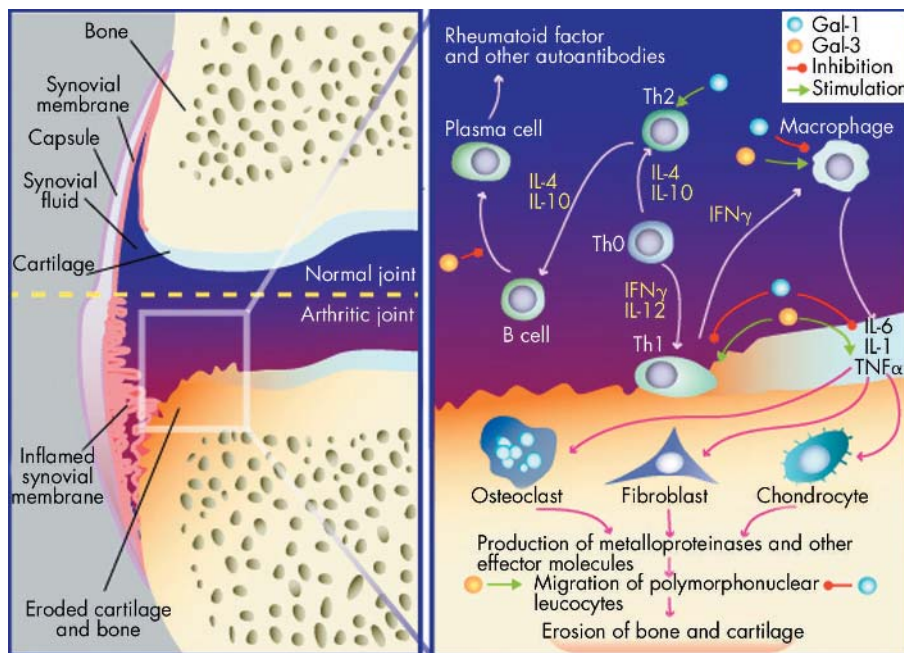


Figure 2 Galectins in immunopathology. Potential effects of galectin-1 (Gal-1) and galectin-3 (Gal-3) in the context of inflamed synovial tissue in rheumatoid arthritis. IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.

results in striking improvement in the clinical and histopathological aspects of the disease.⁵⁷ Furthermore, galectin-1 treatment induced a reduction of the number of hapten activated spleen and mucosal T cells and decreased proinflammatory cytokine production.⁵⁷ These results clearly indicated that galectin-1 exerts protective and immunoregulatory activity in TNBS induced colitis and it also might be effective in the treatment of IBD.

Nephrotoxic nephritis

Nephrotoxic serum nephritis (NSN) represents a group of immune mediated disorders characterised by crescent formation and glomerular influx of CD8+ cells into glomerular capillaries.⁹⁰ The disease can be induced in rats by injecting antiglomerular basement membrane serum.⁹⁰ Tsuchiyama and colleagues⁹⁰ investigated the efficacy of different members of the galectin family in the amelioration of NSN⁹⁰ and found that galectin-9 induces apoptosis of activated CD8+ cells, while galectin-1 and galectin-3 block the accumulation of macrophages in the renal glomeruli.⁹⁰ These findings raises the possibility to ameliorate immune mediated renal diseases by administration of galectins.

Graft versus host disease

Graft versus host disease (GVHD) is a lethal complication of allogeneic haematopoietic stem cell transplantation, where immunocompetent donor T cells attack the genetically disparate host cells. The predominant symptoms of acute graft versus host disease occur in the skin, liver, and intestine.⁹¹ Baum and colleagues⁵⁹ investigated the efficacy of galectin-1 in a murine model of graft versus host disease and found that 68% of mice treated with galectin-1 survived, compared with 3% of vehicle -treated mice. Galectin-1 treatment reduced inflammatory infiltrates in affected tissues and significantly improved reconstitution of normal splenic architecture following transplantation. Similar to the findings in the CIA and IBD models, Th 1 cytokines were markedly reduced whereas production of Th 2 cytokines was similar between galectin-1 treated and control animals.⁵⁹ These findings demonstrated that galectin-1 therapy is capable of suppressing graft versus host disease without

compromising engraftment or immune reconstitution following allogeneic haematopoietic stem cell transplantation.

Cancer: modulation of antitumour T cell mediated responses

The immunoregulatory effects of galectin-1¹ and the correlation between galectin-1 expression in cancer cells and the aggressiveness of these tumours⁹² prompted us to investigate the role of galectin-1 in tumour immune escape. We hypothesised that tumour cells may impair T cell effector functions through secretion of galectin-1 and that this mechanism may contribute in tilting the balance towards an immunosuppressive environment at the tumour site. By a combination of in vitro and in vivo experiments, we established a link between galectin-1-mediated immunoregulation and its contribution to tumour immune escape.²⁰ Blockade of the inhibitory effects of galectin-1 within tumour tissue resulted in reduced tumour mass (an effect which required intact CD4+ and CD8+ T cell responses) and stimulated the generation of a tumour specific T cell response in vivo.²⁰ Our observations supported the idea that galectin-1 may contribute to immune privilege of tumours by modulating survival of effector T cells, and suggest a potential molecular target for manipulation of T cell tolerance and apoptosis with potential implications in immunotherapy.

DISCUSSION AND FUTURE DIRECTIONS

Recent advances in the understanding of the contribution of cell surface glycoconjugates and carbohydrate binding proteins to inflammatory processes have motivated the design of synthetic glycoconjugates or lectins as candidates for future generations of therapeutic drugs.⁹³⁻⁹⁴ Galectins are involved in a variety of biological processes able to modulate immune responses including T cell survival, activation, differentiation, and cytokine secretion. In addition, the tolerogenic properties of galectins render these sugar binding proteins particularly suitable for the treatment of chronic inflammatory disorders and autoimmune diseases. In the present review we illustrated the relevance of different members of the galectin family in the regulation of T cell physiology and chronic inflammatory disorders. Despite considerable progress in elucidating galectin functions within

the immune system, future comparative studies between different galectins will be necessary to address the precise role of individual members of the family and their functional redundancy, the toxicity of these proteins in vivo and the concentrations required to achieve different effects. The accumulating evidence for the multiple pleiotropic mechanisms regulated by galectins should indeed prompt further exploration of their potential in the development of therapies for several autoimmune disorders. In addition, given the influence of galectins in different events of tumour progression and metastasis, a current challenge is the design of specific and potent galectin inhibitors as potential anticancer agents.

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