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# Roles of galectins in chronic inflammatory microenvironments

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Lectins are multifunctional carbohydrate-binding proteins that can recognize various carbohydrates on cell surfaces and extracellular matrix, and are involved in several biological processes. Galectins, a family of animal lectins with affinity for β-galactoside-containing oligosaccharides, are expressed by several cells of the immune system and tissue-resident stromal cells. Increasingly, experimental evidence indicates that galectins might play critical regulatory roles in cancer, fibrosis and chronic inflammatory disorders, such as rheumatoid arthritis. In this review, we summarize recent developments in our understanding of the galectins' roles within particular cells, and in the broader context of the inflammatory or tumor microenvironments. This body of knowledge, documenting the coming-of-age of galectins as potential immunosuppressive agents or targets for anti-inflammatory drugs, represents a sound basis to further explore their immunoregulatory properties in the development of novel therapies for autoimmune diseases and chronic inflammation.

Galectins, a subfamily of the animal lectins, are evolutionarily conserved carbohydrate-binding proteins [1]. 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 [2]. To date, 15 mammalian galectins have been identified, 11 of which have human orthologus (Table 1). These can be subdivided into three groups; single-CRD galectins; tandem-CRD galectins, and the unique 'chimera-type' galectin-3, which contains a single CRD fused to unusual tandem repeats of short amino acid stretches [1,3].

Some single-CRD galectins can exist as dimers, tandem-CRD galectins have two carbohydrate binding sites and galectin-3 can form oligomers when it binds to multivalent carbohydrates [4,5]. Galectins, like antibodies, can therefore establish ordered arrays of complexes with increased avidity when they bind to multivalent glycosylated proteins [6,7].

Galectins can be found inside and outside cells and have distinct functions in each location [8]. Whether endogenously expressed, or rapidly internalized from the cell surface, galectins have been implicated in important intracellular functions, such as pre-mRNA splicing, regulation of cell growth, cell cycle progression and protein sorting [9–14]. Furthermore, the cytoplasmic-nuclear transport of galectin-3 appears to be regulated by unknown chaperone factors [15] and modulated by neighboring leucine-rich nuclear export signals [16,17].

Although galectins do not contain signal peptides to direct them through the classical endoplasmic reticulum (ER)–Golgi apparatus secretory system, they can be secreted by other unorthodox secretory pathways [18–21]. Once outside the cell, galectins bind to and crosslink multiple molecules found on the cell surface or in the extracellular matrix (ECM) that display appropriate galactose-containing oligosaccharides. In this way, galectins may exert autocrine and paracrine effects to regulate the inflammatory response within tissue microenvironments [3,22,23].

## General considerations for extracellular galectin signaling

Cross-linkage of cell-surface receptors by galectins can trigger transmembrane signaling events through which diverse processes such as apoptosis, cytokine secretion and cell migration are modulated. However, highly significant factors that determine the responsiveness of cells to galectin-mediated signals include the repertoire of potentially glycosylated molecules expressed on the cell surface, and the activities of specific glycosyltransferases that are responsible for producing galectin ligands. These variables can dramatically change according to the developmental stage and activation status of cells [24].

In addition to producing galectin ligands, glycosyltransferases can also effectively mask galectin saccharide ligands. For example, the addition of α2,6-linked sialic acids to lactosamine units by the α2,6-sialyltransferase I *(ST6Gal-I)* has been shown to block galectin-1 binding [25].

Keywords: apoptosis, cancer, chronic inflammation, fibroblast, fibrosis, galectin, immunosuppression, neutrophil, rheumatoid arthritis, T cell

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On the other hand, the Core-2-β-1,6-*N*-acetylglucosaminyltransferase (C2GnT), creates branched polysaccharide structures, which galectin-1 recognizes on T-cell surface glycoproteins, such as CD45 [26].

Undoubtedly, one of the most intriguing findings is the fact that individual galectins can exert contrasting effects on the same target cells, depending on the activation or differentiation state of these cells. In this regard, galectins-1 and -3 have been shown to promote survival or death, activation or silencing and differentiation or proliferation on particular leukocyte subsets [27–31].The intimate mechanisms of these contrasting effects still remain to be elucidated.

Some galectins, such as galectins-1 and -3, appear to be expressed ubiquitously whereas others, such as galectins-2, -4, -7 and -13, have a more restricted tissue localization [1,3,32]. The expression of the galectins themselves is modulated during the activation and differentiation of immune cells and changes under different physiological, pathological or *in vitro* conditions [22]. For instance, galectins- 1 and -3 are upregulated following activation of differentiation in macrophages [19,33,34], T cells [35–37] and fibroblasts [38]. In addition, recent evidence indicates that galectin-1 is overexpressed during the expansion of $CD4^+$ $CD25^+$-regulatory T cells [39], suggesting a potential role for this protein in the establishment of peripheral tolerance. Furthermore, galectin-9 expression can be upregulated by proinflammatory cytokines, including interleukin (IL)-1β and interferon (IFN)-γ [40,41], and galectin-12 expression can be downregulated by different stimuli, including isoproterenol, tumor necrosis factor (TNF)-α and dexamethasone [42]. In addition, different members of the family can be up- or downregulated during myeloid differentiation into the monocyte, eosinophil or neutrophil lineages [43].

