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Review

Review

Galectin-8 in the onset of the immune response and inflammation

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Abstract

Galectins (Gals), a family of mammalian lectins, have emerged as key regulators of the immune response, being implicated in several physiologic and pathologic conditions. Lately, there is increasing data regarding the participation of Galectin-8 (Gal-8) in both the adaptive and innate immune responses, as well as its high expression in inflammatory disorders. Here, we focus on the pro- and anti-inflammatory properties of Gal-8 and discuss the potential use of this lectin in order to shape the immune response, according to the context.

Key words: adaptive immune response, autoimmune disorders, B and T cell cooperation, galectin–glycan interaction, innate immune response

Galectins: overview

Galectins (Gals) comprise a family of endogenous mammalian lectins characterized by the presence of conserved carbohydrate recognition domains (CRDs) that bind to poly-LacNac-containing glycans on target cells. Depending on the number of CRDs, Gals are classified as prototype (one CRD, such as Gal-1, -2 and -7), tandem-repeat (two linked CRDs, such as Gal-4, -8 and -9) and chimera (one CRD fused to a nonlectin domain, Gal-3). A remarkable feature of this family is the formation of ordered cell surface Gal-glycan lattices, which engage specific glycoconjugates by traditional ligand-receptor interactions. By building these lattices, Gals induce the aggregation of glycoconjugates that would otherwise scatter on the cell surface (Brewer et al. 2002), leading to the formation of rafts that, in turn, can either facilitate or hamper cell signaling. Gals are synthesized as cytoplasmatic proteins and are secreted as soluble factors by an unresolved mechanism to the extracellular milieu where they exert the majority of their biological functions. Upon secretion, Gals can bind to glycoproteins present at the surface of the same cell that produce them (autocrine), adjacent cells (paracrine) and the extracellular matrix. Therefore, by acting in a paracrine way, Gals can spread their functions to surrounding cells that may not synthesize them. They can also exert some effects at the intracellular compartments, for example, by recognizing glycans exposed in damaged vacuoles during the autophagic process (Thurston et al. 2012).

Different members of the Gals family have been found in primary and secondary lymphoid tissues, as well as in circulating cells. At present, enough evidence has been accumulated as to position Gals among the major mediators of the innate and adaptive immune response, being implicated in many different processes such as tumor escape, autoimmune disorders, tolerance induction and host defense (Elola et al. 2007, Liu and Rabinovich 2010).

Gal-8, which belongs to the tandem-repeat group, is intrinsically a heterodimer with an *N*-terminal CRD (*N*-CRD) and a *C*-terminal CRD (*C*-CRD), each displaying a different glycan affinity and covalently fused by a linker peptide of variable length which defines the isoforms among species (Bidon et al. 2001; Tribulatti et al. 2007). Given its particular heterodimeric structure, Gal-8 can mediate cell–cell and cell–matrix interactions. Gal-8 is widely expressed in many

organs and tissues under physiological or pathological conditions such as inflamed synovia, osteoarthritis and tumors (Bidon-Wagner and Le Pennec 2004; Eshkar Sebban et al. 2007; Hadari et al. 2000; Thijssen et al. 2008; Weinmann et al. 2018; Zick et al. 2004). Endothelial cells from both lymphatic and vascular vessels constitute an important source of secreted Gal-8 in different tissues, with the potential to deliver lectin activity through the surrounding microenvironment and circulating cells (Cattaneo et al. 2014; Cueni 2009; Obino et al. 2018; Stancic et al. 2011; Thijssen et al. 2008). Several reports describe shared effects among Gal-8 and other prominent members of this family, such as Gal-1 in T and B responses (Tribulatti et al. 2012; Tsai et al. 2011) or Gal-4 in bacterial killing (Stowell et al. 2010), just to name a few. Despite that there is some redundancy in Gals roles, in the present review we will only focus on Gal-8 to gain a more comprehensive picture of its function in the adaptive and innate immune responses, as well as its participation in the inflammatory processes.

Adaptive response

T cells

Cellular immunity is accomplished by T lymphocytes that specifically recognize antigens to eliminate intracellular pathogens or infected cells. Within the T cell compartment, $CD4^+$ T helper (Th) are effector cells that, upon antigen encounter, secrete soluble mediators that stimulate B lymphocyte differentiation, and phagocyte and leukocyte activation, thus orchestrating humoral, cellular and inflammatory responses. Gal-8 is found in primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid organs (Obino et al. 2018; Tribulatti et al. 2009; Tribulatti et al. 2007). Gal-8 mRNA is expressed in both purified mouse thymocytes and thymic epithelial cells (TECs), as well as in purified mouse splenocytes, whereas Gal-8 protein is detected in whole homogenates of mouse spleen and thymus (Tribulatti et al. 2009; Tribulatti et al. 2007). In lymph nodes, Gal-8 mRNA is observed in the paracortical T zone area and in the vasculature (Obino et al. 2018). Despite that the presence of the lectin is well documented in these T cell compartments, the precise cellular source in the periphery remains mostly undetermined. Additionally, circulating platelets, dendritic cells (DCs), splenic B lymphocytes and endothelial cells that are shown to produce Gal-8 at steady state and under inflammatory stimulation (Carabelli et al. 2017; Cattaneo et al. 2014; Romaniuk et al. 2010; Tsai et al. 2011) can also represent alternative sources for the extracellular protein in those lymphoid tissues.

