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Review

Re-wiring regulatory cell networks in immunity by galectin–glycan interactions

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ABSTRACT

Programs that control immune cell homeostasis are orchestrated through the coordinated action of a number of regulatory cell populations, including regulatory T cells, regulatory B cells, myeloid-derived suppressor cells, alternatively-activated macrophages and tolerogenic dendritic cells. These regulatory cell populations can prevent harmful inflammation following completion of protective responses and thwart the development of autoimmune pathology. However, they also have a detrimental role in cancer by favoring escape from immune surveillance. One of the hallmarks of regulatory cells is their remarkable plasticity as they can be positively or negatively modulated by a plethora of cytokines, growth factors and co-stimulatory signals that tailor their differentiation, stability and survival. Here we focus on the emerging roles of galectins, a family of highly conserved glycan-binding proteins in regulating the fate and function of regulatory immune cell populations, both of lymphoid and myeloid origins. Given the broad distribution of circulating and tissue-specific galectins, understanding the relevance of lectin–glycan interactions in shaping regulatory cell compartments will contribute to the design of novel therapeutic strategies aimed at modulating their function in a broad range of immunological disorders.

1. Introduction

1.1. Immune cell homeostatic programs regulated by galectin–glycan interactions: the 'sweet' sound of silence

The immune system has evolved to mount an effective defense against pathogens and tumors and to minimize deleterious inflammation caused by commensal microorganisms and immune responses against self and environmental antigens. Programs that safeguard immune cell homeostasis are required for resetting host protective immunity to steady-state conditions, and for preserving immune tolerance, while preventing autoimmune and allergic reactions, maintaining fetal survival during pregnancy and suppressing metabolic inflammation [1]. However, the same regulatory programs may be usurped by tumors or pathogens to evade immune responses [2]. During the past decades a number of regulatory cell populations, belonging to lymphoid or myeloid lineages, have been shown to be instrumental for preserving and restoring immune cell homeostasis by controlling the fate of innate and adaptive effector cells. These include, among others, different types and subsets of naturally-occurring or inducible regulatory T cells (Tregs), regulatory B cells (Bregs), myeloid-derived suppressor cells (MDSCs), M2-type macrophages and tolerogenic dendritic cells (DCs) [3–7]. Moreover, recent studies highlighted the immunosuppressive potential of other cell types including uterine natural killer (uNK) cells and mesenchymal stem cells (MSCs) [8,9]. One of the hallmarks of regulatory cell populations is their remarkable plasticity as they can be positively or negatively modulated by a broad spectrum of cytokines, chemokines, growth factors and co-stimulatory signals that tailor their differentiation, expansion, stability and survival [10]. Moreover, although underappreciated for many years, emerging observations suggest essential roles for endogenous glycan-binding proteins or lectins and their corresponding glycosylated ligands in controlling the fate and function of immune regulatory cells [11].

Among the various lectin families, galectins are probably the most conserved throughout the evolution, with members identified in most animal taxa examined so far [12]. Although galectins do not have the signal sequence required for the classical secretory

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pathway, most of them are externalized through an unconventional pathway that is still poorly understood [13]. Fifteen members of the galectin (Gal) family, divided into three different subfamilies, have been identified in a variety of cells and tissues: a) 'proto-type' galectins (Gal-1, -2, -5, -7, -10, -11, -13, -14 and -15) have one carbohydrate recognition domain (CRD) that can dimerize, b) 'tandem-repeat' galectins (Gal-4, -6, -8, -9 and -12) contain two homologous CRDs in tandem in a single polypeptide chain and c) the 'chimera-type' Gal-3 which contains a CRD connected to a non-lectin N-terminal region that is responsible for oligomerization [14].

Although galectins can recognize complex glycan determinants with relatively high affinity in the submicromolar range [15], it is their ability to dimerize or oligomerize, together with the structure, number and density of glycan epitopes in multivalent glycoproteins, which determine the avidity of galectin–glycan interactions and their signaling potency [16]. Interestingly, galectins play critical roles outside the cells by interacting with a variety of glycosylated ligands on the cell surface and the extracellular matrix [11]. Once in the extracellular milieu, galectins can regulate cell proliferation, signaling, apoptosis and trafficking and modulate critical physiologic and pathologic processes including inflammation, angiogenesis, tumorigenesis and neurodegeneration [17,18]. However, these lectins can also play roles inside the cells including modulation of cell survival, intracellular immunity, clathrin-independent endocytosis, signaling, mRNA splicing and autophagy [19–21].

Interestingly, analysis of the molecular and biochemical determinants of purified galectins and glycans revealed the formation of two- and three-dimensional arrangements of multivalent structures often termed 'lattices' [22]. These multivalent lectin–glycan complexes have been proposed to serve as scaffolds for organizing cell surface domains, which in turn modulate the signaling threshold of relevant surface glycoproteins including the T cell receptor (TCR), B cell receptor (BCR), cytokine receptors (e.g. transforming growth factor (TGF)-β RII), ion channels (e.g. transient receptor potential cation channel subfamily V member 5; TRPV5), membrane transporters (e.g. glucose transporter 2; GLUT-2) and growth factor receptors (e.g. vascular endothelial growth factor receptor 2; VEGFR2) [11,23,24]. Regarding their saccharide specificity, galectins were first defined by their common ability to recognize the disaccharide *N*-acetyllactosamine [Galβ(1–4)-GlcNAc; LacNAc]. However, recent evidence revealed substantial differences in the glycan-binding preferences of individual members of the galectin family [14,25,26]. Illustrating this concept, Gal-1 binds to non-sialylated and α2,3-sialylated, but not α 2,6-sialylated glycans, whereas Gal-3 binds either α 2,3- or α 2,6-sialylated glycans and Gal-2 exhibits reduced binding to all sialylated glycans. Moreover, Gal-8 has higher affinity for 3′-O-sulfated or 3′-O-sialylated glycans and Lewis X-containing glycans than for LacNAc-terminating oligosaccharides, while Gal-10 surprisingly recognizes mannose-containing ligands [11]. Interestingly, different factors may control the biological activity of galectins including: (a) their oligomerization status (monomeric versus dimeric or oligomeric forms); (b) their subcellular compartmentalization (nuclear, cytoplasmic or extracellular localization); (c) their stability in reducing or oxidative microenvironments and (d) the active remodeling of N- and O-glycans on target cells [18,27].

The contribution of galectins and glycosylated ligands to innate and adaptive immune responses, particularly effector T cell responses, has been reviewed elsewhere [28–31]. Here we focus on the relevance of galectins and glycans in regulating the fate and function of lymphoid and myeloid regulatory cell populations including Tregs, Bregs, tolerogenic DCs, M2-type macrophages and MDSCs.

2. Regulatory T cells

Regulatory T cells are key players in maintaining the balance between immune activation and tolerance. They can shut-off exuberant or undesired immune responses by restraining inflammation to self antigens, commensal microbiota, allergens, and pathogens, thus preventing autoimmune and autoinflammatory disorders. The so-called, inducible $CD4^+$ regulatory T cells (iTregs) are generated outside the thymic compartment to regulate peripheral immune tolerance, whereas thymus-derived naturally-occurring $CD4^+$ regulatory T cells (nTregs) are generated in the thymus. Depending on whether they stably express the forkhead box P3 (Foxp3) transcription factor, iTregs may be divided into two subsets: the classical TGF-β-induced $CD4^+Foxp3^+$ Tregs and the $CD4^+Foxp3^-$ type 1 regulatory T (Tr1) cells [32]. From a functional viewpoint, Tregs can suppress T cell responses through different, although potentially overlapping mechanisms including the synthesis of inhibitory cytokines (interleukin (IL)-10, IL-35, TGF-β), suppression by cytolysis (perforin- and granzyme-dependent pathways), inhibition by metabolic disruption (IL-2 deprivation), and modulation of DC function (i.e. tryptophan depletion via induction of indoleamine 2,3-dioxygenase; IDO) [3]. Development of Tregs, either in the thymus or peripheral compartments, as well as their stability and function, all depend on the right combination of intracellular signals and environmental cues, including cytokines, chemokines, microbial products and metabolites [3].

Under this complex scenario, galectins and their ligands have emerged as novel regulators of Tregs biology and mediators of their immunosuppressive activity. In microarray analysis, the *LGALS1* gene, encoding Gal-1, was found to be up-regulated in Tregs compared to activated effector T cells [33]. Garin and colleagues confirmed the abundance of Gal-1 protein in $Foxp3^+$ Tregs and provided evidence of its contribution to the suppressive activity of these cells. Targeted disruption of Gal-1, using biochemical or genetic approaches, attenuated the inhibitory effects of human and mouse $CD4^+CD25^+$ Tregs, suggesting the involvement of this lectin in Treg cell-mediated immunosuppression [34]. Further mechanistic analysis demonstrated that $Foxp3^+$ Tregs utilize Gal-1 to transiently inhibit PI3K/p21ras activity in human $CD8^+$ T cells despite partial activation of TCR proximal signals, such as phosphorylation of CD3ζ, zeta-chain-associated protein kinase 70 (Zap70), linker of activated T cells (LAT) and protein kinase Cϕ (PKCϕ), leading to $CD8^+$ T cell dysfunction [35]. Interestingly, Wang et al. showed that Treg-derived Gal-1 dampens effector T cell responses through cross-linking of the monosialotetrahexosylganglioside (GM1), resulting in activation of the short transient receptor potential channel 5 (TRPC5) and modulation of Ca^{2+} influx [36]. Thus, targeting Gal-1 synthesis in Tregs may contribute to attenuate the suppressive potential of these cells, leading to stimulation of anti-tumor and anti-microbial T cell-mediated immunity. On the other hand, reinforcing Gal-1 expression would lead to generation of Tregs with enhanced immunosuppressive activity in settings of chronic inflammation, autoimmune disease and organ transplantation.

However, this regulatory mechanism does not seem to be limited to Gal-1, as other members of the galectin family have also been shown to be up-regulated in Tregs. In fact, *LGALS3*, the gene encoding Gal-3, is selectively increased in human Tregs, as compared to human T helper (Th) cells through a transcriptional mechanism involving the gene expressing ubiquitin D (UBD), a downstream element of Foxp3 [37]. Interestingly, proteomic analysis of human $CD4^+CD25^+Foxp3^+$ Tregs identified Gal-10 as a novel marker that delineates this cell population from resting and activated $CD4^+$ T cells. Targeted inhibition of Gal-10 restored the proliferative capacity of human Tregs and abrogated their suppressive

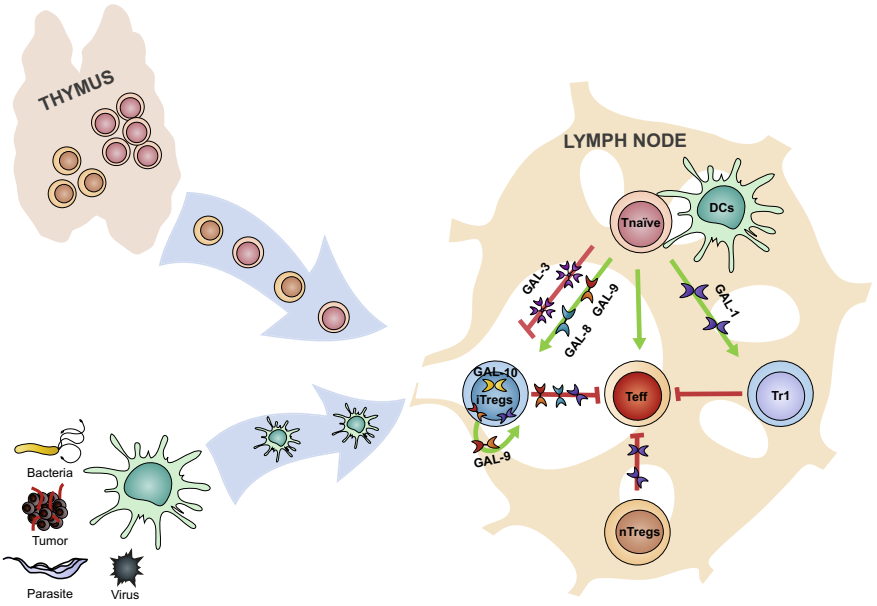

Fig. 1. Impact of galectins in the modulation of Treg cell programs. Naïve T cells (T naïve) and naturally-occurring regulatory T cells (nTregs) emigrate from the thymus to lymph nodes. Dendritic cells (DCs) reach the lymph nodes via afferent lymphatic vessels following processing of microbial and tumor antigens. Upon antigen presentation, TCR signaling and local cytokines contribute to activation and differentiation of effector T cells (Teff), which readily migrate to peripheral tissues to eradicate pathological threats. However, different stimuli, including members of the galectin family instruct differentiation of naïve T cells toward a regulatory phenotype. Galectin (GAL)-1 favors differentiation of naïve T cells into Foxp3$^-$IL-10$^+$ Tr1 regulatory cells. GAL-1 is up-regulated in inducible Tregs (iTregs) and nTregs, and contributes to their immunosuppressive activity. GAL-3 inhibits differentiation of naïve T cells into iTregs, whereas GAL-8 and GAL-9 enhance the iTreg profile and suppress Teff activity. Studies also showed that GAL-10 contributes to the suppressive function of human Tregs.