### Functions of the tandem-CRD galectins

Although galectin-1 (single-CRD galectin) and galectin-3 (chimera-type galectin) are the most extensively studied members of the galectin family, it is gradually becoming evident that other galectins can also modulate innate and acquired immune responses. Examples include the abilities of the tandem-CRD-type galectin-8 to activate microbial killing machinery in neutrophils [44], and of galectin-9 to act as an eosinophil-specific chemoattractant [45]. In addition, galectin-9 can induce the maturation of monocyte-derived human dendritic cells, providing a link between innate and adaptive immunity [46].

Another tandem-CRD-type family member, galectin-4, has been found to play a key role in $CD4^+$ T-cell activation in intestinal inflammation [47]. Epithelial cell-derived galectin-4 stimulates an increase of IL-6 production in $CD4^+$ T cells and exacerbates chronic colitis. Discussion of this finding is worthwhile in terms of the different roles played by individual members of the same galectin subfamily in activating or silencing pathogenic T-cell responses.

**Table 1. Mammalian galectin family and their subgroups.**

| Single-CRD (monomeric or dimeric) | Two-CRDs in tandem | Chimera-type |
|---|---|---|
| Galectin-1 | | |
| Galectin-2 | | |
| | | Galectin-3 |
| | Galectin-4 | |
| Galectin-5 (found in rat)* | | |
| | Galectin-6 (found in chicken and mouse)* | |
| Galectin-7 | | |
| | Galectin-8 | |
| | Galectin-9 | |
| Galectin-10 | | |
| Galectin-11 (found in sheep)* | | |
| | Galectin-12 | |
| Galectin-13 | | |
| Galectin-14 | | |

*These galectins have no reported human ortholog.
*CRD: Carbohydrate recognition domain.*

In this regard, immunosuppressive functions for tandem-CRD galectins have also been described. For example, galectin-9 induces apoptosis of murine thymocytes [48] and peripheral CD4$^+$ and CD8$^+$ T-cell death through a Ca$^{2+}$-calpain-caspase-1 signaling pathway [49]. Interestingly, in a very elegant study, Zhu and colleagues recently showed that galectin-9 is a ligand of Tim-3, a T-helper (Th)1-specific cell-surface molecule. The authors showed that galectin-9 specifically kills Tim-3 expressing IFN-γ-producing cells [50]. Interestingly, this immunosuppressive effect has clear consequences in silencing Th1 responses *in vivo* [50]. This effect will be more extensively discussed in the next sections.

Regarding other members of this family, it has been demonstrated that galectin-8 can induce either growth arrest or apoptosis of tumor cell lines depending upon the activities of cyclin-dependent kinase inhibitors and c-Jun N-terminal kinase (JNK) [51]. Finally, the tightly restricted expression of galectin-12 in adipocytes has also been shown to regulate cell-cycle progression and apoptosis [52,53].

## Pro-inflammatory functions of galectin-3

Studies of acute peritonitis in galectin-3-deficient mice have provided significant support for the pro-inflammatory role of galectin-3 [54,55]. Following thioglycolate administration into the peritoneum, fewer granulocytes could be recovered from galectin-3-deficient mice than from wild-type controls. Furthermore, galectin-3 has been shown to promote neutrophil adhesion to laminin and endothelial cells *in vitro* [56,57]. Karlsson and colleagues showed that galectin-3 is able to induce activation of the superoxide-producing NADPH oxidase in primed neutrophils [58]. In this regard, we have recently demonstrated that galectin-3 and soluble fibrinogen together regulate neutrophil activation, degranulation and survival [59].

An essential role for galectin-3 in the phagocytic function of macrophages has been reported by Liu and coworkers [60]. 3 years ago the same group also demonstrated that galectin-3 can promote chemotaxis of human monocytes through interaction with a G-protein coupled receptor [61].

Consistent with its pro-inflammatory function, galectin-3 promotes dendritic cell-naive T-cell interactions in lymph nodes [62]. This molecule is also a critical intracellular mediator of IL-4-induced survival and differentiation of B cells into a memory phenotype [63]. Therefore, it seems evident that galectin-3 plays a critical role in the regulation of the inflammatory response.