Several functions on T cell populations were ascribed to this lectin. Our group has demonstrated that recombinant Gal-8 (rGal-8) is able to induce apoptosis of the immature $CD4^{high}CD8^{high}$ mouse thymocyte subpopulation in vitro, through caspase pathway activation (Tribulatti et al. 2007). This proapoptotic ability of Gal-8 on immature T cells suggests its involvement in the central maturation process and tolerance induction. In peripheral cells, however, we found that rGal-8 exerts two well-defined effects on mouse naïve $CD4^+$ T cells: At relatively high concentrations, it induces strong antigen-independent proliferation, whereas at lower concentrations, it costimulates T cells in the presence of antigen-presenting cells (APCs) and the corresponding antigen (Tribulatti et al. 2009). Both in vitro activities on peripheral $CD4^+$ T cells differ in their molecular requirements, with the Gal-8 heterodimeric structure being only essential for antigen-independent proliferation but not for costimulation induction, where both single *N*-CRDs and *C*-CRDs are active. Nevertheless, in both Gal-8-induced effects, the *C*-CRD is the main domain involved (Cattaneo et al. 2011). The absolute requirement of the tandem-repeat structure for the induction of T cell proliferation suggests the involvement of lattice formation at the T cell surface, thus providing a plausible explanation for the high lectin concentration required. In strong contrast, the costimulatory effect is achieved with low amounts of the lectin and involves the recognition of high-affinity ligands such as poly-LacNac and blood groups antigens, indicating that this effect is rather elicited through agonistic interactions (Schroeder et al. 2016). In the absence of antigen, the robust Gal-8-induced proliferation of naïve $CD4^+$ T cells was accompanied by an increased expression of IL-2, IFN-γ and IL-4, supporting the polyclonal expansion of Th1 and Th2 populations and its potential involvement in inflammatory and autoimmune processes. Interestingly, the rate of Gal-8-induced proliferation of human naïve $CD4^+$ T cells varied among donors, ranging from responders to nonresponder individuals (Cattaneo et al. 2011). Both Gal-8's proliferative and costimulatory effects on primary $CD4^+$ T cells are mediated by the CD45 phosphotyrosine phosphatase (PTPase) activity and involve the activation of ZAP-70 and ERK1/2 signaling pathways (Cattaneo et al. 2011; Tribulatti et al. 2009). In line with this, other authors demonstrated that Gal-8 is a potent extracellular stimulus for the Jurkat T cell line, which induces lamellipodia formation, cell spreading and adhesion by binding to integrins at the cell surface and triggering ERK1/2 phosphorylation (Carcamo et al. 2006; Yamamoto et al. 2008). Besides the ERK1/2 and CD45PTPase pathways, blockade of other representative T cell receptor (TCR) downstream signal transducers like Lck, PI3K, PKC and p38MAPK prevents Gal-8-induced antigen-specific costimulation on naïve $CD4^+$ T cells. These results demonstrate that Gal-8 is actually enhancing weak signals from borderline antigen-TCR engagement, by lowering the TCR activation threshold (Tribulatti et al. 2012). To completely achieve the costimulatory activity, Gal-8 has to bind APCs and $CD4^+$ T cells simultaneously, which indicates that Gal-8 is likely stabilizing the immune synapses during antigen presentation, thus strengthening TCR-downstream signaling, particularly when antigenic signals are weak (Tribulatti et al. 2012). In fact, the costimulatory effect is evident when the antigen amount is limited, and Gal-8 is present at such low dose that is unable to induce the polyclonal proliferation. Since this effect is reached with lower concentrations of Gal-8, it could be its primary activity in vivo, triggering some otherwise borderline signals. In this regard, we have observed in different immunization models that a single dose of rGal-8 administered in combination with a suboptimal dose of antigen is sufficient to increase the specific cellular response in mice, demonstrating that Gal-8 can prime resting T cells in vivo to enhance borderline immune responses (Carabelli et al. 2017; Schroeder et al. 2016; Tribulatti et al. 2012).

Despite that Gal-8 is able to induce a strong proliferation of resting human or murine T cells, it can promote cell death or antiproliferative activities when these cells become activated. Our group and others have shown that phytohemagglutinin (PHA)- and CD3/CD28-prestimulated human peripheral blood mononuclear cells (PBMCs) become apoptotic after restimulation with rGal-8 (Cattaneo et al. 2011; Norambuena et al. 2009). Similarly, rGal-8 provides antiproliferative signals to concanavalin A (Con A)-preactivated murine T cells (Tribulatti et al. 2012). Therefore, a dual role for Gal-8 on T cell arises, enhancing normal or physiological responses, especially when stimulus is limited, and restraining the effector phase of ongoing or exacerbated responses by controlling T-cell population expansion. In line with this, a regulatory role for Gal-8 in the immune response is further supported by the reported in vivo immunosuppressive activities in different autoimmune models. For example, rGal-8 was shown to ameliorate experimental autoimmune encephalomyelitis (EAE) in

C57BL/6 mice, which is associated with the lectin ability to promote apoptosis of preactivated Th17 but not Th1 cells in vitro (Pardo et al. 2017). Similarly, Sampson et al. (2015) demonstrated that rGal-8 treatment reduces the severity of murine experimental autoimmune uveitis (EAU) pathology by enhancing anti-inflammatory and regulatory T (Treg) cell responses. In this autoimmune model, rGal-8 administration increases the number of CTLA-4$^+$IL-10$^+$CD103$^+$ Treg cells as well as Th2 cells in the draining lymph node and in the inflamed retina and impairs the production of inflammatory cytokines by retinal Th1 and Th17 cells. Mechanistically, Gal-8 seems to promote Treg differentiation by activating both TGF-β and sustained-IL-2 receptor signaling (Sampson et al. 2015; Sampson et al. 2016). Remarkably, rGal-8 treatment affects T cell differentiation only at sites of inflammation, such as retina and draining lymph node, but does not alter the differentiation state of resting T cells systemically, since CD4$^+$ T cell subpopulations in the spleen remained unchanged. These observations indicate that Gal-8 inhibitory effects are specific for tissues undergoing an active immune response, which is in agreement with our previous reports where Gal-8 rather functions as a proinflammatory stimulus for naïve CD4$^+$ T cell responses (Cattaneo et al. 2011; Tribulatti et al. 2009; Tribulatti et al 2012). Therefore, by exerting these dual activities on the T cell compartment, Gal-8 arises as an interesting molecule able to shape the immune response that could potentially be developed as a novel pharmaceutical against immunological disorders.