function [38]. Moreover, Wang et al. found that Gal-9, which serves as a ligand for the T cell immunoglobulin mucin-3 (Tim-3) receptor, was up-regulated on mouse CD4$^+$CD25$^+$Foxp3$^+$ Tregs and positively modulated their function. Blockade of the Tim-3-Gal-9 pathway counteracted the suppressive activity of Tregs, enhanced Th1 cytokine production in vitro and abrogated survival of allogeneic skin grafts induced by Tregs in vivo [39]. Furthermore, recent studies demonstrated that hepatic Foxp3$^+$ Tregs promoted liver T cell homeostasis in a model of concanavalin A-induced hepatitis via up-regulation of Gal-9 [40]. Likewise, Gal-9-expressing Tregs promote the exhaustion of Tim-3$^+$ T cells in patients persistently infected with hepatitis C virus (HCV) [41]. The mechanisms underlying these effects were recently revealed by Wu and colleagues, who showed that expression of Gal-9 was crucial for the generation of iTregs, but not nTregs. The authors found that activation of the Smad3 transcription factor induced expression of this lectin and modulated iTreg cell stability and function through direct association with a molecular complex involving CD44 and TGF-β RI [42]. Thus, distinct members of the galectin family, including Gal-1, -3, -9 and -10, are up-regulated in Tregs and may influence their immunosuppressive function.

In a large number of experimental models of autoimmune disease and chronic inflammation, including collagen-induced arthritis (CIA), experimental autoimmune encephalomyelitis (EAE), experimental autoimmune uveitis (EAU), experimental diabetes, inflammatory bowel disease (IBD), experimental autoimmune orchitis (EAO) and graft versus host disease (GvHD), in vivo administration of recombinant Gal-1 or its genetic delivery attenuated the clinical severity of the disease and tilted the balance toward an anti-inflammatory cytokine profile [43–49]. On the other hand, silencing of Gal-1 gene expression or antibody-mediated Gal-1 blockade suppressed tumor growth and/or metastasis and promoted T cell-mediated tumor rejection in a variety of cancers including melanoma, lung adenocarcinoma, pancreatic adenocarcinoma, Hodgkin's lymphoma, neuroblastoma, mammary adenocarcinoma, Kaposi's sarcoma, glioblastoma and ovary carcinoma [24,50–58]. Seeking for potential mechanisms that could explain the broad immunosuppressive activity of this β-galactoside-binding lectin [59], we found, in a model of autoimmune ocular inflammation, that Gal-1 therapy increased the frequency of Foxp3$^-$ Tr1 cells, which suppressed retinal disease when adoptively transferred into immunized mice [45]. Moreover, Foxp3$^+$ Tregs were also expanded following administration of recombinant Gal-1 in a lupus-like model [60]. In vitro, Gal-1 increased the relative abundance of CD4$^+$CD25$^+$Foxp3$^+$ Tregs in co-cultures with Reed Sternberg cells [53]. Interestingly, in a breast cancer model, silencing tumor-derived Gal-1, using small hairpin ribonucleic acid (shRNA) strategies, reduced the frequency of CD4$^+$CD25$^+$Foxp3$^+$ Tregs within the tumor, draining lymph nodes, spleen, and lung metastases, attenuated the immunosuppressive activity of Tregs and selectively lowered expression of the regulatory molecule LAT [55]. Likewise, in a mouse model of stress-induced failing pregnancies in vivo and in co-cultures of human trophoblasts and T cells in vitro, Gal-1 favored the induction of Foxp3-dependent Treg cell programs [61,62]. Notably, Gal-1 was preferentially expressed either by trophoblast cells or by uNK cells, which play key roles in maintenance of placental immune privilege [63]. These effects were confirmed in studies demonstrating that Gal-1 controls cardiac inflammation and suppresses acute myocardial infarction by increasing the frequency of Tregs in cardiac tissue [64]. Thus, in addition to the well-established immune regulatory roles of this lectin, mediated by induction of Th1 and Th17 cell apoptosis [44], regulation of cytokine secretion [65] and inhibition of T cell trafficking [28], Gal-1 also contributes to create immunosuppressive microenvironments via induction of Foxp3$^+$ Tregs and Foxp3$^-$ Tr1 cells.

Moreover, in a model of autoimmune uveitis, Gal-8 increased the number of Tregs in both the draining lymph node and the inflamed retina. In vivo generated Gal-8-induced Tregs expressed the inhibitory coreceptor cytotoxic T lymphocyte antigen (CTLA)-

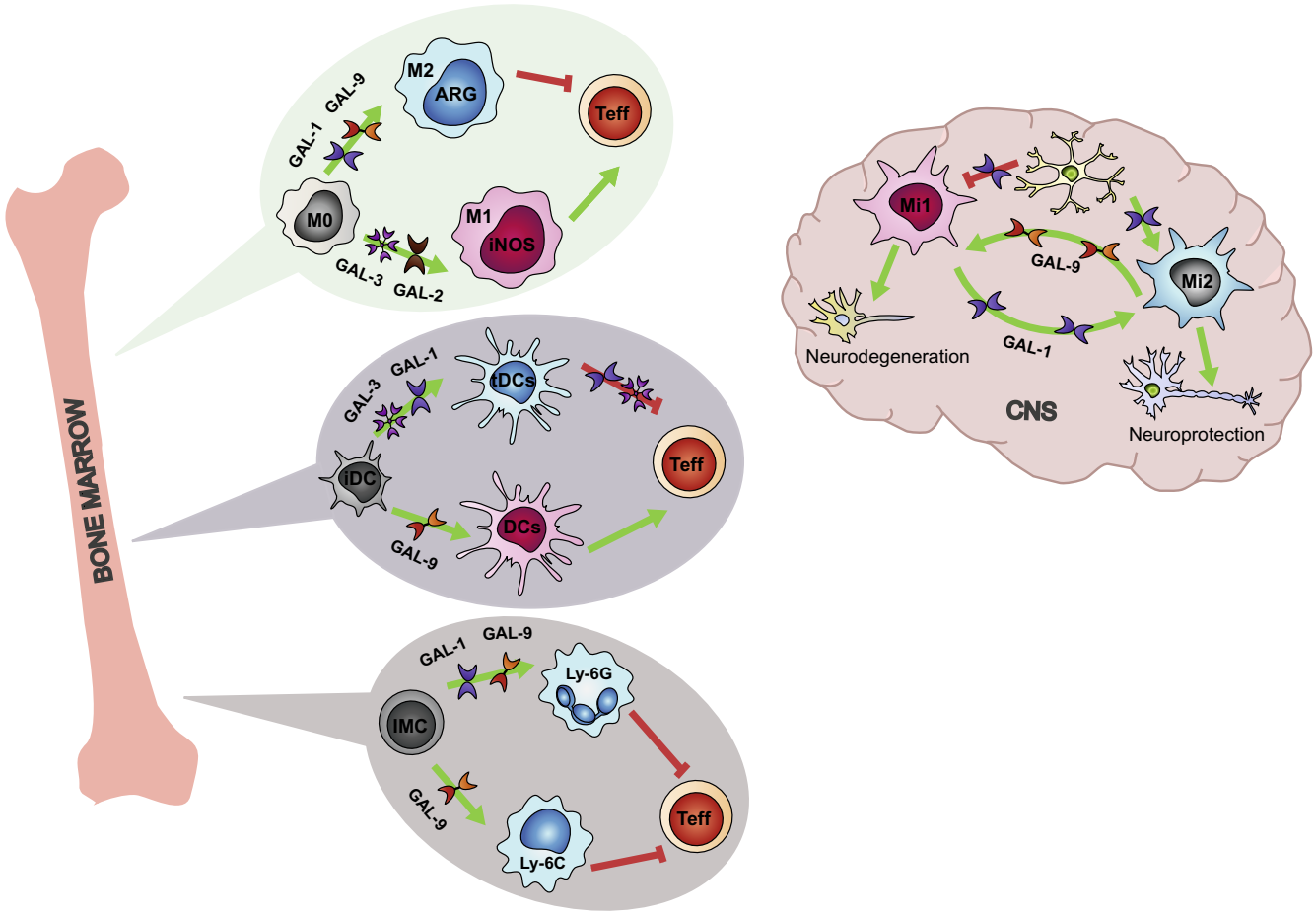

Fig. 2. Impact of galectins in the control of regulatory myeloid cells. Upon interactions with stromal cells, cytokines and growth factors, myeloid cells can differentiate and emigrate from the bone marrow to colonize peripheral tissues and orchestrate innate immune responses. However, given their plasticity, immunogenic myeloid cells (*pink*) can also display regulatory or tolerogenic activity (*blue*). Galectin (GAL)-1 and GAL-9 stimulate M2-type polarization in macrophages, enhancing arginase (ARG) activity and supporting anti-inflammatory functions. On the other hand, GAL-2 and GAL-3 can polarize macrophages toward an M1 phenotype, characterized by high iNOS activity and pro-inflammatory function. Within the DC compartment, GAL-1 and GAL-3 can instruct these cells to become tolerogenic, while GAL-9 favors an immunogenic profile. Whereas GAL-1 increases conversion of immature myeloid cells (IMCs) into Ly-6G$^+$ MDSCs, GAL-9 favors differentiation into Ly-6G$^+$ and Ly-6C$^+$ MDSC profiles. Within the Central Nervous System (CNS), astrocytes synthesize high amounts of GAL-1 which, upon binding to CD45 on microglia cells, favors their polarization into an M2-type profile (Mi2), thereby preventing demyelination and inflammation-induced neurodegeneration. In contrast, GAL-9 shifts the balance toward an M1 (Mi1) pro-inflammatory phenotype.

4, the immunosuppressive cytokine IL-10, and the tissue-homing integrin CD103 [66]. In vitro analysis of the mechanisms underlying Gal-8 effects revealed the ability of this lectin to activate the TGF-β pathway and sustain IL-2 receptor signaling [67]. Interestingly, the Gal-9-Tim-3 axis can also modulate Treg cell biology. Seki and colleagues found that recombinant Gal-9 suppressed clinical signs of arthritis, reduced the number of Tim-3$^+$CD4$^+$ T cells and promoted differentiation of naïve T cells into Tregs in vitro [68]. Moreover, Gal-9 favored the expansion and proliferation of Foxp3$^+$ Tregs in a model of myocarditis [69], and contributed to the immunosuppressive activity of HCV-infected hepatocytes [70]. Furthermore, in response to Toll-like receptor (TLR)9 ligands and non-digestible oligosaccharides, Gal-9 released by intestinal epithelial cells favored Treg cell polarization [71]. These regulatory effects were recently confirmed by intranasal administration of Gal-9-expressing adenoviral associated virus (rAAV) into mice infected with respiratory syncytial virus (RSV). Genetic delivery of Gal-9 decreased viral load, and reduced the severity of lung pathology through mechanisms involving expansion of Tregs [72]. Analysis of the intracellular pathways triggered by Gal-9 revealed the ability of this lectin to enhance TGF-β_1-induced phosphorylation of Smad2/3 and extracellular signal-regulated kinases 1/2 (ERK1/2) [73]. In contrast to the positive role of Gal-1, -8- and -9 in promoting Treg cell differentiation and augmenting their suppressive activity, Gal-3 mostly displayed inhibitory effects within the Treg cell compartment. In a model of autoimmune neuroinflammation, Gal-3 deficiency reduced the clinical symptoms of the disease and increased the frequency of Foxp3$^+$ Tregs in the central nervous system (CNS), suggesting a major pro-inflammatory role for this lectin [74]. Supporting these findings, Gal-3 also reduced the frequency and function of CD4$^+$CD25$^+$Foxp3$^+$ Tregs in a model of *Leishmania major* infection via modulation of the Notch signaling pathway [75].

