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Tolerogenic Dendritic Cells in the Control of Autoimmune Neuroinflammation: An Emerging Role of Protein-Glycan Interactions

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Abstract

During the past decade, a great deal of information has contributed to our understanding of the immunosuppressive pathways that operate during the resolution of autoimmune pathology, including central nervous system (CNS) inflammation. Activation of these pathways is accomplished through the integration of an intricate network of inhibitory signals and immune suppressive cells, including regulatory T cells, myeloid-derived suppressor cells, 'alternatively activated' macrophages and tolerogenic dendritic cells (DCs). During the course of inflammatory diseases, immature or mature DCs may be licensed by different stimuli (e.g. cytokines, neuropeptides and growth factors) to become tolerogenic and suppress pathogenic T cell responses, thus emphasizing the outstanding plasticity of these cells. Recent findings have shed light to an immunoregulatory circuit by which galectin-1, an endogenous glycan-binding protein, favors the differentiation of regulatory DCs which promote T cell tolerance and contribute to resolution of autoimmune pathology through mechanisms involving IL-27 and IL-10. Together with the ability of galectin-1-glycan interactions to selectively blunt T helper (Th)1 and Th17 responses, this effect provides a rational explanation for the broad immunosuppressive effects of this glycan-binding protein in several experimental models of chronic inflammation and cancer. In this mini review, we will summarize the regulatory signals leading to the differentiation of tolerogenic DCs and their participation in CNS inflammation. In addition, we will underscore recent findings on the emerging role of galectin-glycan interactions in the establishment of immunosuppressive networks during the resolution of chronic inflammation.

Dendritic Cells at the Crossroads of Tolerance and Inflammation

During the past years great progress has been made in our understanding of the cellular and molecular pathways that regulate immune tolerance [1]. Active immunosuppression can be achieved through the secretion of anti-inflammatory cytokines or through specialized suppressor cells, including $CD4^+CD25^+FoxP3^+$ T regulatory cells (T_{reg}) cells, type-1 $FoxP3^-$ T_{reg} cells, myeloid-derived suppressor cells, 'alternatively-activated' macrophages and tolerogenic dendritic cells (DCs) [1].

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DCs are the central players in all immune responses, both innate and adaptive [1, 2]. Conventional DC subsets described in humans include myeloid DCs and plasmacytoid DCs [2]. Through antigen recognition, processing and presentation, DCs can orchestrate adaptive immune responses; yet these cells can also attenuate inflammatory reactions irrespective of their maturation status by promoting T cell anergy or favoring the expansion and/or differentiation of T_{reg} cells [2]. During the course of chronic inflammatory reactions, bidirectional interactions take place between dendritic cells (DCs) and T cells, which may initiate either an immunogenic or tolerogenic pathway [2]. Several stimuli may influence the decision of DCs to become tolerogenic, including transforming growth factor-β, interleukin (IL)-10, vasoactive intestinal peptide, hepatocyte growth factor, and 1,25-dihydroxyvitamin D_3 [1, 2]. In addition, interaction with stromal cells, $CD4^+CD25^+FoxP3^+$ T_{reg} cells or apoptotic cells may also drive the differentiation of tolerogenic DCs [2]. In spite of early observations assigning a predominant immunogenic and pro-inflammatory function to mature DCs (which express high levels of major histocompatibility complex II and costimulatory molecules CD80/CD86), recent evidence challenged this paradigm showing that DC maturation itself is neither an immunogenic nor a tolerogenic hallmark of DCs [2]. Supporting this concept, fully mature DCs are abundant throughout the peak and resolution phase of autoimmune inflammation and are capable of promoting the expansion of T_{reg} cells instead of inciting a primary or memory T cell response [1–3]. Hence, it is the flexibility of specialized DCs to respond to selective environmental signals, which may determine the amplification or silencing of Th1-, Th2-, Th17- or Th9-driven effector immunity, which may in turn shape the course of chronic inflammation. The mechanisms and pathways underlying these regulatory processes are subject of an intensive research with still more questions than answers.