Notwithstanding the overlapping functions between Gal-8 and other members of Gal family, Gal-8 knockout (KO) mice have been useful to disclose endogenous lectin contribution, not only in leukocyte biology but also in other relevant physiological effects such as bone mass regulation (Vinik et al. 2018). Splenocytes from Gal-8KO mice and their wild-type (WT) counterparts show similar frequencies of DCs, B cells and CD8$^+$ and CD4$^+$ T lymphocytes including naïve, effector and memory CD4$^+$ T cell subsets. However, splenocytes from Gal-8KO mice display increased CXCR3$^+$ and CCR6$^+$ Tregs frequencies, which are specific suppressors of Th1- and Th17-mediated inflammation respectively. This was associated with a higher polarization towards Th17 in Gal-8KO-derived splenocytes after CD3/CD8 activation (Pardo et al. 2017). In accordance, Gal-8KO mice developed exacerbated EAE, likely involving the imbalance of Th1 and Th17 cell polarization generated by the differentiation of their respective CXCR3$^+$ and CCR6$^+$ Tregs (Pardo et al. 2017). These findings highlight the participation of endogenous Gal-8 in T cell-mediated responses.

B cells

B cells are responsible for humoral immunity by recognizing extracellular antigens and differentiate to antibody-secreting plasma cells. Tsai et al. (2011) first described that mouse splenic B cells secrete Gal-8, which is accumulated along plasma cell differentiation. Exogenous Gal-8 binds with higher affinity to mature B cells than to plasma cells, an event that is associated with stage-specific expression of glycosyltransferase enzymes during plasma cell differentiation (Tsai et al. 2011). At a functional level, these authors demonstrated that ectopic expression of Gal-8, as well as in vitro treatment with the recombinant protein, promotes the generation of B220^{low}CD138$^+$ plasma cells and IgM secretion from both lipopolysaccharide (LPS)-stimulated and naïve B cells (Tsai et al. 2011). The molecular pathways underlying these effects include the induction of Blimp-1 and the spliced form of XBP-1 mRNA, genes that are crucial for plasma cell differentiation and antibody secretion, respectively. Besides, authors also showed that rGal-8 is able to activate splenic B cell proliferation, a crucial event prior to efficient plasma cell differentiation, and to induce the production of IL-6 and IL-10 (Tsai et al. 2011). Taken together, these findings highlight a role for Gal-8 in promoting plasma cell formation and antibody secretion in a T-cell independent model of LPS-induced B cell activation. In a more specific scenario, our group has demonstrated that a single dose of rGal-8 administrated together with the antigen was able to elicit a protective antibody-mediated response in an experimental model of foot-and-mouth disease virus (FMDV) vaccination (Carabelli et al. 2017). BALB/cJ mice immunized with inactivated FMDV in combination with rGal-8 display an incremented specific humoral response compared with animals vaccinated with antigen alone. The Gal-8-induced response is characterized by higher neutralizing IgG titers that efficiently protect against homologous pathogen challenge (Carabelli et al. 2017). These results highlight an in vivo role for Gal-8 in the B cell compartment during the elicitation of the adaptive immune response, supporting the potential use of exogenous Gal-8 as an immunostimulant additive in vaccine preparations. Among the underlying mechanism, the Gal-8-induced anti-FMDV response was preceded by a peak of IL-6 and IFN-γ production as well as enhanced lymphoproliferation at 48 h post-immunization, suggesting that Gal-8 activates the elicitation of the adaptive immune response at an early stage (Carabelli et al. 2017). IL-6 is a pleiotropic cytokine involved in the differentiation of follicular helper T (Tfh) cells and germinal center (GC) formation, which supports high-affinity antibody production by B cells (Eto et al. 2011; Harker et al. 2011). Results from the FMDV-vaccination model and those from Tsai et al. (2011) using LPS-activated B cells unravel the involvement of IL-6 signaling in Gal-8-induced B cell responses. In agreement with this, we have recently reported that IL-6 signaling is triggered during Gal-8-induced costimulation of antigen-specific CD4$^+$ T cells in vitro, as determined by signal transducer and activator of transcription 3 (STAT3) phosphorylation (Carabelli et al. 2018). During the antigen-presentation process, rGal-8 stimulates different splenic cell populations including plasmacytoid and conventional subsets of DCs, macrophages and B cells to produce IL-6. Remarkably, TCR cognate engagement potentiates further Gal-8-induced cytokine secretion by these APCs. The involvement of IL-6 signaling in Gal-8-induced antigen-specific CD4$^+$ T cell costimulation was further demonstrated by two approaches: IL-6 activity neutralization with a specific monoclonal antibody, and use of IL-6-deficient APCs during antigen presentation (Carabelli et al. 2018). Since IL-6 is a key factor in the Tfh cell differentiation and GC formation, these results provide a plausible mechanism by which Gal-8 activates the antigen-specific protective humoral response previously obtained in the viral vaccine model. In support of this notion, it was recently reported that mice immunized with OVA/HEL-particulate antigens in combination with soluble rGal-8 display enhanced numbers of antigen-specific B220$^+$GL7$^+$Fas$^+$ GC B cells and CD4$^+$CXCR5$^+$PD-1$^+$ Tfh cells (Obino et al. 2018). These authors also described increased expression of Gal-8 in the subcapsular sinus within lymph nodes, in a region where B cells acquire and process cell-surface tethered antigens, from mice systemically exposed to inflammatory stimulus (Obino et al. 2018). Interestingly, when Gal-8KO mice were immunized with OVA/HEL-particulate antigens, an impaired generation of GC B and Tfh cells was obtained compared to the WT counterpart, arguing for a role of the endogenous lectin in enhancing antigen presentation by B cells (Obino et al. 2018). Mechanistically, it was proposed that Gal-8 from the extracellular environment, secreted by either B cells or other cells, enhances B cell arrest phases upon antigen recognition and