Collectively, these data imply that a coordinated galectin program may control Treg cell biology by regulating their differentiation, expansion, stability and immunosuppressive activity (see Fig. 1). Whether these glycan-binding proteins are transcriptionally or epigenetically regulated during the lifespan of Tregs in distinct physiologic and/or pathologic settings still remains to be elucidated. Moreover, further studies are warranted to investigate the differential contribution of individual members of the galectin family to Treg cell trafficking, expansion and tissue distribution and their selective involvement in nTregs versus iTregs compartments.

3. Regulatory B cells

In addition to their well-established roles in antigen recognition and processing and their unique function as precursors of immunoglobulin-producing plasma cells, B cells may also become regulatory suppressing immunopathology, silencing the expansion of pathogenic T and B cells and supporting immunological tolerance through the production of IL-10, IL-35, and TGF-β [76]. Studies in experimental animal models, as well as in patients with autoimmune and infectious diseases, have identified multiple Breg subsets induced in response to inflammation, which arise at different stages of B cell development and exhibit diverse mechanisms of immune suppression [76].

We have demonstrated that, upon BCR engagement, B cells synthesize and secrete elevated amounts of Gal-1 to dampen the survival of neighboring activated T cells [77]. Moreover, intracellular Gal-1 has been shown to interact with the B cell-specific transcriptional coactivator Oct binding factor (OBF)-1-OCA-B and negatively regulated B cell proliferation and tyrosine phosphorylation upon BCR stimulation [78], suggesting that this lectin can restrain T cell and B cell signaling. However, exposure of human chronic lymphocytic leukemia (CLL) B cells to extracellular Gal-1 supported tonic B cell signaling [79], an effect that was recently confirmed by phosphoproteomic analysis of mature B cells [80]. Thus, intracellular and extracellular Gal-1 may play different roles in modulating mature B cell fate.

Interestingly, Clark and collaborators identified Gal-1 (*LGALS1*) and Gal-3 (*LGALS3*) as genes that were differentially transcribed in anergic B cells [81]. The authors further demonstrated that mice lacking Gal-1 or Gal-3 showed subtle differences in B cell tolerance [82], suggesting that galectin deficiency may impact B cell tolerogenic circuits in combination with other factors, including epigenetic and/or environmental mechanisms. Moreover, Gal-1 was found to be up-regulated in IgM^+ memory B cells and contributed to B cell apoptosis through inhibition of Akt phosphorylation and up-regulation of the BH3-only protein Bim [83], thus substantiating the key regulatory role of galectins within the mature B cell compartment. Interestingly, although Gal-1 suppressed mature B cell signaling, it increased plasma cell differentiation and survival, suggesting that this lectin may be part of the intracellular signaling machinery that governs B cell decisions [84,85]. This regulatory effect appears to be restricted to Gal-1 as other members of the galectin family have been proposed to play opposite role in plasma cell biology. In fact, Gal-9 promoted plasma cell apoptosis [86] and intracellular Gal-3 has been reported to skew the balance toward a memory B cell phenotype [87]. Following antigen challenge, Gal-3 deficiency led to enhanced plasma cell differentiation and unleashed immunoglobulin production, both in B1 and B2 cells [87,88]. Finally, elegant studies reported that, during B-cell development, Gal-1 secreted by bone marrow stromal cells acts as a pre-B cell receptor ligand to modulate B cell maturation through mechanisms involving displacement of Gal-1/glycan lattices and shift toward a glycosylation-independent Gal-1-mediated protein–protein interaction [89]. Thus, although not investigated in such detail as the T cell compartment, these studies suggest that galectins may play different regulatory roles during the lifespan of B cells, targeting their maturation, differentiation, signaling, tolerance and survival.

4. Tolerogenic dendritic cells

In spite of their traditional role in orchestrating adaptive immune responses, conventional DC subsets, including myeloid DCs and plasmacytoid DCs, can also attenuate inflammatory reactions by promoting T cell anergy or by favoring the expansion, differentiation and/or recruitment of Tregs or Bregs [90]. During the course of chronic inflammatory reactions, bidirectional interactions take place between DCs and T cells, which may initiate either immunogenic or tolerogenic circuits [91,92]. Several stimuli may influence the decision of DCs to become tolerogenic, including IL-10, TGF-β, vasoactive intestinal peptide (VIP) and 1,25-dihydroxyvitamin D3. In addition, interaction with stromal cells, $CD4^+CD25^+Foxp3^+$ Tregs or apoptotic cells may also drive the differentiation of tolerogenic DCs [91].

We found that both human and mouse DCs differentiated or matured in a Gal-1-enriched microenvironment acquired a distinctive regulatory or tolerogenic signature characterized by low expression of CD11c (mouse DCs) or CD1a (human DCs), high expression of the cell surface marker CD45RB, phosphorylation of the transcription factor signal transducers and activators of transcription (STAT)-3 and abundant secretion of IL-27 and IL-10 [93]. When transferred in vivo, these DCs promoted T-cell tolerance in antigen-specific settings, blunted Th1 and Th17 responses and halted autoimmune CNS inflammation through mechanisms involving DC-derived IL-27 and T cell-derived IL-10. Thus, using IL-27 receptor-deficient ($Il27ra^{-/-}$) and IL-10-deficient ($Il10^{-/-}$) mice, our results identified an immunoregulatory circuit linking Gal-1 signaling, IL-27-producing tolerogenic DCs and IL-10 secreting Tregs [93]. Induction of regulatory DCs by Gal-1 also contributed to immune tolerance in settings of cancer and pregnancy [54,61,94]. In fact, Kuo et al. found that lung cancer derived-Gal-1 promoted tolerogenic DCs, which in turn favored the induction of $CD4^+CD25^+Foxp3^+$ Tregs. At the molecular level, this regulatory effect was, at least in part, mediated by induction of the inhibitor of DNA binding 3 (Id3) and up-regulation of IL-10 [94]. Interestingly, this tolerogenic mechanism may be usurped by pathogens to thwart host protective responses as illustrated by a recent study showing that *Trypanosoma cruzi*, the etiological agent of Chagas' disease, instructs differentiation of tolerogenic DCs and $Foxp3^+$ Tregs and blunts Th1 and $CD8^+$-mediated antiparasite immunity [95]. Moreover, Thiemann et al. have recently shown a novel mechanism through which Gal-1 blunts DC responses; the authors demonstrated that this lectin inhibited tissue emigration of immunogenic, but not tolerogenic DCs, through extracellular matrix and across endothelial cells. This selective effect appeared to be related to the differential expression of core-2 O-glycans on CD43 on immunogenic versus tolerogenic DCs, and to reduced phosphorylation of the protein tyrosine kinase 2 (Pyk2) [96]. Notably, up-regulation of glycosyltransferases involved in LacNAc synthesis revealed greater sensitivity of mature versus immature DCs to the immunoregulatory effects of galectins [97]. However, not only exogenous but also endogenous Gal-1 can endow DCs with tolerogenic capacity. In fact, Gal-1-expressing DCs greatly contributed to the resolution of antigen-specific and autoimmune diseases [93]. Although the precise molecular mechanisms involved in this effect still remain to be explored, recent studies identified CD69 as a counter-receptor for DC-derived Gal-1 on T cells, suggesting that a Gal-1-CD69 axis may contribute to DC-mediated suppression of pro-inflammatory T cell responses [98]. Moreover, DC expression of Gal-1 mediates, at least in part, the tolerogenic effects triggered by IL-10 and apoptotic cells [99,100]. Supporting these findings, shRNA-mediated knockdown of human galectins on DCs, revealed the importance of Gal-1 and Gal-3, but not Gal-8, in DC suppression of human T cell responses [101].

Evidence demonstrating the role of endogenous Gal-3 in promoting mouse regulatory DCs was provided by Bernardes et al. who showed that DCs devoid of this lectin synthesize greater amounts of IL-12 in response to *Toxoplasma gondii* infection [102]. However, in the context of helminth infection, targeting Gal-3 on DCs enhanced T-cell cytokine responses both in vitro

and in vivo, without biasing the immune response toward a Th1 or Th2 direction [103]. In line with these findings, recent studies showed that endogenous Gal-3 suppresses Th17 responses during fungal infections by attenuating secretion of IL-23 on DCs via inhibition of c-Rel/nuclear factor (NF)-κ B [104,105]. Thus, at least in infection models, DC-derived Gal-3 contributes to regulate the magnitude of Th cytokine responses, suggesting a major regulatory role of this lectin in regulating the strength and duration of adaptive immune responses. Interestingly, elegant studies aimed at elucidating the role of mucins during gut homeostasis showed that glycans associated with mucin 2 (MUC2) instructed DCs to become tolerogenic by facilitating the formation of a multimeric complex formed by Gal-3, the C-type lectin Dectin-1 and the Fc receptor FcγRIIB. Signaling through this receptor complex activates the β-catenin transcription factor and inhibits transcription of pro-inflammatory cytokines via a NF-κB-dependent pathway [106]. More recently, it has been demonstrated that, in addition to the well-established role of this lectin within conventional DC compartments, tumor-derived Gal-3 can also shape effector responses and defeat cancer immunotherapy strategies by limiting the expansion of plasmacytoid DCs [107].

In contrast to the tolerogenic activities of Gal-1 and Gal-3, exposure of human and mouse DCs to Gal-9 often evoked pro-inflammatory responses. Accordingly, Gal-9-treated DCs secreted higher amounts of IL-12, but not IL-10, elicited the production of Th1 cytokines [108] and increased the number of Tim-3^{+}CD86^{+} mature DCs [109]. A mechanistic analysis successfully dissected the paradoxical T cell regulatory and DC proinflammatory activities of Gal-9, suggesting that the N- and C-terminal CRDs of this lectin contribute differently to its multiple functions. While Gal-9-C is implicated in the regulation of T cell survival, the Gal-9-N is much more effective in activating DCs by inducing higher tumor necrosis factor (TNF) and IL-6 production and greater p38 mitogen-activated protein kinase (p38 MAPK)s and Akt phosphorylation [110]. Collectively, these studies suggest different roles of individual members of the galectin family in DC biology. Whereas Gal-1 and Gal-3 augment the tolerogenic properties of DCs, exposure to the tandem-repeat Gal-9 increases the immunogenic potential of these cells (see Fig. 2). Whether a galectin-specific signature might delineate the tolerogenic or immunogenic function of DCs is still an open question.

5. Alternatively-activated M2-type macrophages and microglia

Cells of the monocyte/macrophage lineage are characterized by their functional diversity and plasticity. In response to a broad spectrum of stimuli (e.g. TLR agonists, microbial products or cytokines), tissue macrophages may undergo M1 (pro-inflammatory or classically-activated) or M2 (anti-inflammatory or alternatively-activated) polarization [111]. While M1-type macrophages are associated with antimicrobial responses, antitumor immunity and pathogenic autoimmune inflammation, M2-type polarization delineates a variety of biological responses including tissue repair, immunosuppression, promotion of tumor angiogenesis and metastasis [111]. The presence of interferon (IFN)-γ or bacterial lipopolysaccharides (LPS) endows macrophages with M1-type activity, whereas IL-4 and IL-13 skew the balance toward an M2-type alternatively-activated profile [112]. Interestingly, M1-type macrophages produce high amounts of nitric oxide (NO) through activation of inducible nitric oxide synthase (iNOS) and secrete greater amounts of pro-inflammatory cytokines such as TNF, IL-1 and IL-6, favoring cytotoxic responses, microbial killing and tissue injury. In contrast, polarization toward an M2-type phenotype involves preferential expression of arginase-1 and synthesis of IL-10, TGF-β and VEGF [112]. This characteristic phenotype of M2-type cells is accompanied by lower expression of major histocompatibility complex (MHC) II and CD11b and up-regulated expression of the chitinase-3-like protein 3, also known as Ym1, a lectin with affinity for glycosaminoglycans such as heparin and heparan sulfate [112]. Interestingly, similar but not identical polarization profiles have been observed in cells of the microglial lineage, and dynamic fluctuations of M1/M2 phenotypes have been associated with different stages of brain inflammatory diseases including multiple sclerosis, Alzheimer's disease, Parkinson's disease and spinal cord injury. Generally, M1-type microglia have been associated with neurodegenerative and demyelinating processes, while M2-type microglia are often a hallmark of immune resolution, neuroprotection and repair [113]. Identification of the mechanisms, pathways and molecules associated with macrophage plasticity and polarization provides an attractive framework for macrophage-centered therapeutic strategies.