Interestingly, depending on whether the protein is found in the intracellular compartment or extracellularly, galectin-3 can have dramatically different functions. Fukumori and colleagues demonstrated that extracellular galectin-3 could induce apoptosis in human T cells, binding mainly to CD7 and β1-integrin and resulting in activation of the mitochondrial death pathway [64]. The proapoptotic effect of galectin-3 was recently confirmed by the groups of Liu and Baum who showed that galectin-3 and galectin-1 can induce cell death through binding to a different set of glycoreceptors [30]. Contrarily, galectin-3 overexpression studies by Yang and colleagues demonstrated that T-cell transfectants exhibited faster growth than control cells and were protected from apoptosis induced through death receptors and mitochondrial routes [11]. Interestingly, over expressed galectin-3 appeared to interact with Bcl-2. Similarly, Hahn and colleagues demonstrated that galectin-1-induced cell death could be inhibited by intracellular expression of galectin-3 [65]. The antiapoptotic activity of intracellular galectin-3 has been implicated in pathological situations, including rheumatoid arthritis (RA) [66], lymphomas [67] and other types of cancer [68].

## Galectin-3 in rheumatoid arthritis, bone development & fibrosis

Two studies have independently found increased expression of galectin-3 and galectin-3-binding protein (G3BP) in cells from RA patients. Using subtractive cDNA hybridization, Seki and colleagues found that G3BP was one of 11 genes that were expressed at significantly higher levels in cultured synovial fibroblasts from RA patients than in osteoarthritis patient fibroblasts [69]. Accordingly, Ohshima and colleagues have reported that both galectin-3 and G3BP are abundantly expressed in RA patient synovia, particularly at sites of cartilage invasion [66]. Both proteins could also be found in synovial fluid. Furthermore, galectin-3 expression appears to be associated with the expression of L1 retrotransposable elements [70] and can be induced by the adhesion of synovial fibroblasts to cartilage oligomeric matrix protein [71].

Interestingly, Colnot and colleagues found that galectin-3-deficient mice displayed accelerated apoptosis or terminal differentiation of chondrocytes in the hypertrophic zones of developing long bones [72]. Furthermore, evidence from Ortega and coworkers suggested that, in matrix metalloproteinase (MMP)-9-deficient mice, excess extracellular galectin-3 can accumulate, potentially leading to increased recruitment of monocytes and the extended survival of osteoclasts [73].

Elegant work by Henderson and colleagues has recently revealed a crucial role of galectin-3 in the fibrotic response to tissue injury [13]. This is consistent with the fact that galectin-3 is an immediate early gene, with a serum responsive element in its promoter [74]. Galectin-3 deficiency in mice drastically reduced renal, pulmonary and hepatic fibrosis by preventing the differentiation of myofibroblasts [13]. Despite comparable levels of transforming growth factor (TGF)-β in injured livers of both wild-type and galectin-3 null animals, and intact Smad-2 and -3 activation in isolated hepatic stellate cells, galectin-3 proved necessary for TGF-β-induced procollagen-I and α-smooth muscle actin expression. Since exogenously added galectin-3 was rapidly internalized, the authors favor a hypothesis in which the essential role of galectin-3 is intracellular, although the protein is likely to be delivered in an autocrine and paracrine fashion.

