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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

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