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Lack of galectin-3 increases Jagged1/Notch activation in bone marrow-derived dendritic cells and promotes dysregulation of T helper cell polarization

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ABSTRACT

Galectin-3, an endogenous glycan-binding protein, is abundantly expressed at sites of inflammation and immune cell activation. Although this lectin has been implicated in the control of T helper (Th) polarization, the mechanisms underlying this effect are not well understood. Here, we investigated the role of endogenous galectin-3 during the course of experimental *Leishmania major* infection using galectin-3-deficient (*Lgals3*$^{-/-}$) mice in a BALB/c background and the involvement of Notch signaling pathway in this process. *Lgals3*$^{-/-}$ mice displayed an augmented, although mixed Th1/Th2 responses compared with wild-type (WT) mice. Concomitantly, lymph node and footpad lesion cells from infected *Lgals3*$^{-/-}$ mice showed enhanced levels of Notch signaling components (Notch-1, Jagged1, Jagged2 and Notch target gene Hes-1). Bone marrow-derived dendritic cells (BMDCs) from uninfected *Lgals3*$^{-/-}$ mice also displayed increased expression of the Notch ligands Delta-like-4 and Jagged1 and pro-inflammatory cytokines. In addition, activation of Notch signaling in BMDCs upon stimulation with Jagged1 was more pronounced in *Lgals3*$^{-/-}$ BMDCs compared to WT BMDCs; this condition resulted in increased production of IL-6 by *Lgals3*$^{-/-}$ BMDCs. Finally, addition of exogenous galectin-3 to *Lgals3*$^{-/-}$ BMDCs partially reverted the increased sensitivity to Jagged1 stimulation. Our results suggest that endogenous galectin-3 regulates Notch signaling activation in BMDCs and influences polarization of T helper responses, thus increasing susceptibility to *L. major* infection.

1. Introduction

Protein-glycan interactions have recently attracted the attention of immunologists as novel regulators of immune cell homeostasis and host–pathogen interactions (Kooyk and Rabinovich, 2008). Galectin-3 is a member of a family of endogenous lectins that bind β-galactoside-containing glycoconjugates and share structural homology in their carbohydrate recognition domains (CRDs) (Rabinovich and Toscano, 2009; Sato et al., 2009; Norling et al., 2009; Dumic et al., 2006). Besides its C-terminal CRD, galectin-3 presents an N-terminal domain, which enables its self-oligomerization leading to the formation of multimeric galectin-3

Abbreviations: Th, T helper; WT, wild-type; *Lgals3*$^{-/-}$, galectin-3-deficient mice; BMDC, bone marrow-derived dendritic cell; DCs, dendritic cells; NICD, Notch intracellular domain; APC, antigen presenting cell; Treg, T regulatory cell; qPCR, quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate buffered saline; LPS, lipopolysaccharide; DLL4, Delta-like-4; JAG1/JAG2, Jagged1/Jagged2.

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molecules (Hsu and Liu, 2004; Sato et al., 2009). Interestingly, galectin-3 is an ubiquitously expressed lectin, occurring in both intracellular and extracellular compartments, despite the lack of a signal peptide required for classical secretion (Hughes, 1997). This lectin is produced by stromal and immune cells, and is widely expressed at sites of tissue inflammation (Chen et al., 2005; Acosta-Rodríguez et al., 2004; Sundblad et al., 2011), where it plays essential roles in host responses to pathogens by modulating glycan-mediated pathogen recognition and subsequent development of innate and adaptive immune responses. In fact, galectin-3 can modulate immune cell activation and differentiation and control acute and chronic inflammatory responses (Esteban et al., 2011; Argüeso et al., 2009; Ferraz et al., 2008; Kohatsu et al., 2006; Silva-Monteiro et al., 2007; Bernardes et al., 2006; Nieminen et al., 2008; Farnworth et al., 2008; Breuilh et al., 2007; Jiang et al., 2009; Demetriou et al., 2001; Chen et al., 2009).

In previous studies, we used different experimental infection models in galectin-3-deficient (*Lgals3*$^{-/-}$) mice to investigate the immunoregulatory properties of galectin-3. We have demonstrated that galectin-3 suppresses the production of IL-12 by dendritic cells (DCs), limits the secretion of IL-6 and IL-1β by macrophages and negatively regulates the number and function of regulatory T cells (Tregs) (Bernardes et al., 2006; Ferraz et al., 2008; Fermino et al., 2013). Additionally, galectin-3 interferes with mice susceptibility to *Paracoccidioides brasiliensis* infection by increasing the inflammatory responses and favoring development of Th2-polarized immune responses (Ruas et al., 2009). However, in spite of considerable progress in elucidating galectin-3 functions, the mechanisms underlying the immune regulatory roles of this lectin during T helper cell differentiation still remain to be elucidated.