## Immunosuppressive & anti-inflammatory functions of galectins

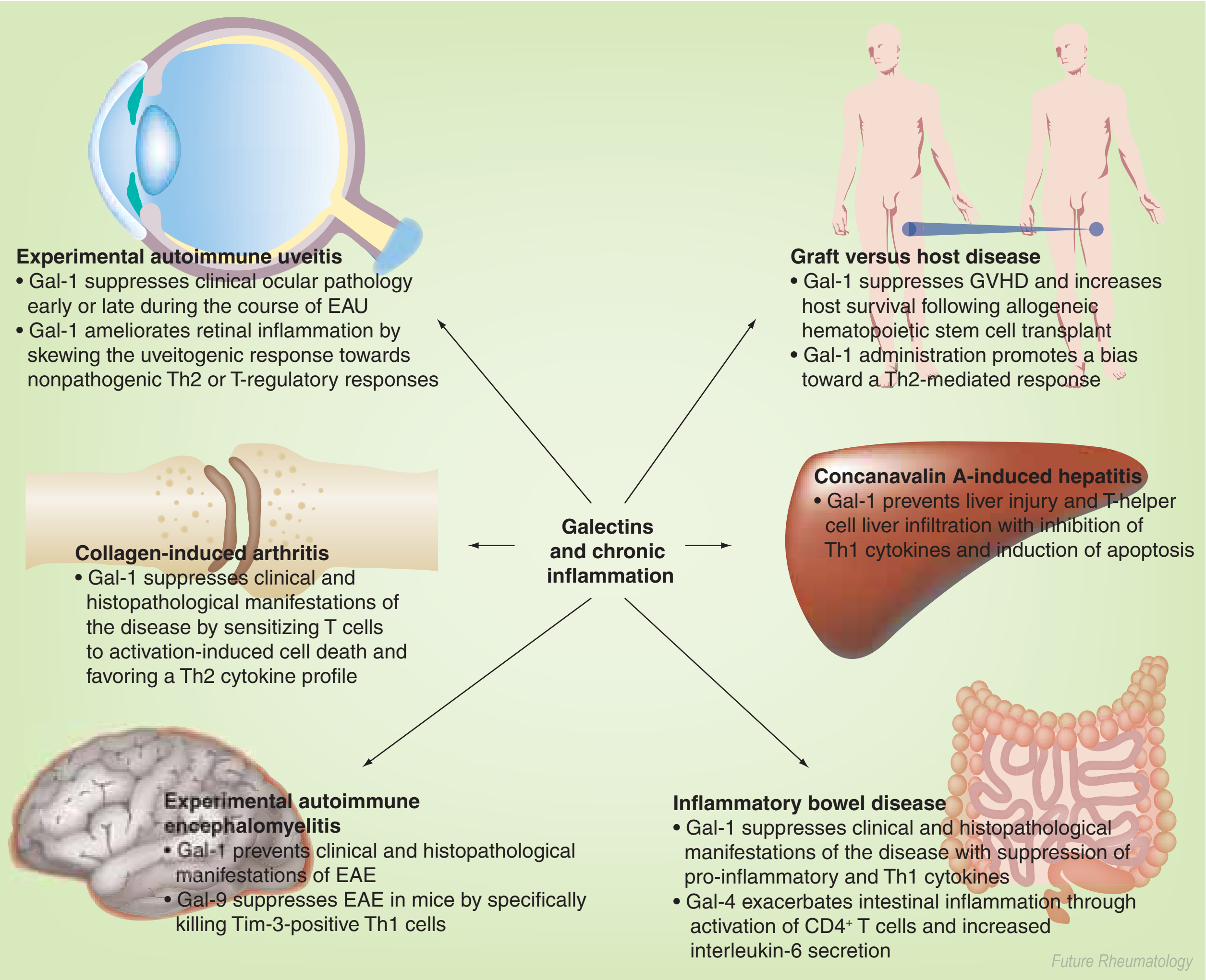
In general, galectin-1, a prototypical single-CRD galectin, displays pro-apoptotic and anti-inflammatory properties (Figure 1). Accordingly, we found that pretreatment of rats with galectin-1 suppressed the acute inflammatory response and inhibited neutrophil extravasation induced by bee venom phospholipase $A_2$ [75]. Furthermore, arachidonic acid release and nitric oxide production from activated macrophages was inhibited [75,76]. In this regard, La and colleagues demonstrated that, at remarkably low doses (20 pmol/mouse), galectin-1 could inhibit neutrophil chemotaxis and transendothelial migration [77]. The authors speculate that local galectin-1 release from endothelial cells at inflammatory sites may be a crucial negative feedback mechanism to prevent excessive neutrophil recruitment. In addition, it has been reported that exogenous galectin-1 causes phosphatidylserine exposure and phagocytic uptake of activated neutrophils [78]. Therefore, it seems that galectin-1 can display a wide variety of anti-inflammatory effects on different immune cell types.

In this regard, accumulating evidence suggests that galectins might have particularly important roles in the regulation of cell survival in the immune system [79]. Recent work by Endharti and colleagues showed that secretion of galectin-1 by stromal cells supports the survival of naive T cells without promoting proliferation [29]. However, galectin-1 has been reported to induce apoptosis and regulate cell growth in developing thymocytes and activated T cells [34,80,81,82]. In this regard, recent evidence indicates that dendritic cells engineered to overexpress galectin-1 can promote contrasting effects on resting and activated T cells, either promoting activation or apoptosis [31]. The fact that galectin-1 is expressed by activated but not resting T cells may point towards an autocrine suicide mechanism, similar to that reported for Fas ligand expression, to prevent excessive T-cell clone expansion after the completion of an ongoing immune response [83].

Perhaps intringuingly, many reports of the pro-apoptotic effect of galectin-1 have used micromolar concentrations of the protein, which are unlikely to exist in biological fluids *in vivo*. However, recent evidence indicates that the more moderate amounts of galectin-1 secreted by most T cells is, in fact, sufficient to kill T cells when the galectin is displayed in the context of ECM glycoproteins [84]. In this regard, the same group recently showed that endothelial cell expression of galectin-1 can also inhibit T-cell transendothelial migration in a manner independent of its pro-apoptotic properties [85].

Several glycosylated proteins on the surface of activated T cells are reported to be crucial receptors for galectins, including CD2, 7, 43 and 45 [86–88]. Galvan and coworkers showed that T cells expressing CD45, but lacking the C2GnT glycosyltransferase, become resistant to galectin-1-induced cell death [26]. Furthermore, a recent report by Lanteri and colleagues is noteworthy in this context – the authors conclude that, during HIV-1 infection, T cells become increasingly susceptible to galectin-1-induced cell death due to altered cell-surface molecule glycosylation [89]. CD7 appears to be a particularly critical receptor for galectin-1-induced cell death. T lymphocytes from patients with mycosis fungoides/Sezary syndrome that lack CD7 expression were demonstrated to be insensitive to galectin-1-triggered death [90]. Interestingly, very recent evidence in neoplastic T-cell lymphoma indicates that haploinsufficiency of C2GnT results in altered