We have identified the presence of Gal-1 in macrophages [114], its up-regulated expression in response to macrophage activation [114,115] and its ability to shift the balance toward an M2-type profile, as shown by suppression of iNOS expression and NO production and up-regulation of arginase activity [116]. This inhibitory function was confirmed in human monocytes, which upon exposure to Gal-1, exhibited a dose-dependent reduction of IFN-γ-induced MHC II expression and MHC II-dependent antigen presentation [117]. Supporting these findings, exposure to Gal-1 inhibited arachidonic acid release and prostaglandin E2 (PGE_2) synthesis [118] and favored the conversion of macrophages toward a pro-resolving phenotype, characterized by CD11b^{low} surface expression, up-regulated activity of the 12/15-lipoxygenase (a pro-resolving enzyme), loss of phagocytic capacity (efferocytic satiation) and diminished TNF and IL-1 secretion [119], suggesting that exogenous Gal-1 may favor alternative M2 activation, de-activation or promotion of a pro-resolving state within the monocyte/macrophage compartment. In this regard, one might speculate that phagocytic clearance of dying cells or cells exposing phosphatidylserine as a result of Gal-1 treatment, might also contribute to the generation of M2-type macrophages and restoration of immune cell homeostasis [120,121]. Supporting these findings, macrophages from mice lacking the β1,3-*N*-acetylglucosaminyltransferase 2 (β3GnT2), an enzyme required for extending poly-LacNAc residues (specific ligands for galectins), showed a greater activation profile upon challenge with inflammatory stimuli compared with their wild-type counterpart [122].

Interestingly, in a model of autoimmune neuroinflammation, we found that astrocytes produce high amounts of Gal-1 which was sufficient to convert inflammatory M1-type microglia toward a neuroprotective anti-inflammatory M2-type phenotype. Mechanistically, astrocyte-derived Gal-1 bound to core 2-O-glycans on CD45, retained this glycoprotein on the surface of microglia cells and increased its phosphatase activity, thereby promoting microglia de-activation. This effect involved modulation of the p38 MAPK, cyclic adenosine monophosphate (cAMP) response element-binding (CREB) and NF-κB-dependent pathways and suppression of pro-inflammatory cascades mediated by iNOS, TNF and the chemokine (C–C motif) ligand 2 (CCL2) [123]. As a result, alternatively-activated M2 microglia prevented inflammation-induced neurodegeneration and abrogated the demyelination process [123]. This inhibitory effect, together with the direct neuroprotective role of Gal-1, mediated by the neuropilin-1 (NRP1)-Plexin A4 complex [124], contributes to promote axonal regeneration, tissue repair and CNS homeostasis. Notably, although exogenous Gal-1 favored an M2 activation phenotype, polarization of human monocytes into M1 or M2 profile was followed by profound changes in intracellular Gal-3, but not Gal-1 expression [125], sug-

gesting divergent, although complementary roles of galectins in tailoring macrophage polarization and function.

Within the immune system, Gal-3 was originally defined as Mac-2 antigen or IgE-binding protein (εBP), and found to be preferentially expressed in tissue macrophages and mast cells [126–128]. Research over the past few years revealed that, in addition to the well-established roles of Gal-3 in controlling macrophage survival and phagocytosis [129,130], this lectin also serves to induce alternative activation of these cells [131]. Accordingly, Gal-3 null (*Lgals3*$^{-/-}$) mutant mice showed defects in IL-4/IL-13-induced alternative macrophage activation in bone marrow-derived macrophages in vitro and in resident lung and recruited peritoneal macrophages in vivo, without affecting IFN-γ/LPS-induced classical activation or IL-10-induced deactivation [131]. In contrast, in a model of liver hepatotoxicity, Gal-3 deficiency resulted in suppression of M1-type pro-inflammatory, but not M2-type macrophage infiltrates [132]. Likewise, high Gal-3 expression was found to be a hallmark of M1-type macrophages in pancreatic inflammatory infiltrates in a model of type-2 diabetes [133]. Moreover, in human peripheral blood monocytes, Gal-3 enhanced IL-10 production, thus contributing to de-activation of these cells [134]. Taken together, these results suggest context-dependent regulation of monocyte/macrophage polarization by Gal-3, which could result from the differential expression of pro- versus anti-inflammatory cytokines in target tissues or the in vivo evaluation of fresh liver macrophages versus in vitro differentiated bone marrow-derived macrophages.

Interestingly, recent studies by Burguillos and colleagues elegantly showed that Gal-3 serves as an endogenous paracrine ligand for TLR4 on microglia cells, shifting the balance toward an M1-type phenotype, which accentuates CNS inflammation [135]. In accordance with these findings, cuprizone (CPZ)-treated mice displayed heightened M1 microglial activation associated with ectodermal dysplasia protein 1 (ED1) expression and pronounced upregulation of the phagocytic triggering receptor expressed on myeloid cells 2 (TREM-2b), while this effect was not observed in CPZ-treated *Lgals3*$^{-/-}$ mice [136]. The pro-inflammatory effects of Gal-3 on microglial cells were found to be mediated by modulation of the Janus kinase (JAK)-STAT signaling pathway [137]. Likewise, Gal-2 was found to function as a pro-inflammatory factor that skews human macrophages toward an M1-like phenotype through binding to CD14 and activating the TLR4 signaling [138]. This pro-inflammatory profile was also suggested in earlier studies demonstrating that Gal-2 expression in macrophages links the lymphotoxin-α cascade with myocardial infarction [139]. Finally, although Gal-9 treatment ameliorated autoimmune arthritis, inhibited FcγR expression and expanded immunosuppressive CD11b^{+}Ly-6C^{+} macrophages [140], association of Gal-9 with Tim-3 on microglia cells promoted tissue inflammation in a model of CNS autoimmunity [141]. These discrepancies could be explained, at least partly, by *cis*-association of Gal-9 to Tim-3 within the macrophage surface, which amplifies TLR signaling and favors pro-inflammatory responses, as opposed to the inhibitory effects of Tim-3 upon delivery of this lectin in *trans* [142]. Thus, distinct members of the galectin family, function intracellularly or extracellularly, to regulate macrophage polarization and activation thresholds, thereby influencing the magnitude and resolution of acute and chronic inflammatory processes (see Fig. 2).

6. Myeloid-derived suppressor cells

Myeloid-derived suppressor cells represent a heterogeneous population of immature and mature myeloid cells that expand and accumulate under pathological conditions, such as cancer, acute and chronic infections, trauma and autoimmune diseases. These cells have been identified in most patients and experimental tumor models based on their ability to dampen effector T cell responses [143]. Recently, two major subtypes of MDSCs have been identified in mice and human, namely granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs) based on morphology, phenotype and functional differences. In mice G-MDSCs have a CD11b^{+}Ly-6G^{+}Ly-6C^{low} phenotype, whereas M-MDSCs display a CD11b^{+}Ly-6G^{-}Ly-6C^{high} phenotype as the Ly-6G molecule is known to be expressed primarily on granulocytes, whereas Ly-6C is typically highly expressed on monocytes. On the other hand, recent studies have implicated CD15 and CD66b as potential markers allowing discrimination of granulocytic and monocytic MDSCs in humans. Interestingly, G-MDSCs primarily use reactive oxygen species (ROS) as the predominant mechanism of immune suppression, whereas M-MDSCs primarily use NO, arginase and inhibitory cytokines to blunt T cell responses [144]. Investigation of the stimuli that regulate expansion and accumulation of MDSCs in the blood, lymph nodes and tumor sites, revealed multiple pro-inflammatory factors including PGE_2, IL-1β, IL-6, VEGF and HYPERLINK "https://en.wikipedia.org/wiki/S100_protein" \o "S100 protein" S100 calcium-binding protein A8/A9 (S100A8/A9), suggesting a link between inflammation, immunosuppression and tumor growth [4]. Interestingly, the immunoregulatory activity of MDSCs can be positively or negatively influenced by a myriad of tumor-derived cytokines and growth factors or by treatment with pharmacological agents [143,145]. Thus, targeting MDSCs either in combination with cancer immunotherapy or vaccination strategies appears to be a clinically promising strategy to overcome immunosuppression and potentiate immune responses. In contrast, promotion of their expansion or accumulation in inflamed tissues, may contribute to alleviate undesired responses in settings of autoimmune inflammation and graft rejection.

Although the impact of galectins within the MDSC compartment has not been studied in such detail as other regulatory cell populations, Dardalhon and colleagues found that activation of the Gal-9/Tim-3 pathway increased the number of CD11b^{+}Ly-6G^{+} G-MDSCs which negatively controlled T cell responses in vivo [146]. Similarly, in a model of myocarditis, Gal-9 remarkably increased the frequency of CD11b^{+}Gr-1^{+} MDSCs in cardiac tissue and spleens of treated mice, although in this case, a Ly-6C^{+} M-MDSC population was favored [147]. Alterations in the frequency of granulocytic and monocytic MDSCs were recently observed under Gal-3 deficiency in a mouse model of lung inflammation, suggesting that Gal-3 also controls the fate of this immunoregulatory cell population [148]. Likewise, silencing Gal-1 expression significantly decreased the frequency of brain-infiltrating MDSCs in models of glioblastoma [149]. Finally, a circuit linking microbiota, tumor-promoting inflammation and immunosuppression via a galectin-dependent mechanism was recently identified. In TLR5-responsive tumors, systemic IL-6 induced by commensal bacteria drove the mobilization of G-MDSCs, which in turn promoted expansion of Gal-1-secreting γδ T lymphocytes, which dampened antitumor immunity and accelerated malignant progression [58]. Although further evidence is still required, these results suggest that a coordinated galectin network may act in concert to differentially control MDSC programs (see Fig. 2).

7. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells with the potential to regenerate tissues and differentiate into a variety of cell types. They have generated great interest because of their broad immunomodulatory potential, mainly due to secretion of soluble factors such as TGF-β, IDO and PGE_2, which could be exploited for the treatment of autoimmune disorders and

prevention of GvHD and graft rejection [150]. Seeking for novel mediators of the immunoregulatory activity of these cells, Gieseke and colleagues identified Gal-1 as a major contributor to MSCs-induced immunosuppression. Knocking down Gal-1 in human MSCs resulted in a significant loss of their immunoregulatory activity compared with MSCs infected with non-targeting sequences. Whereas Gal-1 silencing partially restored proliferation of $CD4^+$ and $CD8^+$ T cells and augmented the release of T-cell derived cytokines, MSC-induced inhibition of natural killer (NK) cell activity was unaffected [151]. Interestingly, Gal-1 and Gal-3 were constitutively expressed in MSCs [152], whereas Gal-9 was readily induced upon exposure to pro-inflammatory or activating stimuli to inhibit T cell and B cell proliferation [153,154]. In vivo, Gal-1 also contributed to suppression of acute liver injury induced by tonsil-derived MSCs [155], and, together with Gal-3, influenced the immunoregulatory activity of human umbilical cord blood-derived MSCs [156,157]. These findings suggest that independently of their tissue origin, MSCs may blunt T-cell responses through galectin-dependent mechanisms.

8. Conclusions and perspectives

Understanding the environmental stimuli, soluble factors and molecular networks that govern the induction, differentiation, accumulation, function and stability of regulatory immune cell populations is critical to defining the pathophysiology of immune-mediated diseases and to developing new therapeutic interventions. In the present review we focus on the relevance of galectins, an endogenous family of immunoregulatory glycan-binding proteins, in the fate and function of lymphoid and myeloid regulatory cells including Tregs ($Foxp3^+$ nTregs, $Foxp3^+$ iTregs and $Foxp3^-$ Tr1 cells), Bregs, tolerogenic DCs, M2-type macrophages, MDSCs and MSCs. Like many cytokines and growth factors, it is not surprising that galectins may exhibit a 'double-edge sword' effect with opposing biological outcomes depending on different intrinsic factors such as their concentrations in local tissues, their extracellular versus intracellular localization, their subcellular compartmentalization, as well as biochemical features including the degree of multimerization and their stability in oxidative versus reducing microenvironments. Moreover, as galectin–glycan lattices can regulate the dynamics of glycosylated binding partners and control receptor segregation, internalization and downstream signaling [18], biochemical factors that govern these cellular events may also play a role in dictating the stimulatory or inhibitory effects of individual members of the galectin family. Furthermore, since glycosylation patterns and expression of galectin-specific ligands may differ substantially among different regulatory cell populations, the overall effect of individual galectins may also depend on extracellular factors including the activation and differentiation state of target cells and their preferential localization in different tissues.