Figure 1

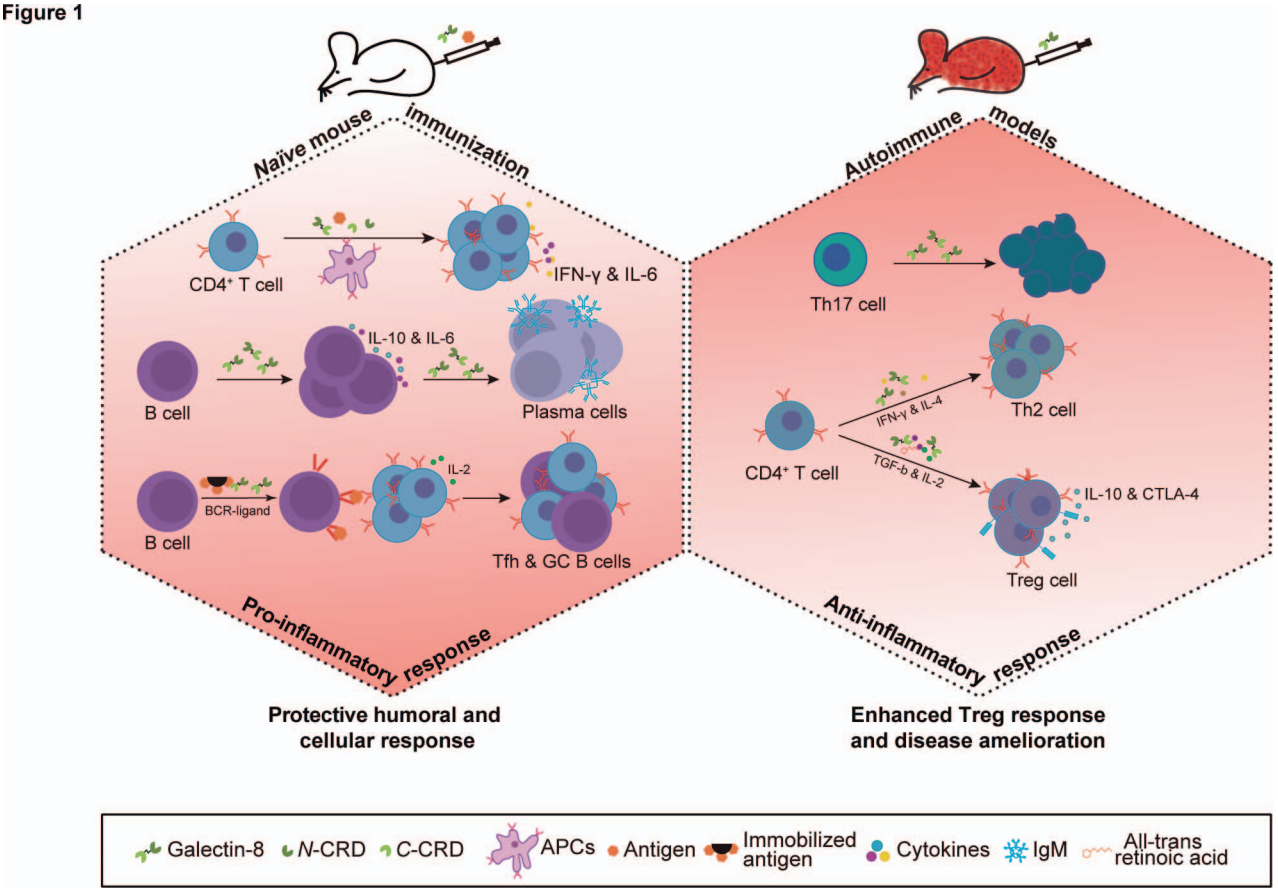

Fig. 1. Dual role of Gal-8 on the adaptive immune response. Gal-8 enhances the elicitation of primary responses (left panel). *Naïve* mice immunized with antigen plus rGal-8 elicit a protective cellular and humoral response. Different effects exerted by Gal-8 on *naïve* cells can be postulated as the underlying mechanisms: costimulation of $CD4^+$ T cells in the presence of both APCs and cognate antigen; B cell proliferation and differentiation to antibody-secreting plasma cell and stabilization of BCR synapse favoring proteolytic extraction of immobilized antigen and the subsequent presentation to specific $CD4^+$ helper T cells, leading to the emergence of GC. Gal-8 restrains the effector phase of exacerbated responses (right panel). Administration of rGal-8 attenuates the pathology of experimental autoimmune diseases by inducing an enhanced Treg cell response in draining lymph nodes. Apoptosis induction of preactivated Th17 cells, as well as an increased polarization towards Th2, can also be associated with the Gal-8 anti-inflammatory effect.

promotes synapse formation. In vitro data revealed that exogenous Gal-8 triggers a rapid recruitment and secretion of lysosome content in the B cell-antigen contact site, resulting in the efficient extraction of immobilized antigens through proteolysis (Obino et al. 2018). In addition, rGal-8 induces Btk and Akt phosphorylation, suggesting that a direct activation of B cell receptor (BCR) signaling via the PI3K pathway is also involved in the modulation of B cell functions (Obino et al. 2018).

Altogether, the data summarized until here unveils a prominent role for Gal-8 in the cooperative link between B and helper T cells during the adaptive immune response (refer to Figure 1 for a schematic view).