**Figure 1. Immunosuppressive effects of galectins in different experimental models of T-cell dependent chronic inflammation and autoimmunity.**



The effects of galectins in different murine models of autoimmune disease and inflammation, including collagen-induced arthritis (CIA), EAE, inflammatory bowel disease (IBD), concanavalin A-induced hepatitis, EAU and GVHD. Gal-1 exerts immunosuppressive effects through induction of T-cell apoptosis, modulation of the Th1/Th2 cytokine balance, inhibition of leukocyte migration and generation of regulatory T cells. On the other hand, Gal-9 negatively regulates Th1-mediated responses through selective killing of Tim-3-positive IFN-γ-producing Th1 cells. In addition, Gal-9 can modulate nephrotoxic nephritis through regulation of cell cycle-dependent kinases. EAE: Experimental autoimmune encephalomyelitis; EAU: Experimental autoimmune uveitis, GVHD: Graft versus host disease; Th: T helper.

cellular glycosylation and resistance to galectin-1-induced cell death. These results identify a potentially novel escape mechanism displayed by T-lymphoma cells to survive in galectin-1 enriched microenvironments [91].

Whilst several classical apoptotic signal transduction events have been documented during galectin-1-induced cell death, including caspase activation and cytochrome c release [28], alternative death pathways and apoptotic end points appear to be triggered in different T-cell types [65]. Indeed, apoptosis may only partially explain the immunosuppressive properties of galectin-1: the T cells that escape apoptosis may instead be subject to suppression of proinflammatory cytokine secretion [92,93] or even targeted for phagocytic removal [78].

Miceli and collaborators demonstrated that galectin-1 induces partial T-cell receptor (TCR)-γ chain phosphorylation, antagonizing full signals through the TCR and costimulatory receptors, but allowing partial TCR-mediated responses, such as CD69 upregulation and apoptosis [94,95]. Furthermore, in a very elegant study, Demetriou and colleagues demonstrated that galectin-3 may also restrict signal transduction initiated by TCR complexes [96]. Hypothetically, the authors argued, the lateral mobility of TCR complexes might be restrained by multivalent complexes of galectin-3 and TCR. Mice deficient in a crucial enzyme in the N-glycosylation pathway (β1,6 N-acetylglucosaminiltransferase or Mgat5), showed an increased susceptibility to autoimmunity [96]. Thus, galectin-1 and galectin-3 may share an ability to suppress T-cell activation.

*In vitro*, galectin-1 is also known to block secretion of pro-inflammatory cytokines including IL-2, IFNγ, and TNF-α [92,94]. *In vivo* studies using inflammatory disease models concur; galectin-1 tends to skew the cytokine response to a balance towards the Th2-type [97–102] (Figure 1). In addition, treatment of both nonactivated and activated $CD4^+$ and $CD8^+$ T cells with recombinant galectin-1 has been reported to cause a significant increase in IL-10 mRNA and protein [103]. IL-10 is known to suppress Th1-type responses, and galectin-1 may therefore employ this mechanism for its immunoregulatory activity. In contrast to galectin-1, galectin-3 suppresses Th2 cytokine secretion in antigen-specific T-cell lines [104]. Furthermore, galectin-3 gene therapy has recently been shown to inhibit both inflammation and stromal remodeling when delivered into the chronically inflamed lungs of mice [105]. Paradoxically, galectin-3-deficient mice also appear to recruit fewer eosinophils than wild-type controls and to display a Th1 cytokine profile in an experimental model of asthma [106]. This apparent discrepancy might be explained by the different experimental strategies used by the authors of these studies (i.e., therapy versus analysis of the susceptibility of galectin-3 gene knock-out mice).

Despite the multiple effects of the exogenously added protein, galectin-1 gene disruption in mice did not apparently cause major spontaneous phenotypic abnormalities [107]. This observation suggests that different members of the galectin family can at least partially compensate for the lack of galectin-1. However, galectin-2, which is structurally related to galectin-1 and is known to share its pro-apoptotic function, clearly operates through a different pathway to galectin-1 [108]. Careful examination of *gal-1* gene deficient mice is beginning to reveal subtle but critical differences in the regulation of inflammatory responses.

Notwithstanding such functional differences, some similarities between the activities of galectins-1 and -2 do exist. For example, galectin-2 and can shift the balance of T-cell-derived cytokines towards a Th2 profile, an *in vitro* property shared by galectin-1 [108]. Interestingly, galectin-2 appears to regulate lymphotoxin-α secretion, and subtle genetic variants of galectin-2 are reported to differentially influence the extent of inflammation during myocardial infarction [109]. In this regard, a cross-sectional genetic study performed in a British population indicated a striking correlation between plasma glucose, serum insulin and the galectin-2 genotype [110].