In light of the broad spectrum of immunosuppressive effects and their relevance in re-wiring immune regulatory cell networks, challenges for the future will embrace a rational manipulation of galectin–glycan interactions toward attenuating immune responses in autoimmune diseases, allergic reactions, graft rejection and recurrent fetal loss. However, before galectin-based therapeutic agents can be extrapolated to clinical settings, a more thorough understanding of the mechanisms involved in galectin functions, their distribution and stability in inflamed tissues and their potential 'off-target' effects is required. In this regard, it will be critical to evaluate the results of side-by-side studies of the immunomodulatory activities of individual family members on particular regulatory cell types and to examine their function in pro-inflammatory and tolerogenic microenvironments.

As a reverse side of the same coin, interrupting galectin-mediated signaling pathways, using glycomimetics, peptidomimetics, small molecule inhibitors, glycodendrimers or neutralizing antibodies, may contribute to overcome immunosuppression and to potentiate anti-cancer and anti-microbial host immunity. Finally, a definitive proof-of-concept of the relevance of galectins in different regulatory compartments will arise from tissue-specific deletion of individual galectins or relevant glycosyltransferases and their connection with specific physiologic or pathologic conditions. Further studies should also be conducted to identify individuals with relevant primary genetic defects or polymorphisms associated to inflammatory or neoplastic settings, similarly to those found for Gal-2 gene (*LGALS2*) in myocardial infarction [139] and for Gal-3 gene (*LGALS3*) in breast cancer [158]. Given the complexity of the galectin network and their glycosylated and non-glycosylated counter-receptors, as well as the multiple parameters governing their localization and molecular interactions, further work is warranted to achieve a global comprehensive view of the relevance of these proteins within regulatory cell compartments and to dissect their individual roles in physiologically- and clinically-relevant settings.

Conflict of interest

None declared.

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References

[1] Abbas, A.K.L., A., H. and Pillai, S. (2014) Basic Immunology: Functions and Disorders of the Immune System, fourth ed, Elsevier, Philadelphia.
[2] Roychoudhuri, R., Eil, R.L. and Restifo, N.P. (2015) The interplay of effector and regulatory T cells in cancer. Curr. Opin. Immunol. 33, 101–111.
[3] Chaudhry, A. and Rudensky, A.Y. (2013) Control of inflammation by integration of environmental cues by regulatory T cells. J. Clin. Invest. 123, 939–944.
[4] Ostrand-Rosenberg, S. and Sinha, P. (2009) Myeloid-derived suppressor cells: linking inflammation and cancer. J. Immunol. 182, 4499–4506.
[5] Steinman, R.M. (2012) Decisions about dendritic cells: past, present, and future. Ann. Rev. Immunol. 30, 1–22.
[6] Mills, C.D. (2015) Anatomy of a discovery: M1 and M2 macrophages. Front. Immunol. 6, 212.
[7] Miyagaki, T., Fujimoto, M. and Sato, S. (2015) Regulatory B cells in human inflammatory and autoimmune diseases: from mouse models to clinical research. Int. Immunol. (in press).
[8] Uccelli, A., Moretta, L. and Pistoia, V. (2006) Immunoregulatory function of mesenchymal stem cells. Eur. J. Immunol. 36, 2566–2573.
[9] Terness, P., Kallikourdis, M., Betz, A.G., Rabinovich, G.A., Saito, S. and Clark, D. A. (2007) Tolerance signaling molecules and pregnancy: IDO, galectins, and the renaissance of regulatory T cells. Am. J. Reprod. Immunol. 58, 238–254.
[10] Hoeppli, R.E., Wu, D., Cook, L. and Levings, M.K. (2015) The environment of regulatory T cell biology: cytokines, metabolites, and the microbiome. Front. Immunol. 6, 61.
[11] Rabinovich, Gabriel A. and Croci, Diego O. (2012) Regulatory circuits mediated by lectin–glycan interactions in autoimmunity and cancer. Immunity 36, 322–335.
[12] Vasta, G.R. (2012) Galectins as pattern recognition receptors: structure, function, and evolution. Adv. Exp. Med. Biol. 946, 21–36.
[13] Mehul, B. and Hughes, R.C. (1997) Plasma membrane targetting, vesicular budding and release of galectin 3 from the cytoplasm of mammalian cells during secretion. J. Cell Sci. 110, 1169–1178.

[14] Rabinovich, G.A. and Toscano, M.A. (2009) Turning 'sweet' on immunity: galectin–glycan interactions in immune tolerance and inflammation. Nat. Rev. Immunol. 9, 338–352.

[15] Cummings, R.D. and E., J. (2009) in: Principles of Glycan Recognition in Essentials of Glycobiology (Varki, A., Ed.), Cold Spring Harbor Laboratory Press, Ney York.

[16] Dam, T.K. and Brewer, C.F. (2010) Lectins as pattern recognition molecules: the effects of epitope density in innate immunity. Glycobiology 20, 270–279.

[17] Croci, D.O., Cerliani, J.P., Pinto, N.A., Morosi, L.G. and Rabinovich, G.A. (2014) Regulatory role of glycans in the control of hypoxia-driven angiogenesis and sensitivity to anti-angiogenic treatment. Glycobiology 24, 1283–1290.

[18] Elola, María T., Blidner, Ada G., Ferragut, F., Bracalente, C. and Rabinovich, Gabriel A. (2015) Assembly, organization and regulation of cell-surface receptors by lectin–glycan complexes. Biochem. J. 469, 1–16.

[19] Liu, F.-T., Patterson, R.J. and Wang, J.L. (2002) Intracellular functions of galectins. Biochim. Biophys. Acta (BBA) – Gen. Subj. 1572, 263–273.

[20] Thurston, T.L.M., Wandel, M.P., von Muhlinen, N., Foeglein, A. and Randow, F. (2012) Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482, 414–418.

[21] Johannes, L., Parton, R.G., Bassereau, P. and Mayor, S. (2015) Building endocytic pits without clathrin. Nat. Rev. Mol. Cell Biol. 16, 311–321.

[22] Fred Brewer, C. (2002) Binding and cross-linking properties of galectins. Biochim. Biophys. Acta (BBA) – Gen. Subj. 1572, 255–262.

[23] Dennis, J.W., Nabi, I.R. and Demetriou, M. (2009) Metabolism, cell surface organization, and disease. Cell 139, 1229–1241.

[24] Croci, D.O., Cerliani, J.P., Dalotto-Moreno, T., Mendez-Huergo, S.P., Mascanfroni, I.D., Dergan-Dylon, S., Toscano, M.A., Caramelo, J.J., Garcia-Vallejo, J.J., Ouyang, J., Mesri, E.A., Junttila, M.R., Bais, C., Shipp, M.A., Salatino, M. and Rabinovich, G.A. (2014) Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. Cell 156, 744–758.

[25] Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Urashima, T., Oka, T., Futai, M., Muller, W.E.G., Yagi, F. and Kasai, K.-I. (2002) Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. Biochim. Biophys. Acta (BBA) – Gen. Subj. 1572, 232–254.

[26] Stowell, S.R., Arthur, C.M., Mehta, P., Slanina, K.A., Blixt, O., Leffler, H., Smith, D.F. and Cummings, R.D. (2008) Galectin-1, -2, and -3 exhibit differential recognition of sialylated glycans and blood group antigens. J. Biol. Chem. 283, 10109–10123.

[27] Di Lella, S., Sundblad, V., Cerliani, J.P., Guardia, C.M., Estrin, D.A., Vasta, G.R. and Rabinovich, G.A. (2011) When galectins recognize glycans: from biochemistry to physiology and back again. Biochemistry 50, 7842–7857.

[28] Cooper, D., Ilarregui, J.M., Pesoa, S.A., Croci, D.O., Perretti, M. and Rabinovich, G.A. (2010) Multiple functional targets of the immunoregulatory activity of galectin-1: control of immune cell trafficking, dendritic cell physiology, and t-cell fate. Meth. Enzymol. 480, 199–244.

[29] Cedeno-Laurent, F. and Dimitroff, C.J. (2012) Galectins and their ligands: negative regulators of anti-tumor immunity. Glycoconj. J. 29, 619–625.

[30] Mendez-Huergo, S.P., Maller, S.M., Farez, M.F., Mariño, K., Correale, J. and Rabinovich, G.A. (2014) Integration of lectin–glycan recognition systems and immune cell networks in CNS inflammation. Cytokine Growth Factor Rev. 25, 247–255.

[31] Cerliani, J., Stowell, S., Mascanfroni, I., Arthur, C., Cummings, R. and Rabinovich, G. (2011) Expanding the universe of cytokines and pattern recognition receptors: galectins and glycans in innate immunity. J. Clin. Immunol. 31, 10–21.

[32] Zeng, H., Zhang, R., Jin, B. and Chen, L. (2015) Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. Cell. Mol. Immunol. (in press).

[33] Sugimoto, N., Oida, T., Hirota, K., Nakamura, K., Nomura, T., Uchiyama, T. and Sakaguchi, S. (2006) Foxp3-dependent and -independent molecules specific for $CD25^+CD4^+$ natural regulatory T cells revealed by DNA microarray analysis. Int. Immunol. 18, 1197–1209.

[34] Garín, M.I., Chu, C.-C., Golshayan, D., Cernuda-Morollón, E., Wait, R. and Lechler, R.I. (2006) Galectin-1: a key effector of regulation mediated by $CD4^+CD25^+$ T cells. Blood 109, 2058–2065.

[35] Baatar, D., Olkhanud, P.B., Wells, V., Indig, F.E., Mallucci, L. and Biragyn, A. (2009) Tregs utilize β-galactoside-binding protein to transiently inhibit PI3K/p21(ras) activity of human CD8(+) T cells to block their TCR-mediated ERK activity and proliferation. Brain Behav. Immun. 23, 1028–1037.

[36] Wang, J., Lu, Z.-H., Gabius, H.-J., Rohowsky-Kochan, C., Ledeen, R.W. and Wu, G. (2009) Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. J. Immunol. 182, 4036–4045.

[37] Ocklenburg, F., Moharregh-Khiabani, D., Geffers, R., Janke, V., Pfoertner, S., Garritsen, H., Groebe, L., Klempnauer, J., Dittmar, K.E.J., Weiss, S., Buer, J. and Probst-Kepper, M. (2006) UBD, a downstream element of FOXP3, allows the identification of LGALS3, a new marker of human regulatory T cells. Lab. Invest. 86, 724–737.

[38] Kubach, J., Lutter, P., Bopp, T., Stoll, S., Becker, C., Huter, E., Richter, C., Weingarten, P., Warger, T., Knop, J., Müllner, S., Wijdenes, J., Schild, H., Schmitt, E. and Jonuleit, H. (2007) Human $CD4^+CD25^+$ regulatory T cells: proteome analysis identifies galectin-10 as a novel marker essential for their anergy and suppressive function. Blood 110, 1550–1558.

[39] Wang, F., Wan, L., Zhang, C., Zheng, X., Li, J. and Chen, Z.K. (2009) Tim-3-Galectin-9 pathway involves the suppression induced by $CD4^+CD25^+$ regulatory T cells. Immunobiology 214, 342–349.

[40] Ju, Y., Shang, X., Liu, Z., Zhang, J., Li, Y., Shen, Y., Liu, Y., Liu, C., Liu, B., Xu, L., Wang, Y., Zhang, B. and Zou, J. (2014) The Tim-3/galectin-9 pathway involves in the homeostasis of hepatic Tregs in a mouse model of concanavalin A-induced hepatitis. Mol. Immunol. 58, 85–91.

[41] Kared, H., Fabre, T., Bédard, N., Bruneau, J. and Shoukry, N.H. (2013) Galectin-9 and IL-21 mediate cross-regulation between Th17 and Treg cells during acute hepatitis C. PLoS Pathog. 9, e1003422.

[42] Wu, C., Thalhamer, T., Franca, Rafael F., Xiao, S., Wang, C., Hotta, C., Zhu, C., Hirashima, M., Anderson, Ana C. and Kuchroo, Vijay K. (2014) Galectin-9-CD44 interaction enhances stability and function of adaptive regulatory T cells. Immunity 41, 270–282.

[43] Rabinovich, G.A., Daly, G., Dreja, H., Tailor, H., Riera, C.M., Hirabayashi, J. and Chernajovsky, Y. (1999) Recombinant galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. J. Exp. Med. 190, 385–398.

[44] Toscano, M.A., Bianco, G.A., Ilarregui, J.M., Croci, D.O., Correale, J., Hernandez, J.D., Zwirner, N.W., Poirier, F., Riley, E.M., Baum, L.G. and Rabinovich, G.A. (2007) Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. Nat. Immunol. 8, 825–834.