Innate response

DCs, macrophages and neutrophils

DCs are the professional APCs that participate in the elicitation of T cell responses, controlling the magnitude and type of the elicited adaptive immune response (Banchereau et al. 2000). Gal-8 protein is expressed by GM-CSF/IL-4 monocyte-derived human DCs (Mobergslien and Sioud 2012) and bone-marrow-derived mouse DCs (BMDCs), and, notably, lectin production is upregulated in LPS-matured BMDCs (Carabelli et al. 2017). Exogenous Gal-8 binds to both splenic mouse DCs, as well as monocyte-derived human DCs (Bax et al. 2007; Carabelli et al. 2017). Interestingly, Gal binding is incremented after DC maturation due to a major exposure of high-affinity Gal-8-ligands such as LacNAc and sialylated glycans (Bax et al. 2007). We have demonstrated that in vitro treatment with rGal-8 led to the activation of BMDCs as well as endogenous splenic DCs, evidenced by high expression of costimulatory molecules CD80, CD86 and MHCII, increased ability to present antigen to naïve $CD4^+$ T cells and an enhanced secretion of inflammatory cytokines IL-3, IL-2, IL-6, IL-13, TNF-α, MCP-1 and MCP-5 and the growth factors G-CSF and GM-CSF (Carabelli et al. 2017). Moreover, Gal-8-activated conventional and plasmacytoid splenic DCs produce high levels of IL-6 during antigen presentation, which signaling synergizes the TCR activation during antigen recognition on $CD4^+$ T cells, resulting in the previously described Gal-8 costimulatory activity (Carabelli et al. 2018). The participation of endogenous Gal-8 in DC functions was also determined by differentiating BMDCs from Gal-8KO mice (*Lgals8*$^{-/-}$ BMDCs). *Lgals8*$^{-/-}$BMDCs display a more immature phenotype, compared to the WT counterpart, characterized by a reduced expression of CD86 and IL-6 and an impaired ability to induce antigen-specific T cell response after maturation (Carabelli et al. 2017). Considering that differences between *Lgals8*$^{-/-}$ and WT

BMDCs become more evident after activation with LPS, together with the fact that this activation induces Gal-8 expression, a physiological autocrine role for the endogenous lectin in the development of mature and functional DC is then postulated. Taken together, this data demonstrates that Gal-8 induces a full DC activation, which is probably one of the mechanisms involved in the elicitation of the adaptive immune responses. Moreover, the increment in Gal-8 expression may represent an endogenous mechanism to fuel the innate response at the inflammation site.

Regarding other APCs, we have reported that the MHCII^{int}CD1 1b^{high} BMDC subpopulation, which resembles monocyte-derived macrophages rather than conventional DCs (Helft et al. 2015), is actually activated under rGal-8 treatment (Carabelli et al. 2017). Furthermore, splenic F4/80^{+} cells were activated to secrete IL-6 during antigen presentation, indicating that Gal-8 stimulates different splenic populations of APCs, including endogenous macrophages, to prime and sustain the immune response (Carabelli et al. 2018).

Neutrophils are highly specialized phagocytes endowed with bactericidal properties that are rapidly recruited to an affected site by sequential steps of attachment, rolling and firm adhesion to endothelial cells and transendothelial migration. A stimulating role in neutrophil function has been ascribed to Gal-8 (Nishi et al. 2003). Integrin αM and pro-matrix metalloproteinase-9 (pro-MMP-9) were identified as putative Gal-8 counter-receptors in purified neutrophils as well as in splenocytes (Nishi et al. 2003; Tribulatti et al. 2009). Nishi et al. (2003) observed that soluble rGal-8 interacts with integrin αMβ2/Mac1 by means of its *C*-CRD, to induce firm adhesion of human peripheral blood neutrophils and to trigger superoxide production. Moreover, rGal-8 was shown to accelerate MMP-3-mediated pro-MMP-9 processing. Since Mac-1 and MMPs play central roles in the firm attachment of circulating cells to the endothelium and the subsequent transendothelial migration respectively, Gal-8 is proposed as an important modulator of neutrophil migration. It is unclear whether neutrophils express Gal-8 themselves; nevertheless, the endothelium can be a plausible source for the secreted lectin in this cellular context. During the early stages of apoptosis, cells externalize phosphatidylserine (PS), which is normally confined to the inner leaflet of the plasma membrane. Besides being an apoptotic marker, PS exposure also serves as a key ligand for macrophage-mediated phagocytosis (Fadok et al. 2000). Some members of the Gals family induce PS reversible exposure in activated neutrophils, in the absence of apoptosis, resulting in phagocytic engulfment and clearance of living cells (Stowell et al. 2009; Stowell et al. 2007). This particular mode of cellular turnover, where cells are prepared for phagocytic removal without inducing apoptosis, is termed preaperesis and represents a key regulatory process in inflammation resolution. Stowell et al. (2008) demonstrated that rGal-8 induces PS exposure in human leukocytes by recognition of surface poly-LacNAc glycans by its *C*-CRD. Interestingly, in this work authors provided mechanistic insight into Gal-8 signaling properties by demonstrating that this Gal can dimerize through its *N*-CRDs, exposing a functionally bivalent *C*-CRD (Stowell et al. 2008). This particular observation further explains the predominant activity of the *C*-terminal domain, not only in neutrophils but also in other cell types, such as T cells. Taken together, the summarized data underscore a modulating role for Gal-8 in neutrophil function, being involved in cell adhesion, migration, microbial killing and the preaperesis process.