Although no evidence exists for the ability of other exogenously-added single-CRD galectins to affect cell survival, transfection of galectin-7 (p53-induced gene 1) in epithelial tumor cell lines did reveal its potential intracellular proapoptotic activity [111].

Regarding the immunosuppressive activities of tandem-CRD galectins, recent evidence indicates that galectin-9 can suppress the progression of experimental autoimmune encephalomyelitis by selectively killing Tim-3-positive IFN-γ-producing cells [50]. Interestingly, T-cell-mediated neuroinflammation was exacerbated in mice treated with galectin-9 small interfering (si)RNA suggesting that knocking-down galectin-9 expression during disease induction may affect the progression of the disease (Figure 1). In addition, Tsuchiyama and colleagues reported tha the effect of galectin-9 inhibits the infiltration of $CD8^+$ T cells in an experimental rat model of nephritis [112]. Furthermore, recent evidence indicates that galectin-9 may inhibit glomerular hypertrophy in db/db diabetic mice via inhibition of cyclin-dependent kinase inhibitors [113]. Thus, future studies are warranted to investigate the different mechanisms involved in the immunosuppressive activities of individual members of the galectin family.

## Galectins & tumor-immune escape

Galectins have been shown to modulate different events of tumor progression [3]. Interestingly, expression of galectin-1 (as well as other galectins) in cancer cells positively correlates with the aggressiveness of tumors [114,115]. This suggested that secretion of galectin-1 by tumor cells may be a mechanism by which immunosuppressive microenvironments at tumor sites can be created. This hypothesis was supported using a combined *in vitro* and *in vivo* strategy: galectin-1-mediated immunoregulation clearly had an important role in tumor immune escape [116]. Local galectin-1 blockade allowed $CD4^+$ and $CD8^+$ tumor-specific T-cell responses to be mounted, causing a reduction in tumor mass.

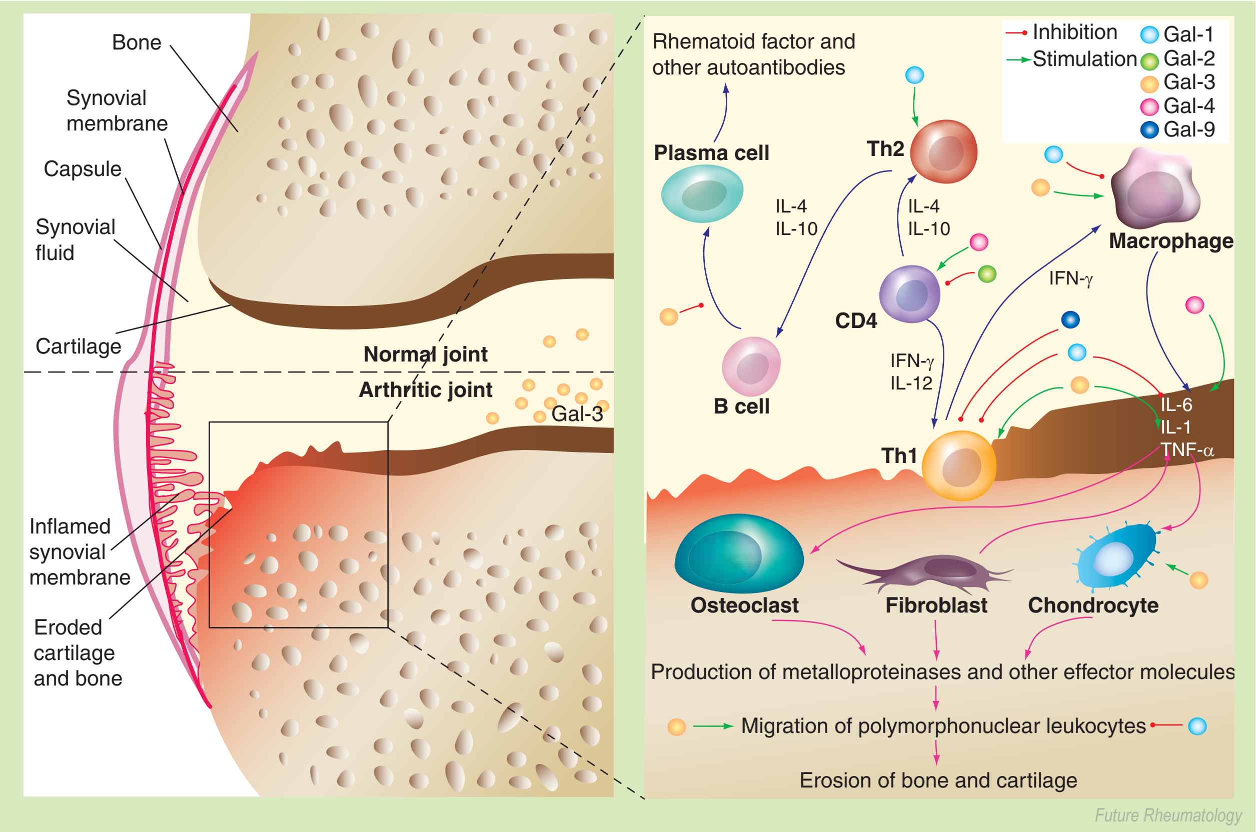
Given its potent immunosuppressive effects, galectin-1 may be a useful target for therapeutic intervention in cancer.