[45] Toscano, M.A., Commodaro, A.G., Ilarregui, J.M., Bianco, G.A., Liberman, A., Serra, H.M., Hirabayashi, J., Rizzo, L.V. and Rabinovich, G.A. (2006) Galectin-1 suppresses autoimmune retinal disease by promoting concomitant Th2- and T regulatory-mediated anti-inflammatory responses. J. Immunol. 176, 6323–6332.

[46] Perone, M.J., Larregina, A.T., Shufesky, W.J., Papworth, G.D., Sullivan, M.L.G., Zahorchak, A.F., Stolz, D.B., Baum, L.G., Watkins, S.C., Thomson, A.W. and Morelli, A.E. (2006) Transgenic galectin-1 induces maturation of dendritic cells that elicit contrasting responses in naive and activated T CELLS. J. Immunol. 176, 7207–7220.

[47] Santucci, L., Fiorucci, S., Rubinstein, N., Mencarelli, A., Palazzetti, B., Federici, B., Rabinovich, G.A. and Morelli, A. (2003) Galectin-1 suppresses experimental colitis in mice. Gastroenterology 124, 1381–1394.

[48] Pérez, C.V., Gómez, L.G., Gualdoni, G.S., Lustig, L., Rabinovich, G.A. and Guazzone, V.A. (2015) Dual roles of endogenous and exogenous galectin-1 in the control of testicular immunopathology. Sci. Rep. 5.

[49] Baum, L.G., Blackall, D.P., Arias-Magallano, S., Nanigian, D., Uh, S.Y., Browne, J. M., Hoffmann, D., Emmanouilides, C.E., Territo, M.C. and Baldwin, G.C. (2003) Amelioration of graft versus host disease by galectin-1. Clin. Immunol. 109, 295–307.

[50] Rubinstein, N., Alvarez, M., Zwirner, N.W., Toscano, M.A., Ilarregui, J.M., Bravo, A., Mordoh, J., Fainboim, L., Podhajcer, O.L. and Rabinovich, G.A. (2004) Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: a potential mechanism of tumor-immune privilege. Cancer Cell 5, 241–251.

[51] Banh, A., Zhang, J., Cao, H., Bouley, D.M., Kwok, S., Kong, C., Giaccia, A.J., Koong, A.C. and Le, Q.T. (2011) Tumor galectin-1 mediates tumor growth and metastasis through regulation of T-cell apoptosis. Cancer Res. 71, 4423–4431.

[52] Martínez-Bosch, N., Fernández-Barrena, M.G., Moreno, M., Ortiz-Zapater, E., Munné-Collado, J., Iglesias, M., André, S., Gabius, H.-J., Hwang, R.F. and Poirier, F. (2014) Galectin-1 drives pancreatic carcinogenesis through stroma remodeling and Hedgehog signaling activation. Cancer Res. 74, 3512–3524.

[53] Juszczynski, P., Ouyang, J., Monti, S., Rodig, S.J., Takeyama, K., Abramson, J., Chen, W., Kutok, J.L., Rabinovich, G.A. and Shipp, M.A. (2007) The AP1-dependent secretion of galectin-1 by Reed-Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. Proc. Natl. Acad. Sci. U.S.A. 104, 13134–13139.

[54] Soldati, R., Berger, E., Zenclussen, A.C., Jorch, G., Lode, H.N., Salatino, M., Rabinovich, G.A. and Fest, S. (2012) Neuroblastoma triggers an immunoevasive program involving galectin-1-dependent modulation of T cell and dendritic cell compartments. Int. J. Cancer 131, 1131–1141.

[55] Dalotto-Moreno, T., Croci, D.O., Cerliani, J.P., Martinez-Allo, V.C., Dergan-Dylon, S., Méndez-Huergo, S.P., Stupirski, J.C., Mazal, D., Osinaga, E., Toscano, M.A., Sundblad, V., Rabinovich, G.A. and Salatino, M. (2013) Targeting galectin-1 overcomes breast cancer-associated immunosuppression and prevents metastatic disease. Cancer Res. 73, 1107–1117.

[56] Croci, D.O., Salatino, M., Rubinstein, N., Cerliani, J.P., Cavallin, L.E., Leung, H.J., Ouyang, J., Ilarregui, J.M., Toscano, M.A., Domaica, C.I., Croci, M.C., Shipp, M.A., Mesri, E.A., Albini, A. and Rabinovich, G.A. (2012) Disrupting galectin-1 interactions with N-glycans suppresses hypoxia-driven angiogenesis and tumorigenesis in Kaposi's sarcoma. J. Exp. Med. 209, 1985–2000.

[57] Baker, G.J., Chockley, P., Yadav, V.N., Doherty, R., Ritt, M., Sivaramakrishnan, S., Castro, M.G. and Lowenstein, P.R. (2014) Natural killer cells eradicate galectin-1 deficient glioma in the absence of adaptive immunity. Cancer Res. 74, 5079–5090.

[58] Rutkowski, Melanie R., Stephen, Tom L., Svoronos, N., Allegrezza, Michael J., Tesone, Amelia J., Perales-Puchalt, A., Brencicova, E., Escovar-Fadul, X., Nguyen, Jenny M., Cadungog, Mark G., Zhang, R., Salatino, M., Tchou, J., Rabinovich, Gabriel A. and Conejo-Garcia, Jose R. (2015) Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. Cancer Cell 27, 27–40.

[59] Salatino, M., Croci, D.O., Bianco, G.A., Ilarregui, J.M., Toscano, M.A. and Rabinovich, G.A. (2007) Galectin-1 as a potential therapeutic target in autoimmune disorders and cancer. Exp. Opin. Biol. Ther. 8, 45–57.

[60] Liu, S.D., Lee, S., Cava, A.L., Motran, C.C., Hahn, B.H. and Miceli, M.C. (2011) Galectin-1-induced down-regulation of T lymphocyte activation protects (NZB × NZW) F1 mice from lupus-like disease. Lupus 20, 473–484.

[61] Blois, S.M., Ilarregui, J.M., Tometten, M., Garcia, M., Orsal, A.S., Cordo-Russo, R., Toscano, M.A., Bianco, G.A., Kobelt, P., Handjiski, B., Tirado, I., Markert, U.R., Klapp, B.F., Poirier, F., Szekeres-Bartho, J., Rabinovich, G.A. and Arck, P.C. (2007) A pivotal role for galectin-1 in fetomaternal tolerance. Nat. Med. 13, 1450–1457.

[62] Ramhorst, R.E., Giribaldi, L., Fraccaroli, L., Toscano, M.A., Stupirski, J.C., Romero, M.D., Durand, E.S., Rubinstein, N., Blaschitz, A., Sedlmayr, P., Genti-Raimondi, S., Fainboim, L. and Rabinovich, G.A. (2012) Galectin-1 confers immune privilege to human trophoblast: implications in recurrent fetal loss. Glycobiology 22, 1374–1386.

[63] Kopcow, H.D., Rosetti, F., Leung, Y., Allan, D.S.J., Kutok, J.L. and Strominger, J.L. (2008) T cell apoptosis at the maternal–fetal interface in early human pregnancy, involvement of galectin-1. Proc. Natl. Acad. Sci. U.S.A. 105, 18472–18477.

[64] Seropian, I.M., Cerliani, J.P., Toldo, S., Van Tassell, B.W., Ilarregui, J.M., González, G.E., Matoso, M., Salloum, F.N., Melchior, R., Gelpi, R.J., Stupirski, J. C., Benatar, A., Gómez, K.A., Morales, C., Abbate, A. and Rabinovich, G.A. (2013) Galectin-1 controls cardiac inflammation and ventricular remodeling during acute myocardial infarction. Am. J. Path. 182, 29–40.

[65] Stowell, S.R., Qian, Y., Karmakar, S., Koyama, N.S., Dias-Baruffi, M., Leffler, H., McEver, R.P. and Cummings, R.D. (2008) Differential roles of galectin-1 and galectin-3 in regulating leukocyte viability and cytokine secretion. J. Immunol. 180, 3091–3102.

[66] Sampson, J.F., Hasegawa, E., Mulki, L., Suryawanshi, A., Jiang, S., Chen, W.-S., Rabinovich, G.A., Connor, K.M. and Panjwani, N. (2015) Galectin-8 ameliorates murine autoimmune ocular pathology and promotes a regulatory T cell response. PLoS ONE 10, e0130772.

[67] Sampson, J.F.S., Amol, Chen, Wei-Sheng, Rabinovich, Gabriel, A. and Panjwani, Noorjahan (2015) Galectin-8 promotes regulatory T-cell differentiation by modulating IL-2 and TGFβ signaling. Immunol. Cell Biol. (in press).

[68] Seki, M., Oomizu, S., Sakata, K.-M., Sakata, A., Arikawa, T., Watanabe, K., Ito, K., Takeshita, K., Niki, T., Saita, N., Nishi, N., Yamauchi, A., Katoh, S., Matsukawa, A., Kuchroo, V. and Hirashima, M. (2008) Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin. Immunol. 127, 78–88.

[69] Lv, K., Xu, W., Wang, C., Niki, T., Hirashima, M. and Xiong, S. (2011) Galectin-9 administration ameliorates CVB3 induced myocarditis by promoting the proliferation of regulatory T cells and alternatively activated Th2 cells. Clin. Immunol. 140, 92–101.

[70] Ji, X.J., Ma, C.J., Wang, J.M., Wu, X.Y., Niki, T., Hirashima, M., Moorman, J.P. and Yao, Z.Q. (2013) Immunomodulatory role of the hepatocyte during HCV infection: driving CD4(+)CD25(+)Foxp3(+) regulatory T cell development through the Tim-3/Gal-9 pathway. Eur. J. Immunol. 43, 458–467.

[71] de Kivit, S., Kraneveld, A.D., Knippels, L.M.J., van Kooyk, Y., Garssen, J. and Willemsen, L.E.M. (2013) Intestinal epithelium-derived galectin-9 is involved in the immunomodulating effects of nondigestible oligosaccharides. J. Innate Immun. 5, 625–638.

[72] Lu, X., McCoy, K.S., Xu, J., Hu, W., Chen, H., Jiang, K., Han, F., Chen, P. and Wang, Y. (2015) Galectin-9 ameliorates respiratory syncytial virus-induced pulmonary immunopathology through regulating the balance between Th17 and regulatory T cells. Virus Res. 195, 162–171.

[73] Lv, Zhang, Zhang, Zhong and Suo (2013) Galectin-9 promotes TGF-β-dependent induction of regulatory T cells via the TGF-β/Smad signaling pathway. Mol. Med. Rep. 7, 205–210.

[74] Jiang, H.-R., Al Rasebi, Z., Mensah-Brown, E., Shahin, A., Xu, D., Goodyear, C.S., Fukada, S.Y., Liu, F.-T., Liew, F.Y. and Lukic, M.L. (2009) Galectin-3 deficiency reduces the severity of experimental autoimmune encephalomyelitis. J. Immunol. 182, 1167–1173.

[75] Fermino, M.L., Dias, F.C., Lopes, C.D., Souza, M.A., Cruz, Â.K., Liu, F.-T., Chammas, R., Roque-Barreira, M.C., Rabinovich, G.A. and Bernardes, E.S. (2013) Galectin-3 negatively regulates the frequency and function of $CD4^+CD25^+Foxp3^+$ regulatory T cells and influences the course of Leishmania major infection. Eur. J. Immunol. 43, 1806–1817.

[76] Rosser, Elizabeth C. and Mauri, C. (2015) Regulatory B cells: origin, phenotype, and function. Immunity 42, 607–612.

[77] Zuñiga, E., Rabinovich, G.A., Iglesias, M.M. and Gruppi, A. (2001) Regulated expression of galectin-1 during B-cell activation and implications for T-cell apoptosis. J. Leukoc. Biol. 70, 73–79.

[78] Yu, X., Siegel, R. and Roeder, R.G. (2006) Interaction of the B cell-specific transcriptional coactivator OCA-B and galectin-1 and a possible role in regulating BCR-mediated B cell proliferation. J. Biol. Chem. 281, 15505–15516.

[79] Croci, D.O., Morande, P.E., Dergan-Dylon, S., Borge, M., Toscano, M.A., Stupirski, J.C., Bezares, R.F., Avalos, J.S., Narbaitz, M., Gamberale, R., Rabinovich, G.A. and Giordano, M. (2013) Nurse-like cells control the activity of chronic lymphocytic leukemia B cells via galectin-1. Leukemia 27, 1413–1416.

[80] Tsai, C.-M., Wu, H.-Y., Su, T.-H., Kuo, C.-W., Huang, H.-W., Chung, C.-H., Chen, C.-S., Khoo, K.-H., Chen, Y.-J. and Lin, K.-I. (2014) Phosphoproteomic analyses reveal that galectin-1 augments the dynamics of B-cell receptor signaling. J. Proteome 103, 241–253.