Pathogen clearance

Innate immunity constitutes the first line of host defense against infections. Discrimination of self from non-self is achieved by the selective recognition of pathogen-associated molecular patterns (PAMPs). In this regard, Gals have emerged as soluble factors able to engage multiple glycosylated ligands on microbes and decode glycan information to instruct innate immune cell programs (revised in Cerliani et al. 2011). However, several studies indicate that Gals might directly behave as innate immune effectors. In this regard, it was demonstrated that rGal-8 is able to selectively kill bacteria expressing human blood group antigens, both in vivo and in vitro (Stowell et al. 2010). Engagement of blood group B^{+} *Escherichia coli* by the isolated C-CRD or entire Gal-8 resulted in rapid loss of motility, significant alterations in membrane architecture and loss of membrane integrity (Stowell et al. 2010). Later on, Gal-8 bactericidal activity was extended to other gram-negative and -positive organisms expressing alternative autoantigen-like epitope structures (Stowell et al. 2014). These results position Gal-8, among other Gals, as an important innate immune factor, providing the host with a plausible mechanism of broad protection against microbial mimicry.

Autophagy has broad functions in immunity, from cell-autonomous defense to the control of inflammatory signaling pathways and the coordination of complex multicellular immune responses (revised in Cadwell 2016). In recent years, an exponential number of reports have focused on the intracellular activities of cytoplasmatic Gal-8, particularly regarding its ability to behave as a "danger signal" by labeling pathogen-invaded vacuoles for their destruction by autophagy. Pioneer work from Randow's group (Thurston et al. 2012) demonstrated that intracellular Gal-8 binds, by its *N*-CRD, to host glycans that become exposed on the luminal side of damaged *Salmonella*-containing vacuoles. By virtue of this, Gal-8 restricts bacteria proliferation by selectively recruiting the autophagy adaptor protein NDP52 that in turn binds to the autophagic machinery to deliver cargo to autophagosomes (Li et al. 2013; Thurston et al. 2012). Since then, several studies have shown that intracellular Gal-8 targets invading microbes for selective autophagy, thus postulating a Gal ability to sense and control a wide range of pathogen infections (Cheng et al. 2017; Jia et al. 2018; Li et al. 2019; Meunier et al. 2014; Montespan et al. 2017; O'Seaghdha 2013; Staring et al. 2017; Weng et al. 2018; detailed information is depicted in Table I). Recently, Jia et al. (2018) further elucidated the mechanism of Gal-8-induced autophagy by demonstrating that, under infective or sterile lysosomal damage, endogenous Gal-8 associates with the Ser/Thr protein kinase mTOR (which is an upstream repressor of autophagy), through interaction with the Ragulator-Rag/SLC389A apparatus, resulting in the inhibition of mTOR-downstream transducing signals. Altogether, these studies highlight a role for intracellular Gal-8 in the induction of selective autophagy, being involved not only in the cell-autonomous innate defense against invading microbes, but also to other exogenous or endogenous agents that cause sterile endosome membrane injury and inflammation.

Inflammatory response

An exacerbated inflammatory response is involved in the progression of many diseases; hence, the fine-tuning mechanisms that modulate the duration and extension of the local inflammatory process are vital to preventing chronic inflammation while preserving host defense. Over the last few years, many groups have been proposing the participation of Gal-8 in the inflammatory process. Several findings support this hypothesis, particularly the activating role on platelets and the endothelium. We have reported that rGal-8 interacts with integrin αIIbβ3 and GPIb at the human platelet surface and induces Src, PI3K/Akt and PLCΥ2 signaling pathways. This interaction triggers

Table I. Gal-8 promotes defense against different intracellular pathogens by inducing selective autophagy

Pathogen model	References	Mechanism of selective autophagy
Salmonella typhimurium	(Meunier et al. 2014; Thurston et al. 2016; Thurston et al. 2012)	Gal-8 binds to host glycans exposed on damaged pathogen-infected vacuoles, acting as a danger receptor that recruits NDP52, ubiquitin, TBK1 and WIPI2.
Shigella flexneri	(Thurston et al. 2012)	Gal-8 acts as a danger receptor and recruits NDP52.
Listeria monocytogenes	(Thurston et al. 2012; Weng et al. 2018)	
Helicobacter pylori	(Li et al. 2019)	
Adenoviruses	(Montespan et al. 2017)	Gal-8 acts as a danger receptor.
Picornavirus (Coxsackievirus B1 and B3, and poliovirus type 1)	(Staring et al. 2017)	
Streptococcus pyogenes	(Cheng et al. 2017; O'Seaghdha 2013)	Gal-8 acts as a danger receptor and recruits E3 ligase parkin to promote ubiquitination.
Mycobacterium tuberculosis	(Jia et al. 2018)	Upon infective-lysosomal damage, Gal-8 interacts with Ragulator-Rag/SLC38A9 and inhibits mTOR activity through its signaling machinery.

different platelet functional responses, including spreading, activation of integrin αIIbβ3, aggregation and release of both dense and α- granule content such as thromboxane B2 (TXB_2), ATP and von Willebrand factor (vWF), as well as P-selectin exposure (Romaniuk et al. 2010). Interestingly, the single *N*-CRD of Gal-8 is sufficient to trigger platelet activation, which is in agreement with the sialylated glycan preference of this particular domain (Schroeder et al. 2016; Stowell et al. 2008) and the abundant sialic acid content on the platelet surface (Tribulatti et al. 2005). Besides being critical for hemostasis and vascular repair, platelet secretion also promotes the interaction with other vascular cells through the expression of P-selectin, thus being implicated in the progression of the inflammatory condition, the metastatic spread and the immune response to bacterial challenge (McNicol and Israels 2008; Polgar et al. 2005). Therefore, these findings unravel a novel function for Gal-8 as a strong platelet agonist, with potential implications not only in the physiopathology of thrombus formation but also in fueling the inflammatory response. Western blot and flow cytometry analysis revealed that human platelets express the medium (M) and long (L) splice variants of Gal-8 and, moreover, expose it on the membrane only after thrombin stimulation (Romaniuk et al. 2010). Therefore, in the vascular system, platelets represent an alternative source of Gal-8 that may be accessible upon activation to eventually promote further thrombus growth and, at the same time, to activate endothelial cells and/or leukocytes by paracrine action.