Since galectins-2, -3 and -9 have also been shown to affect T-cell survival, future studies are warranted to investigate the potential role of these proteins in tumor-cell evasion of immune responses *in vivo*.

## Therapeutic potential of galectins as novel immunosuppressive agents

In a study using the DAB/1 collagen-induced arthritis model, we found a strong correlation between the apoptotic properties of galectin-1 *in vitro* and its therapeutic potential *in vivo* [97]. We demonstrated that, at the day of disease onset, a single injection of syngeneic DAB/1 fibroblasts engineered to secrete galectin-1 could abrogate clinical and histopathological manifestations of arthritis. This process involved an increased susceptibility of lymph-node cells to antigen-induced apoptosis and a shift from a Th1 to a Th2-polarised immune response. Interestingly, galectin-1-expressing fibroblasts also inhibited antigen-dependent IL-2 production in a collagen type-II-specific T-cell clone (Figure 1). In addition, in synovial tissue from juvenile RA patients, we found an interesting correlation between the levels of galectins-1 and -3 and the regulation of apoptosis [117]. Given the contrasting immunoregulatory functions of different members of the galectin family, we speculate that individual members of the galectin family might play different roles in the context of an inflamed joint during the development and resolution of RA (Figure 2).

**Figure 2. Potential role of galectins in the immunopathology of rheumatoid arthritis.**



Future Rheumatology

The potential role of individual members of the galectin family in the context of inflamed synovial tissue. Galectins are expressed by a number of different inflammatory, stromal cells and synovial fibroblasts, and may regulate the function of these cells, thereby affecting the development of inflammatory responses. As illustrated in this diagram, galectins can behave as pro- or anti-inflammatory mediators by modulating the physiology and responses of immune cells, including macrophages, synovial fibroblasts, Th1 and Th2 cells, B cells, neutrophils and mast cells. By positively or negatively affecting the inflammatory response, galectins may indirectly influence the clinical course of rheumatoid arthritis.

GAL: Galactin; IFN: Interferon; IL: Interleukin; Th: T helper; TNF: Tumor necrosis factor.

Similarly, in T-cell-dependent animal models of liver injury [98] and inflammatory bowel disease [99], galectin-1 pretreatment has been shown to abrogate tissue damage and T-cell infiltration (Figure 1). Again the immunosuppressive mechanism involved the selective culling of antigen-activated T cells and inhibition of pro-inflammatory cytokine secretion from T cells and macrophages. Remarkably, even in a murine model of graft-versus-host disease, treatment with galectin-1 substantially suppressed inflammation without compromising the engraftment of donor cells [100]. Finally, given the potential role of galectin-1 in the maintenance of immune privilege in organs such as the eye, we have recently investigated the immunoregulatory effects of this protein in experimental autoimmune uveitis (EAU), a Th1-mediated model of retinal disease [101]. Interestingly, treatment with galectin-1 either early or late in the course of EAU was sufficient to suppress clinical ocular pathology, inhibit leukocyte infiltration and counteract pathogenic Th1 cells [101]. Administration of galectin-1 ameliorated retinal inflammation by skewing the uveitogenic response towards non-pathogenic Th2 or T regulatory (IL-10 and TGF-β)-mediated anti-inflammatory responses (Figure 1). These results highlight the ability of this endogenous lectin to counteract Th1-mediated responses through different, but potentially overlapping, anti-inflammatory mechanisms. In addition, a striking correlation was found between the levels of antiretinal galectin-1 autoantibodies in sera from uveitic patients and the severity of autoimmune retinal inflammation [118].

In addition, the ability of galectin-9 to negatively regulate Th1 responses [50], to suppress neuro-inflammation [50] and to inhibit glomerular hypertrophy and nephritis [112,113] suggest that this tandem-repeat galectin may also be used as a potent target in autoimmunity and chronic inflammation.

## Conclusions & future perspective

As we have seen, galectins can modulate both innate and adaptive immune responses by acting intracellularly and extracellularly, as chemokines, adhesion molecules, differentiation factors, death triggers and survival inducers. However, before the use of galectin-based therapeutic agents can be fully realized, a more thorough understanding of the lesser studied galectins is required. To what extent is there functional redundancy and specificity of action within the galectin family? What doses are required to achieve immunosuppression and what are the tolerated 'nontoxic' doses of these sugar-binding proteins *in vivo*? What are the optimal vehicles for galectin-1 delivery and the most efficient administration routes?

The tolerogenic potency of galectin-1 is such that tumors dysregulate its expression to attain immune privilege [116]. Indeed, specific galectin-1 inhibitors might prove to be potent anticancer agents [119]. Future studies are warranted to investigate the immunosuppressive effects of other members of the galectin family, including galectins-2, -3 and -9, within tumor microenvironment.