Tolerogenic Dendritic Cells in CNS Autoimmune Inflammation

Multiple sclerosis is a major inflammatory and demyelinating disorder of the CNS characterized by a relapsing-remitting stage followed by a secondary progressive phase [3]. Animal models of experimental autoimmune encephalomyelitis (EAE) recapitulate the clinical and immunological features of the disease and have been of crucial importance for the validation of many therapeutic agents [3]. From an immunological standpoint, the activity of the disease is controlled through a delicate balance of Th1, Th2, Th17 and T_{reg} cells [3]. While DCs producing high amounts of IL-12 favor the differentiation of Th1 effector cells, IL-23-secreting DCs favor a Th17 pathogenic phenotype and those producing high amounts of IL-10 and IL-27 determine the differentiation of $FoxP3^-$ T_{reg} cells [1, 3, 4]. In this regard, alterations in DC activation and cytokine production have been shown to contribute to the transition from the relapsing-remitting to the progressive phase of multiple sclerosis [3]. Hence, targeting pro-inflammatory Th1 or Th17 effector cells, promoting the expansion of T_{reg} cells or fine-tuning the tolerogenic function of DCs have emerged as rational therapeutic strategies aimed at tempering autoimmune neuroinflammation and protecting the CNS from immune-mediated damage [3].

Galectin-Glycan Interactions in the Regulation of CNS Autoimmunity

Endogenous glycan-binding proteins or lectins control a variety of biological processes by deciphering information encoded by cell surface glycoproteins and glycolipids [4]. Galectins are a family of ubiquitous lectins defined by a conserved carbohydrate-recognition domain and a common structural fold [4]. Galectins preferentially bind to β-galactoside-containing glycans comprised of repeating units of N-acetyllactosamine (Galβ1,4GlcNAc), either as disaccharide units at the termini of complex N-glycans or as repeating units in a poly-N-acetyllactosamine chain on N-glycans or O-glycans [4]. As many as 15 galectins have been identified in mammals and proposed to mediate diverse biological roles in the regulation of innate and adaptive immune responses, including cytokine receptor endocytosis, host-pathogen interactions and homeostasis of immune cells [4]. Galectin-1, a prototype member of this family, can modulate T cell functions by controlling the proliferation and survival of effector T cells, antagonizing T cell activation or modulating the balance of pro-inflammatory and anti-inflammatory cytokines [4].

Compelling evidence indicates that galectin-1 treatment suppresses chronic inflammation and skews the balance of the immune responses towards a Th2-type cytokine profile in several experimental models of autoimmune inflammation, including arthritis, uveitis, inflammatory bowel disease, diabetes, hepatitis and autoimmune encephalomyelitis [reviewed in 4]. In addition, selective blockade of galectin-1 expression in tumor tis-

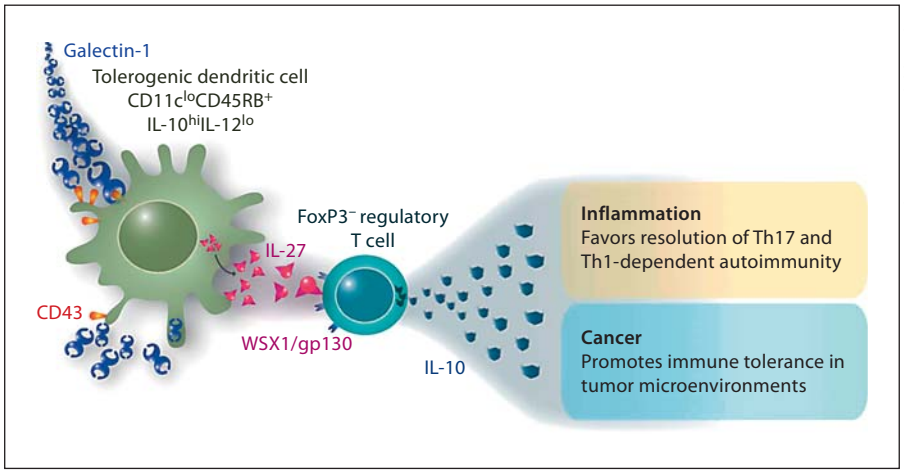

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Fig. 1. Identification of an immunoregulatory circuit linking galectin-1 signaling, IL-27-producing DCs and IL-10-secreting T_{reg} cells. During the peak and resolution stages of autoimmune neuroinflammation, galectin-1 expression augments and promotes the differentiation of $CD11c^{lo}$, $CD45RB^{+}$ tolerogenic DCs which express high levels of phosphorylated STAT3 (pSTAT3) and IL-10. These DCs also secrete high amounts of IL-27, a heterodimeric cytokine composed of the p28 and the EBI3 subunits, which interacts with its specific receptor (gp130/WSX1) and promotes the expansion of IL-10-producing $FoxP3^{-}$ regulatory T cells. Delivery of these tolerogenic signals from dendritic cells to T cells blunt Th1 and Th17 responses and promote the resolution of autoimmune neuroinflammation. On the other hand, hierarchical expression of these immunoregulatory signals may contribute to create a tolerogenic microenvironment at sites of tumor growth, suggesting that targeting the gears of this tolerogenic circuit may have broad therapeutic implications in immunopathology.