Gal-8, as other Gal members, are expressed and secreted by endothelial cells, virtually spreading their activity throughout the organism (Thijssen et al. 2008). Thijssen et al. (2008) first described Gal-8 expression in quiescent human umbilical vein endothelial cells (HUVECS), and its translocation to the outer surface upon activation, suggesting Gal-8 involvement in endothelial functioning. In line with these previous observations, our group demonstrated that the inflammatory stimulus LPS induces Gal-8 exposure at the surface of human microvascular endothelial cells (HMEC-1), an event that preceded the subsequent secretion of Gal-8 to the inflamed microenvironment (Cattaneo et al. 2014). These findings indicate that the increased secretion of Gal-8 could be involved in amplifying the inflammatory signals to the neighboring cells, acting in a paracrine way. Upon rGal-8 in vitro treatment, endothelial cells become proadhesive to human resting platelets, a phenotype that constitutes a hallmark of the activated endothelium in an inflammatory context (Cattaneo et al. 2014). Additional proadhesive roles have been proposed for human Gal-8 that may account for its proinflammatory activity: (i) immobilized rGal-8 triggers platelet adhesion and spreading (Romaniuk et al. 2010); (ii) rGal-8 induces a firm but reversible adhesion of peripheral blood neutrophils (Nishi et al. 2003); and (iii) it also induces adhesion of different peripheral blood leukocytes such as eosinophils, monocytes and T and B cells to HUVECs, by acting as a linker between the cells (Yamamoto et al. 2008). In particular, the platelet proadhesive phenotype displayed by Gal-8-stimulated endothelial cells is likely mediated by an increased exposure of vWF, which interacts with GPIb from platelets, rather than Gal-8 acting as a bridge between platelet and endothelium. Another characteristic of the vascular endothelium is the expression and release of diverse inflammatory molecules. Besides vWF exposition, Gal-8 also triggers NFκB p65 subunit phosphorylation and the production of various cytokines and chemokines, such as CCL2, CXCL3, CXCL8, CXCL1, GM-CSF, IL-6 and CCL5 (Cattaneo et al. 2014). Altogether, these molecules are involved in the recruitment and differentiation of inflammatory cells, principally monocytes and granulocytes further supporting that Gal-8 induces an activation state on the endothelium in the inflammatory context. Very recently, (Zamorano et al. 2019) demonstrated that similar to other proinflammatory agonists like PAF (platelet-activator factor) and TNF-α, Gal-8 is also able to generate endothelial permeability in vitro by inducing S-nitrosylation of p120-catenin and dissociation of adherence junctions. Therefore, by inducing a plethora of proinflammatory molecules as well as promoting hyperpermeability in the endothelium, Gal-8 may orchestrate the inflammatory interaction among leukocytes, platelets and endothelium, providing a direct linkage among hemostasis, infection and chronic inflammation (participation of Gal-8 in the inflammatory foci is outlined in Figure 2).

Angiogenesis is a process tightly regulated by a continuous interplay of stimulators and inhibitors, and their imbalance contributes to numerous neoplastic, ischemic and immune disorders (Carmeliet 2005). Many proinflammatory proteins possess proangiogenic properties and vice versa, thus denoting a link between inflammation and angiogenesis processes (Kreuger and Phillipson 2016; Mohr et al. 2017). In this regard, immobilized rGal-8 promotes vascular endothelial cell morphogenesis in vitro and the formation of an extensive capillary network in vivo (Delgado et al. 2011). Furthermore, we described that Gal-8-stimulated platelets release the vascular endothelial growth factor (VEGF) from α-granules that, in concert with additional proangiogenic factors present in these releasates,

Figure 2

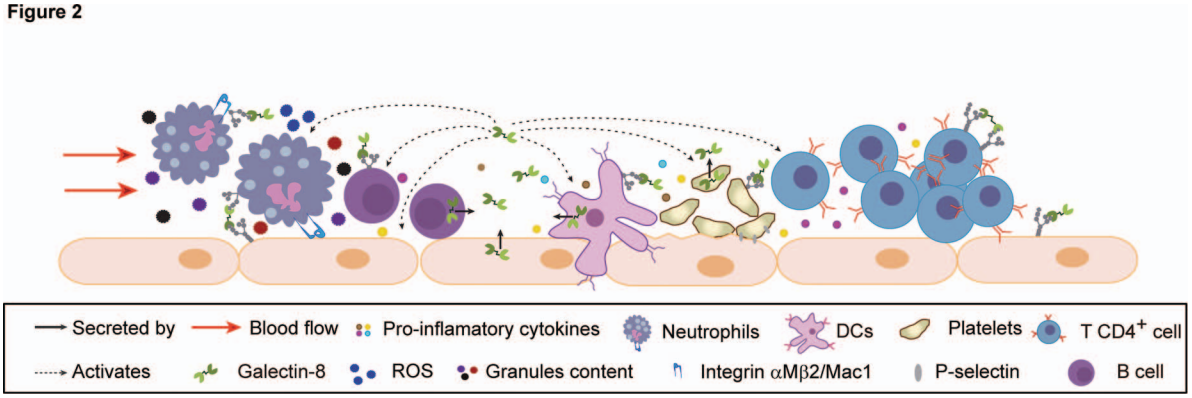

Figure 2. Participation of Gal-8 in the inflammatory process. Under inflammatory stimulation, the endothelium expresses, secretes and exposes Gal-8 on its surface. In turn, Gal-8 itself activates endothelial cells by increasing its permeability and the release of diverse inflammatory molecules and inducing resting platelet adhesion. Platelets also express Gal-8 and expose it on the membrane upon activation. Gal-8 interacts with the integrins (αIIbβ3 and GPIb) of the platelet surface, triggering adhesion, spreading, aggregation, release of granules content and P-selectin exposure. Gal-8 also interacts with integrin αMβ2/Mac1 on neutrophils to induce firm adhesion and subsequent transendothelial migration, and to trigger superoxide production. Activated DCs and B cells constitute another source of Gal-8. In turn, these cells can be stimulated by the lectin to produce several proinflammatory cytokines, such as IL-6. On the other hand, Gal-8 promotes a strong antigen-independent proliferation of $CD4^+$ T lymphocytes and their adhesion to the endothelium.