Contrarily, synthetic glycoconjugates or lectins may prove to be excellent therapeutic immunosuppressive drugs for treating chronic inflammatory and autoimmune conditions [120–122]. Furthermore, galectin-3 gene silencing may be a very effective treatment for preventing fibrosis [13]. Our current knowledge promises a future scenario in which individual members of the galectin family may be use as immunoregulatory agents (e.g., galectins-1 and -9) or targets for anti-inflammatory drugs in autoimmune disorders, including RA. Future studies should be focussed on the careful examination of galectin-1 or -9-based immunosuppressive agents [123] or specific galectin-3 inhibitors [124] for the treatment of chronic inflammation *in vivo.*

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## Executive summary

### Galectin family

- Structurally distinct subgroups; galectins with a single-carbohydrate recognition domain (CRD), galectins with two CRDs in tandem and chimera-type galectins that contain a single CRD fused to unusual tandem repeats of short amino acid stretches.
- Galectins are secreted by nonclassical mechanisms.
- Galectins can act in an autocrine and paracrine manner to positively or negatively regulate the inflammatory response within tissue microenvironments.

### General considerations for extracellular galectin signaling

- The repertoire of glycosylated cell-surface molecules available for galectin binding is determined by the specific activities of glycosyltransferase enzymes within the cell. Some create and expose galectin ligands, whilst others mask them.

### Functions of the tandem-CRD galectins

- Members of the tandem-CRD galectin subgroup have not been investigated as widely as galectin-1 (the prototypical single-CRD subgroup member) and galectin-3 (the only chimera-type subgroup member). However recent findings suggest intriguing roles for two-CRD ‘tandem-repeat’ galectins in the regulation of inflammation.
- Pro-inflammatory functions have been described for galectins -8 and -9 in the innate arm of the immune response.
- In the context of adaptive immunity, galectin-4 has been shown to affect T-cell activation and interleukin (IL)-6 production and galectin-9 modulates dendritic cell maturation.
- A pro-apoptotic function has also been reported for galectin-9.
- Recent evidence indicates that galectin-9 is a ligand of Tim-3, a T helper (Th)1-specific cell-surface molecule. Galectin-9 negatively regulates Th1 responses, through binding to Tim-3,

### Regulation of the inflammatory response by galectin-3 (the only chimera-type galectin)

- Galectin-3-deficient mice mount a poor inflammatory response to intraperitoneal thioglycollate injection.
- Galectin-3 activates superoxide burst in neutrophils.
- This chimera-type lectin acts as a chemoattractant for monocytes and macrophages.
- It has been demonstrated that this protein is critical for phagocytic function of macrophages.
- Interestingly, galectin-3 acts intracellularly to prevent apoptosis, while exogenously added galectin-3 promotes T-cell apoptosis.

### Galectin-3 in rheumatoid arthritis, bone development & fibrosis

- Galectin-3 is highly expressed together with galectin-3-binding protein (Mac-2BP/90K) at the sites of joint erosion in rheumatoid arthritis (RA) patients.
- Potentially, this protein has an antiapoptotic role in chondrocytes and osteoclasts during embryonic osteogenesis.
- Galectin-3 is a necessary factor for transforming growth factor-β-induced myofibroblast differentiation in the fibrotic response to tissue damage.

### Immunosuppressive & proapoptotic functions of one-CRD galectins

- Galectin-1 can suppress acute inflammation *in vivo* by preventing neutrophil extravasation.
- Galectin-1 induces thymocyte and activated peripheral T-cell apoptosis through binding to a variety of glycoreceptors, including CD2, 7, 43 and 45.
- Galectin-1 suppresses proximal signals through the T-cell receptor and downregulates the release of pro-inflammatory cytokines from T cells.
- Galectin-2 promotes apoptosis of activated T cells and induces a bias toward a Th2 response *in vitro*.

### Galectin-1 & tumor immune escape

- Tumors dysregulate the expression of galectin-1 to attain an immune-privileged microenvironment.
- The ability of other members of the galectin family (e.g., galectins-2, -3 and -9) to suppress T-cell responses and their high levels in certain tumor types suggest the potential contribution of these proteins to tumor-cell evasion of immune responses.

### Therapeutic potential of galectins as novel immunosuppressants

- Galectin-1 suppresses pathology in several T-cell-dependent animal models of disease, including collagen-induced arthritis, experimental autoimmune encephalomyelitis, experimental autoimmune uveitis, concanavalin a-induced hepatitis, inflammatory bowel disease and graft-versus-host disease.
- In most cases, galectin-1 achieved immunosuppression by specific culling of activated T cells, together with immune deviation to a Th2-type response.
- Interestingly, galectin-9 suppresses experimental autoimmune encephalomyelitis by specifically killing Tim-3-positive Th1 pathogenic cells.

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