[81] Clark, A.G., Chen, S., Zhang, H., Brady, G.F., Ungewitter, E.K., Bradley, J.K., Sackey, F.N. and Foster, M.H. (2007) Multifunctional regulators of cell growth are differentially expressed in anergic murine B cells. Mol. Immunol. 44, 1274–1285.

[82] Clark, A.G., Weston, M.L. and Foster, M.H. (2013) Lack of galectin-1 or galectin-3 alters B cell deletion and anergy in an autoantibody transgene model. Glycobiology 23, 893–903.

[83] Tabrizi, S.J., Niiro, H., Masui, M., Yoshimoto, G., Iino, T., Kikushige, Y., Wakasaki, T., Baba, E., Shimoda, S., Miyamoto, T., Hara, T. and Akashi, K. (2009) T cell leukemia/lymphoma 1 and galectin-1 regulate survival/cell death pathways in human naive and IgM^+ memory B cells through altering balances in Bcl-2 family proteins. J. Immunol. 182, 1490–1499.

[84] Tsai, C.-M., Chiu, Y.-K., Hsu, T.-L., Lin, I.Y., Hsieh, S.-L. and Lin, K.-I. (2008) Galectin-1 promotes immunoglobulin production during plasma cell differentiation. J. Immunol. 181, 4570–4579.

[85] Anginot, A., Espeli, M., Chasson, L., Mancini, S.J.C. and Schiff, C. (2013) Galectin 1 modulates plasma cell homeostasis and regulates the humoral immune response. J. Immunol. 190, 5526–5533.

[86] Moritoki, M., Kadowak, T., Niki, T., Nakano, D., Soma, G., Mori, H., Kobara, H., Masaki, T., Kohno, M. and Hirashima, M. (2013) Galectin-9 ameliorates clinical severity of MRL/lpr Lupus-Prone mice by inducing plasma cell apoptosis independently of Tim-3. PLoS ONE 8, 1–10.

[87] Acosta-Rodríguez, E.V., Montes, C.L., Motrán, C.C., Zuniga, E.I., Liu, F.-T., Rabinovich, G.A. and Gruppi, A. (2004) Galectin-3 mediates IL-4-induced survival and differentiation of B cells: functional cross-talk and implications during *Trypanosoma cruzi* infection. J. Immunol. 172, 493–502.

[88] Oliveira, F.L., Chammas, R., Ricon, L., Fermino, M.L., Bernardes, E.S., Hsu, D.K., Liu, F.-T., Borojevic, R. and El-Cheikh, M.C. (2009) Galectin-3 regulates peritoneal B1-cell differentiation into plasma cells. Glycobiology 19, 1248–1258.

[89] Bonzi, J., Bornet, O., Betzi, S., Kasper, B.T., Mahal, L.K., Mancini, S.J., Schiff, C., Sebban-Kreuzer, C., Guerlesquin, F. and Elantak, L. (2015) Pre-B cell receptor binding to galectin-1 modifies galectin-1/carbohydrate affinity to modulate specific galectin-1/glycan lattice interactions. Nat. Commun. 6, 6194.

[90] Kim, S.J. and Diamond, B. (2015) Modulation of tolerogenic dendritic cells and autoimmunity. Semin. Cell Dev. Biol. 41, 49–58.

[91] Rutella, S., Danese, S. and Leone, G. (2006) Tolerogenic dendritic cells: cytokine modulation comes of age. Blood 108, 1435–1440.

[92] Ilarregui, J.M. and Rabinovich, G.A. (2010) Tolerogenic dendritic cells in the control of autoimmune neuroinflammation: an emerging role of protein–glycan interactions. NeuroImmunoModulation 17, 157–160.

[93] Ilarregui, J.M., Croci, D.O., Bianco, G.A., Toscano, M.A., Salatino, M., Vermeulen, M.E., Geffner, J.R. and Rabinovich, G.A. (2009) Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. Nat. Immunol. 10, 981–991.

[94] Kuo, P.-L., Hung, J.-Y., Huang, S.-K., Chou, S.-H., Cheng, D.-E., Jong, Y.-J., Hung, C.-H., Yang, C.-J., Tsai, Y.-M., Hsu, Y.-L. and Huang, M.-S. (2011) Lung cancer-derived galectin-1 mediates dendritic cell anergy through inhibitor of DNA binding 3/IL-10 signaling pathway. J. Immunol. 186, 1521–1530.

[95] Poncini, C.V., Ilarregui, J.M., Batalla, E.I., Engels, S., Cerliani, J.P., Cucher, M.A., van Kooyk, Y., González Cappa, S.M. and Rabinovich, G.A. (2015) *Trypanosoma cruzi* infection imparts a regulatory program in dendritic cells and T cells via galectin-1-dependent mechanisms. J. Immunol. (in press).

[96] Thiemann, S., Man, J.H., Chang, M.H., Lee, B. and Baum, L.G. (2015) Galectin-1 regulates tissue exit of specific dendritic cell populations. J. Biol. Chem. (in press).

[97] Bax, M., García-Vallejo, J.J., Jang-Lee, J., North, S.J., Gilmartin, T.J., Hernández, G., Crocker, P.R., Leffler, H., Head, S.R., Haslam, S.M., Dell, A. and van Kooyk, Y. (2007) Dendritic cell maturation results in pronounced changes in glycan expression affecting recognition by siglecs and galectins. J. Immunol. 179, 8216–8224.

[98] de la Fuente, H., Cruz-Adalia, A., Martinez del Hoyo, G., Cibrián-Vera, D., Bonay, P., Pérez-Hernández, D., Vázquez, J., Navarro, P., Gutierrez-Gallego, R., Ramirez-Huesca, M., Martín, P. and Sánchez-Madrid, F. (2014) The leukocyte activation receptor CD69 controls T cell differentiation through its interaction with galectin-1. Mol. Cell. Biol. 34, 2479–2487.

[99] Zhou, F., Ciric, B., Li, H., Yan, Y., Li, K., Cullimore, M., Lauretti, E., Gonnella, P., Zhang, G.-X. and Rostami, A. (2012) IL-10 deficiency blocks the ability of LPS to regulate expression of tolerance-related molecules on dendritic cells. Eur. J. Immunol. 42, 1449–1458.

[100] Zhou, F., Lauretti, E., di Meco, A., Ciric, B., Gonnella, P., Zhang, G.-X. and Rostami, A. (2013) Intravenous transfer of apoptotic cell-treated dendritic cells leads to immune tolerance by blocking Th17 cell activity. Immunobiology 218, 1069–1076.

[101] Mobergslien, A. and Sioud, M. (2012) Galectin-1 and -3 gene silencing in immature and mature dendritic cells enhances T cell activation and interferon-γ production. J. Leukoc. Biol. 91, 461–467.

[102] Bernardes, E.S., Silva, N.M., Ruas, L.P., Mineo, J.R., Loyola, A.M., Hsu, D.K., Liu, F.-T., Chammas, R. and Roque-Barreira, M.C. (2006) *Toxoplasma gondii* infection reveals a novel regulatory role for galectin-3 in the interface of innate and adaptive immunity. Am. J. Pathol. 168, 1910–1920.

[103] Breuilh, L., Vanhoutte, F., Fontaine, J., van Stijn, C.M.W., Tillie-Leblond, I., Capron, M., Faveeuw, C., Jouault, T., van Die, I., Gosset, P. and Trottein, F. (2007) Galectin-3 modulates immune and inflammatory responses during helminthic infection: impact of galectin-3 deficiency on the functions of dendritic cells. Infect. Immun. 75, 5148–5157.

[104] Fermin Lee, A., Chen, H.-Y., Wan, L., Wu, S.-Y., Yu, J.-S., Huang, A.C., Miaw, S.-C., Hsu, D.K., Wu-Hsieh, B.A. and Liu, F.-T. (2013) Galectin-3 modulates Th17 responses by regulating dendritic cell cytokines. Am. J. Pathol. 183, 1209–1222.

[105] Wu, S.-Y., Yu, J.-S., Liu, F.-T., Miaw, S.-C. and Wu-Hsieh, B.A. (2013) Galectin-3 negatively regulates dendritic cell production of IL-23/IL-17–axis cytokines in infection by *Histoplasma capsulatum*. J. Immunol. 190, 3427–3437.

[106] Shan, M., Gentile, M., Yeiser, J.R., Walland, A.C., Bornstein, V.U., Chen, K., He, B., Cassis, L., Bigas, A., Cols, M., Comerma, L., Huang, B., Blander, J.M., Xiong, H., Mayer, L., Berin, C., Augenlicht, L.H., Velcich, A. and Cerutti, A. (2013) Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. Science 342, 447–453.

[107] Kouo, T., Huang, L., Pucsek, A.B., Cao, M., Solt, S., Armstrong, T. and Jaffee, E. (2015) Galectin-3 shapes antitumor immune responses by suppressing $CD8^+$ T cells via LAG-3 and inhibiting expansion of plasmacytoid dendritic cells. Cancer Immunol. Res. 3, 412–423.

[108] Dai, S.-Y., Nakagawa, R., Itoh, A., Murakami, H., Kashio, Y., Abe, H., Katoh, S., Kontani, K., Kihara, M., Zhang, S.-L., Hata, T., Nakamura, T., Yamauchi, A. and Hirashima, M. (2005) Galectin-9 induces maturation of human monocyte-derived dendritic cells. J. Immunol. 175, 2974–2981.

[109] Nagahara, K., Arikawa, T., Oomizu, S., Kontani, K., Nobumoto, A., Tateno, H., Watanabe, K., Niki, T., Katoh, S., Miyake, M., Nagahata, S.-I., Hirabayashi, J., Kuchroo, V.K., Yamauchi, A. and Hirashima, M. (2008) Galectin-9 increases $Tim\text{-}3^+$ dendritic cells and $CD8^+$ T cells and enhances antitumor immunity via galectin-9-Tim-3 interactions. J. Immunol. 181, 7660–7669.

[110] Li, Y., Feng, J., Geng, S., Geng, S., Wei, H., Chen, G., Li, X., Wang, L., Wang, R., Peng, H., Han, G., Shen, B. and Li, Y. (2011) The N- and C-terminal carbohydrate recognition domains of galectin-9 contribute differently to its multiple functions in innate immunity and adaptive immunity. Mol. Immunol. 48, 670–677.

[111] Sica, A. and Mantovani, A. (2012) Macrophage plasticity and polarization: in vivo veritas. J. Clin. Invest. 122, 787–795.

[112] Martinez, F.O. and Gordon, S. (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000 Prime Rep. 6, 13.

[113] Franco, R. and Fernández-Suárez, D. (2015) Alternatively activated microglia and macrophages in the central nervous system. Prog. Neurobiol. 131, 65–86.

[114] Rabinovich, G., Castagna, L., Landa, C., Riera, C.M. and Sotomayor, C. (1996) Regulated expression of a 16-kd galectin-like protein in activated rat macrophages. J. Leukoc. Biol. 59, 363–370.

[115] Rabinovich, G.A., Iglesias, M.M., Modesti, N.M., Castagna, L.F., Wolfenstein-Todel, C., Riera, C.M. and Sotomayor, C.E. (1998) Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells: biochemical and functional characterization. J. Immunol. 160, 4831–4840.

[116] Correa, S.G., Sotomayor, C.E., Aoki, M.P., Maldonado, C.A. and Rabinovich, G.A. (2003) Opposite effects of galectin-1 on alternative metabolic pathways of L-arginine in resident, inflammatory, and activated macrophages. Glycobiology 13, 119–128.

[117] Barrionuevo, P., Beigier-Bompadre, M., Ilarregui, J.M., Toscano, M.A., Bianco, G.A., Isturiz, M.A. and Rabinovich, G.A. (2007) A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. J. Immunol. 178, 436–445.

[118] Rabinovich, G.A., Sotomayor, C.E., Riera, C.M., Bianco, I. and Correa, S.G. (2000) Evidence of a role for galectin-1 in acute inflammation. Eur. J. Immunol. 30, 1331–1339.

[119] Rostoker, R., Yaseen, H., Schif-Zuck, S., Lichtenstein, R.G., Rabinovich, G.A. and Ariel, A. (2013) Galectin-1 induces 12/15-lipoxygenase expression in murine macrophages and favors their conversion toward a pro-resolving phenotype. Prostaglandins Other Lipid Mediat. 107, 85–94.

[120] Pang, M., He, J., Johnson, P. and Baum, L.G. (2009) CD45 mediated fodrin cleavage during galectin-1 mediated T cell death promotes phagocytic clearance of dying cells. J. Immunol. 182, 7001–7008.