promotes endothelial cell proliferation and tubulogenesis in vitro (Etulain et al. 2014). Altogether, these findings reveal that Gal-8 plays an important role in angiogenesis, on the one hand by acting as a matricellular protein but also indirectly by triggering the release of platelet-derived proangiogenic factors. Regarding the lymphatic vasculature, rGal-8 interacts with podoplanin at the surface of lymphatic endothelial cells (LECs) to induce adhesion and haptotaxis of these cells (Cueni and Detmar 2009). A role for Gal-8 in the development of pathological lymphangiogenesis, acting as a key mediator of crosstalk among VEGF-C/VEGFR-3, podoplanin and integrin pathways, was demonstrated by using two in vivo models: (i) mouse corneal allogeneic transplantation, where lymphangiogenesis induced upon rGal-8 administration is associated with an increased rate of corneal graft rejection, and (ii) herpes simplex virus (HSV-1) keratitis, where corneal pathology and lymphangiogenesis are ameliorated in Gal-8KO mice (Chen et al. 2016). An endogenous role in inflammatory lymphangiogenesis was further proposed since Gal-8 expression was found markedly upregulated in inflamed human and mouse corneas and, moreover, the use of Gal-8 inhibitors ameliorated experimental pathological lymphangiogenesis (Chen et al. 2016).

The upregulation of endogenous Gal-8 upon cellular inflammatory activation has been largely reported. For instance, human platelets treated with thrombin expose higher levels of Gal-8 on their surface (Romaniuk et al. 2010), and LPS-activated endothelia translocate and secrete higher amounts of Gal-8, potentially spreading its activity all over the organism (Cattaneo et al. 2014; Thijssen et al. 2008). Toll-like receptor 4 (TLR-4) signaling also leads to an augmented expression of Gal-8 by DCs and B cells (Carabelli et al. 2017; Tsai et al. 2011). The existence of incremented Gal-8 levels in the inflammatory environments supports the participation of the endogenous lectin in the onset and/or progression of some pathological disorders such as autoimmunity. In this sense, in active lesions from multiple sclerosis (MS) patients, Gal-8 is found markedly incremented in activated endothelial cells of blood vessels surrounded by perivascular inflammatory infiltrates (Stancic et al. 2011). Since endothelium activation plays an important role in the early onset of MS, together with the reported Gal-8 activity in this tissue (Cattaneo et al. 2014; Thijssen et al. 2008), these observations suggest that Gal-8 produced locally can fuel the pathogenesis of MS. In the same line of evidence, high levels of Gal-8 were found in the synovial fluid of rheumatoid arthritis (RA) patients, which was associated with a modulating role in the joint inflammation (Eshkar Sebban et al. 2007). Likewise, Gal-8 expression in chondrocytes from osteoarthritis (OA) patients positively correlated with the severity of articular cartilage degeneration (Toegel et al. 2014; Weinmann et al. 2018). Expression of several prodegradative/inflammatory molecules such as IL-1β, TNF, IL-6, MMP-1, MMP-3 and MMP-13 was induced in OA chondrocytes upon rGal-8 treatment in vitro, via the NFκB signaling pathway (Weinmann et al. 2018). All these data underscore a functional role for endogenous Gal-8 in shaping the inflammatory microenvironment of different chronic autoimmune disorders.

Perspectives

Data reviewed hereby underscores a dual behavior of Gal-8, acting mainly as a proinflammatory-like molecule in different resting cells of the immune system but also displaying anti-inflammatory properties when these cells became activated. This can be readily explained by the differential glycosylation profile exhibited by naïve vs. activated cells, thus displaying selective Gal-8-ligands depending on the cellular status, which ultimately defines the intracellular signaling and the outcome response. In virtue of the presented bibliography, it is reasonable to assume that in a physiological context, endogenous Gal-8 stimulates the elicitation of both the innate and adaptive immune responses thus being involved in host defense during pathogen infections. A potential pharmacological application of this molecule as a stimulant of weak or borderline responses exhibits special interest.

Endogenous Gal-8 is upregulated in various pathological processes such as autoimmune disorders and tumors, even though the effects exerted by the chronic elevated levels of Gal-8 appear to be more diverse than in normal conditions. (Note that Gal-8 expression and its potential effects in tumorigenesis are not addressed in the present review but were carefully revised in Elola et al. 2014). For instance, in the EAE and EAU murine models, Gal-8 seems to control the disease progression, by inducing the expansion of regulatory T cell compartment, while in the OA chondrocytes Gal-8 induces disease markers and positively correlated with cartilage degeneration. Therefore, whether the persistent high level of locally

produced Gal-8 is actually restraining an exacerbated response or, by the opposite, it is fueling a given ongoing inflammatory response is, at present, debatable. Nevertheless, the pharmacological benefit of using exogenous Gal-8 (or stimulate its expression) or inhibit the endogenous activity, as to prevent or limit inflammation, will ultimately depend on the specific pathological context.

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Conflict of interest statement

None declared.

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