Notch signaling pathway is highly conserved from Drosophila to mammals and was originally identified in differentiation and developmental processes (Bray, 2006). In mammals, the signaling is triggered when one of the five ligands (Delta-1, -3, -4 and Jagged1 and 2) binds to one of the four Notch receptors (Notch-1-4) present in a neighboring cell, thus inducing Notch receptor proteolysis and release of the Notch intracellular domain (NICD). Next, NICD translocates to the nucleus where it initiates a signaling cascade culminating in the transcriptional regulation of Notch target genes (*Hes* and *Hey* family) (Ito et al., 2012). In the past years, Notch signaling has also emerged as a critical component of immune system regulation (Shang et al., 2016; Ito et al., 2012), controlling multiple steps of T and B cell development in both central and peripheral lymphoid organs (Shang et al., 2016). The expression of Jagged or Delta-like ligands in antigen presenting cells (APCs) has been shown to regulate the differentiation of naïve T helper cells into Th1 or Th2 effector subsets. While APCs expressing Delta-like ligands induced Th1 differentiation, expression of Jagged ligands was associated with Th2-skewed responses (Radtke et al., 2013).

Recently, interactions of galectin-3 with the Notch-1 receptor and Notch signaling activation was shown to inhibit osteoblast differentiation (Nakajima et al., 2014). Moreover, our group has shown that Tregs isolated from *Lgals3*$^{-/-}$ mice exhibit altered expression of Notch signaling components during *L. major* infection (Fermino et al., 2013). In the present study, we show that DCs from *Lgals3*$^{-/-}$ mice display an altered expression of Notch ligands and receptors which contribute to augment Th2 responses in the BALB/c model of *L. major* infection. Our results suggest that a cross-talk between galectin-3 and the Jagged1/Notch signaling pathways may contribute, at least in part, to the control of T helper polarization programs.

2. Material and methods

2.1. Mice

Galectin-3-deficient (*Lgals3*$^{-/-}$) mice were generated and backcrossed to BALB/c background for nine generations as previously described (Hsu et al., 2000). Age-matched wild-type (WT) mice in BALB/c background were used as control in all the experiments. Mice were housed and cared under approved conditions at the Animal Research Facilities of Faculdade de Medicina de Ribeirão Preto-USP and the London School of Hygiene and Tropical Medicine (London, UK). All of the animals used in the experiments were 6- to 8-week-old males.

2.2. L. major *infection*

Experiments were performed with *L. major* strain LV 39. The strain was maintained *in vivo* in BALB/c mice to keep its infectivity. For experimental infection, parasites were obtained from lymph nodes of infected mice and grown *in vitro* as described (Launois et al., 1997; Zimmermann et al., 1998). Promastigote forms were washed twice in PBS before infection. Mice were infected subcutaneously in one hind footpad with 1×10^7 stationary phase *L. major* promastigotes in a final volume of 50 μL. Lesion development was monitored weekly with a vernier caliper and lesion size expressed as the difference in thickness between the infected footpad and the non-infected contralateral footpad.

2.3. Real time quantitative PCR analysis

For quantification of mRNA relative expression we utilized the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Total RNA was isolated using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), following the manufacturer's instructions. cDNA synthesis was performed in a final volume of 20 μL, using ImProm-II Reverse Transcriptase (Promega Corporation, Madison, WI, USA). The reaction mixture contained 4 μg of total RNA, 20 pmol of oligodT primer (Invitrogen Life Technologies, Carlsbad, CA, USA), 40 U of RNAsin, 1 mM of dNTP mix, and 1 U of reverse transcriptase buffer. It was then immediately used or stored at −20 °C. PCR amplification and analysis were achieved using an ABI Prism 7500 sequence detector (Applied Biosystems, Foster City, CA, USA). All reactions were performed with SYBR Green PCR Master Mix (Applied Biosystems) using a 10 μL final volume in each reaction, which contained 1 μL of template cDNA, 2.5 pmol of each primer and 5 μL of SYBR Green. The cycles were processed according to the manufacturer's instructions. Each sample was tested in duplicate and all quantifications were normalized using β-actin as endogenous control. The primers used for all PCR amplification are described in Table 1.

2.4. In vitro *restimulation*

For *in vitro* restimulation after infection with *L. major*, total lymph node cells (5×10^6) were collected 14 days post-infection and stimulated with 20 μg/mL of *L. major* antigen for 72 h at 37 °C/5% CO_2/95% humidity in RPMI-1640 medium supplemented with 10% heat-inactivated FCS, 0.1% 2-ME (Sigma-Aldrich), 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM l-glutamine, and 5 mM HEPES in a final volume of 1 mL. Culture supernatants were collected after 72 h of stimulation and stored at −20 °C for cytokine measurement.