[121] Dias-Baruffi, M., Zhu, H., Cho, M., Karmakar, S., McEver, R.P. and Cummings, R.D. (2003) Dimeric galectin-1 induces surface exposure of phosphatidylserine and phagocytic recognition of leukocytes without inducing apoptosis. J. Biol. Chem. 278, 41282–41293.

[122] Togayachi, A., Kozono, Y., Ishida, H., Abe, S., Suzuki, N., Tsunoda, Y., Hagiwara, K., Kuno, A., Ohkura, T., Sato, N., Sato, T., Hirabayashi, J., Ikehara, Y., Tachibana, K. and Narimatsu, H. (2007) Polylactosamine on glycoproteins influences basal levels of lymphocyte and macrophage activation. Proc. Natl. Acad. Sci. U.S.A. 104, 15829–15834.

[123] Starossom, Sarah C., Mascanfroni, Ivan D., Imitola, J., Cao, L., Raddassi, K., Hernandez, Silvia F., Bassil, R., Croci, Diego O., Cerliani, Juan P., Delacour, D., Wang, Y., Elyaman, W., Khoury, Samia J. and Rabinovich, Gabriel A. (2012) Galectin-1 deactivates classically activated microglia and protects from inflammation-induced neurodegeneration. Immunity 37, 249–263.

[124] Quinta, H.R., Pasquini, J.M., Rabinovich, G.A. and Pasquini, L.A. (2014) Glycan-dependent binding of galectin-1 to neuropilin-1 promotes axonal regeneration after spinal cord injury. Cell Death Differ. 21, 941–955.

[125] Novak, R., Dabelic, S. and Dumic, J. (2012) Galectin-1 and galectin-3 expression profiles in classically and alternatively activated human macrophages. Biochim. Biophys. Acta (BBA) – Gen. Subj. 1820, 1383–1390.

[126] Sato, S. and Hughes, R.C. (1994) Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. J. Biol. Chem. 269, 4424–4430.

[127] Frigeri, L.G. and Liu, F.T. (1992) Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages. J. Immunol. 148, 861–867.

[128] Sundblad, V., Croci, D.O. and Rabinovich, G.A. (2011) Regulated expression of galectin-3, a multifunctional glycan-binding protein, in haematopoietic and non-haematopoietic tissues. Histol. Histopathol. 26, 1–19.

[129] Hsu, D.K., Yang, R.-Y., Pan, Z., Yu, L., Salomon, D.R., Fung-Leung, W.-P. and Liu, F.-T. (2000) Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. Am. J. Pathol. 156, 1073–1083.

[130] Sano, H., Hsu, D.K., Apgar, J.R., Yu, L., Sharma, B.B., Kuwabara, I., Izui, S. and Liu, F.-T. (2003) Critical role of galectin-3 in phagocytosis by macrophages. J. Clin. Invest. 112, 389–397.

[131] MacKinnon, A.C., Farnworth, S.L., Hodkinson, P.S., Henderson, N.C., Atkinson, K.M., Leffler, H., Nilsson, U.J., Haslett, C., Forbes, S.J. and Sethi, T. (2008) Regulation of alternative macrophage activation by galectin-3. J. Immunol. 180, 2650–2658.

[132] Dragomir, A.-C.D., Sun, R., Choi, H., Laskin, J.D. and Laskin, D.L. (2012) Role of galectin-3 in classical and alternative macrophage activation in the liver following acetaminophen intoxication. J. Immunol. 189, 5934–5941.

[133] Cucak, H., Grunnet, L.G. and Rosendahl, A. (2014) Accumulation of M1-like macrophages in type 2 diabetic islets is followed by a systemic shift in macrophage polarization. J. Leukoc. Biol. 95, 149–160.

[134] Chung, A.W., Sieling, P.A., Schenk, M., Teles, R.M.B., Krutzik, S.R., Hsu, D.K., Liu, F.-T., Sarno, E.N., Rea, T.H., Stenger, S., Modlin, R.L. and Lee, D.J. (2013) Galectin-3 regulates the innate immune response of human monocytes. J. Infect. Dis. 207, 947–956.

[135] Burguillos, Miguel A., Svensson, M., Schulte, T., Boza-Serrano, A., Garcia-Quintanilla, A., Kavanagh, E., Santiago, M., Viceconte, N., Oliva-Martin, Maria J., Osman, Ahmed M., Salomonsson, E., Amar, L., Persson, A., Blomgren, K., Achour, A., Englund, E., Leffler, H., Venero, Jose L., Joseph, B. and Deierborg, T. (2015) Microglia-secreted galectin-3 acts as a toll-like receptor 4 ligand and contributes to microglial activation. Cell Rep. 10, 1626–1638.

[136] Hoyos, H.C., Rinaldi, M., Mendez-Huergo, S.P., Marder, M., Rabinovich, G.A., Pasquini, J.M. and Pasquini, L.A. (2014) Galectin-3 controls the response of microglial cells to limit cuprizone-induced demyelination. Neurobiol. Dis. 62, 441–455.

[137] Jeon, S.-B., Yoon, H.J., Chang, C.Y., Koh, H.S., Jeon, S.-H. and Park, E.J. (2010) Galectin-3 exerts cytokine-like regulatory actions through the JAK–STAT pathway. J. Immunol. 185, 7037–7046.

[138] Yıldırım, C., Vogel, D.Y.S., Hollander, M.R., Baggen, J.M., Fontijn, R.D., Nieuwenhuis, S., Haverkamp, A., de Vries, M.R., Quax, P.H.A., Garcia-Vallejo, J.J., van der Laan, A.M., Dijkstra, C.D., van der Pouw Kraan, T.C.T.M., van Royen, N. and Horrevoets, A.J.G. (2015) Galectin-2 induces a proinflammatory, anti-arteriogenic phenotype in monocytes and macrophages. PLoS ONE 10, e0124347.

[139] Ozaki, K., Inoue, K., Sato, H., Iida, A., Ohnishi, Y., Sekine, A., Sato, H., Odashiro, K., Nobuyoshi, M., Hori, M., Nakamura, Y. and Tanaka, T. (2004) Functional variation in LGALS2 confers risk of myocardial infarction and regulates lymphotoxin-[alpha] secretion in vitro. Nature 429, 72–75.

[140] Arikawa, T., Saita, N., Oomizu, S., Ueno, M., Matsukawa, A., Katoh, S., Kojima, K., Nagahara, K., Miyake, M., Yamauchi, A., Kohrogi, H. and Hirashima, M. (2010) Galectin-9 expands immunosuppressive macrophages to ameliorate T-cell-mediated lung inflammation. Eur. J. Immunol. 40, 548–558.

[141] Anderson, A.C., Anderson, D.E., Bregoli, L., Hastings, W.D., Kassam, N., Lei, C., Chandwaskar, R., Karman, J., Su, E.W., Hirashima, M., Bruce, J.N., Kane, L.P., Kuchroo, V.K. and Hafler, D.A. (2007) Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science 318, 1141–1143.

[142] Ma, C.J., Li, G.Y., Cheng, Y.Q., Wang, J.M., Ying, R.S., Shi, L., Wu, X.Y., Niki, T., Hirashima, M., Li, C.F., Moorman, J.P. and Yao, Z.Q. (2013) Cis association of galectin-9 with Tim-3 differentially regulates IL-12/IL-23 expressions in monocytes via TLR signaling. PLoS ONE 8, e72488.

[143] Gabrilovich, D.I., Ostrand-Rosenberg, S. and Bronte, V. (2012) Coordinated regulation of myeloid cells by tumours. Nat. Rev. Immunol. 12, 253–268.

[144] Youn, J.I. and Gabrilovich, D.I. (2010) The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. Eur. J. Immunol. 40, 2969–2975.

[145] Blidner, A.G., Salatino, M., Mascanfroni, I.D., Diament, M.J., de Kier, Bal., Joffé, E., Jasnis, M.A., Klein, S.M. and Rabinovich, G.A. (2015) Differential response of myeloid-derived suppressor cells to the nonsteroidal anti-inflammatory agent indomethacin in tumor-associated and tumor-free microenvironments. J. Immunol. 194, 3452–3462.

[146] Dardalhon, V., Anderson, A.C., Karman, J., Apetoh, L., Chandwaskar, R., Lee, D. H., Cornejo, M., Nishi, N., Yamauchi, A., Quintana, F.J., Sobel, R.A., Hirashima, M. and Kuchroo, V.K. (2010) Tim-3/galectin-9 pathway: regulation of Th1 immunity through promotion of CD11b(+)Ly-6G(+) myeloid cells. J. Immunol. 185, 1383–1392.

[147] Zhang, Y., Jiang, L., Zhang, M. and Lv, K. (2014) Galectin-9 induced myeloid suppressor cells expand regulatory T cells in an IL-10-dependent manner in CVB3-induced acute myocarditis. Int. J. Mol. Sci. 15, 3356–3372.

[148] Sunil, V.R., Francis, M., Vayas, K.N., Cervelli, J.A., Choi, H., Laskin, J.D. and Laskin, D.L. (2015) Regulation of ozone-induced lung inflammation and injury by the β-galactoside-binding lectin galectin-3. Toxicol. Appl. Pharm. 284, 236–245.

[149] Verschuere, T., Toelen, J., Maes, W., Poirier, F., Boon, L., Tousseyn, T., Mathivet, T., Gerhardt, H., Mathieu, V., Kiss, R., Lefranc, F., Van Gool, S.W. and

Vleeschouwer, A. (2008) Glioma-derived galectin-1 regulates innate and adaptive antitumor immunity. Int. J. Cancer 134, 873–884.
[150] Uccelli, A. and de Rosbo, N.K. (2015) The immunomodulatory function of mesenchymal stem cells: mode of action and pathways. Ann. N.Y. Acad. Sci..
[151] Gieseke, F., Böhringer, J., Bussolari, R., Dominici, M., Handgretinger, R. and Müller, I. (2010) Human multipotent mesenchymal stromal cells use galectin-1 to inhibit immune effector cells. Blood 116, 3770–3779.
[152] Sioud, M., Mobergslien, A., Boudabous, A. and Floisand, Y. (2011) Mesenchymal stem cell-mediated T cell suppression occurs through secreted galectins. Int. J. Oncol. 38, 385–390.
[153] Gieseke, F., Kruchen, A., Tzaribachev, N., Bentzien, F., Dominici, M. and Müller, I. (2013) Proinflammatory stimuli induce galectin-9 in human mesenchymal stromal cells to suppress T-cell proliferation. Eur. J. Immunol. 43, 2741–2749.
[154] Ungerer, C., Quade-Lyssy, P., Radeke, H.H., Henschler, R., Königs, C., Köhl, U., Seifried, E. and Schüttrumpf, J. (2014) Galectin-9 is a suppressor of T and B cells and predicts the immune modulatory potential of mesenchymal stromal cell preparations. Stem Cells Dev. 23, 755–766.
[155] Ryu, K.-H., Kim, S.-Y., Kim, Y.-R., Woo, S.-Y., Sung, S.H., Kim, H.S., Jung, S.-C., Jo, I. and Park, J.-W. (2014) Tonsil-derived mesenchymal stem cells alleviate concanavalin A-induced acute liver injury. Exp. Cell Res. 326, 143–154.
[156] Suila, H., Hirvonen, T., Kotovuori, A., Ritamo, I., Kerkelä, E., Anderson, H., Natunen, S., Tuimala, J., Laitinen, S., Nystedt, J., Räbinä, J. and Valmu, L. (2014) Human umbilical cord blood-derived mesenchymal stromal cells display a novel interaction between P-selectin and galectin-1. Scan. J. Immunol. 80, 12–21.
[157] Liu, G., Wang, L., Pang, T., Zhu, D., Xu, Y., Wang, H., Cong, X. and Liu, Y. (2014) Umbilical cord-derived mesenchymal stem cells regulate thymic epithelial cell development and function in Foxn1$^{-/-}$ mice. Cell. Mol. Immunol. 11, 275–284.
[158] Balan, V., Nangia-Makker, P., Schwartz, A.G., Jung, Y.S., Tait, L., Hogan, V., Raz, T., Yi, W., Yang, Z.Q., Wu, G.S., Guo, Y., Li, H., Abrams, J., Couch, F.J., Lingle, W. L., Lloyd, R.V., Ethier, S.P., Tainsky, M. and Raz, A. (2008) Racial disparity in breast cancer and functional germ line mutation in galectin-3 (rs4644): a pilot study. Cancer Res. 68, 10045–10050.