sue results in reduced tumor growth and unleashed Th1-mediated antitumor responses in experimental settings of melanoma and Hodgkin lymphoma, suggesting the involvement of this glycan-binding protein in tumor-immune escape [5, 6]. Recent work provided a molecular explanation for galectin-1-mediated Th2 skewing, demonstrating that Th1 and Th17 effector cells express the repertoire of cell surface glycans that are essential for the formation of galectin-glycoprotein lattices, and hence are cellular targets of the immunoregulatory activity of galectin-1. By contrast, Th2 cells are protected from galectin-1 binding through differential α2,6 sialylation of cell surface glycoproteins [7]. Consistent with these findings, galectin-1-deficient *(Lgals1*$^{-/-}$*)* mice challenged with encephalitogenic stimuli developed greater antigen-specific Th1 and Th17 responses compared to their wild-type counterpart [7].

To further understand the cellular and molecular mechanisms underlying the broad immunosuppressive activity of galectin-1 in autoimmunity and tumor settings, we investigated the impact of this glycan-binding protein on human and mouse DCs using different experimental models in vivo [8]. Notably, DCs differentiated or matured in a galectin-1-enriched microenvironment acquired a distinctive 'regulatory signature' characterized by high expression of the cell surface marker CD45RB, phosphorylation of the transcription factor STAT3 and abundant secretion of IL-27 and IL-10 [8]. More importantly, when transferred in vivo, these DCs promoted T-cell tolerance in antigen-specific and neoplastic settings, blunted Th1 and Th17 responses and halted autoimmune neuroinflammation through mechanisms involving DC-derived IL-27 and T cell-derived IL-10 [8]. Thus, using IL-27 receptor-deficient *(Il27ra*$^{-/-}$*)* and IL-10-deficient *(Il10*$^{-/-}$*)* mice, we have identified an immunoregulatory circuit linking galectin-1 signaling, IL-27-producing tolerogenic DCs and IL-10 secreting T_{reg} cells (fig. 1) [8]. Interestingly, other studies have found that DCs engineered to overexpress galectin-1 can induce contrasting effects on naïve and stimulated T cells similar to direct exposure of T cells to soluble recombinant galectin-1 [9], suggesting that these cells could also be used as vehicles of immunomodulatory target genes. Moreover, exposure to galectin-1 also promoted the migration of DCs through mechanisms involving Syk and PKC signaling [10], suggesting that DCs exposed to galectin-1 may acquire a distinctive immunomodulatory program characterized by a 'mature' or 'semi-mature' cell surface phenotype, increased migration profile and greatly enhanced tolerogenic potential.

Given its tolerogenic effects, we also investigated the relevance of endogenous galectin-1 during the evolution of CNS autoimmune inflammation. Remarkably, galectin-1 expression augmented during the peak and recovery phases of EAE and was dramatically upregulated by tolerogenic stimuli including vasoactive intestinal peptide, vitamin D_3 and IL-10, but significantly downregulated by pro-inflammatory cytokines (TNF and IFN-γ) and most Toll-like receptor agonists [8]. Moreover, DCs lacking *Lgals1* gene had lower expression of IL-27, higher expression of IL-23 and less STAT3 phosphorylation and were not capable of promoting T cell tolerance during ongoing EAE [8]. In contrast, galectin-1-sufficient DCs restored T cell tolerance and contributed to the resolution of autoimmune neuroinflammation [8], suggesting a crucial role of endogenous galectin-1 in 'fine-tuning' the immunogenicity of DCs.

Conclusions and Future Directions

Research over the past decade has identified a number of upstream and downstream signaling events on DCs which can be manipulated in a selective manner to amplify either an immunogenic or a tolerogenic response [1–3]. In this context, tolerogenic DCs may be employed as 'Trojan horses' to propagate immune tolerance and silence CNS inflammation. Hence, galectin-1-differentiated DCs producing IL-27 can be harnessed to silence Th1- and Th17-mediated responses and promote the differentiation of IL-10-producing T_{reg} cells, suggesting a hierarchy of tolerogenic signals which may represent potential targets in T cell-mediated demyelinating disease. However, before galectin-based therapeutic strategies can be embraced, a more thorough understanding of the mechanisms underlying the function of galectin-glycan lattices is required, including the extent of redundancy in galectin functions in vivo and the regulation of galectin expression in healthy, inflamed and neoplastic tissue.

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