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Review Article

Galectin-3 Controls Inflammatory Responses during Schistosomiasis

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Abstract

Schistosomiasis affects 240 million people around the world. *Schistosoma mansoni* is the major infecting form and evokes a severe immune response after egg-deposition in the mesenteric venous system. In the liver, eggs induce a powerful fibrogranulomatous reaction by macrophage-dependent manner. These cells drive a robust T_H1 immune response that progresses to acute T_H2 response. Subsequently, chronic phase is hallmarked by liver fibrosis, hepato/splenomegaly and humoral immune response. *S.mansoni* synthesizes glycoconjugates which interact with galectin-3, a β-galactoside-binding protein that regulates cell-cell and cell-matrix interactions and this binding elicits inflammation and humoral response against the parasite. Using galectin-3-deficient mice (gal-3-/- mice), we have investigate the involvement of galectin-3 on the pathogenesis of schistosomiasis. Here, we discussed the possibility to use galectin-3 to interfere with distinct phases of schistosomiasis and proposed some targets: macrophage and B lymphocyte differentiation, IL-5 and TGF-β signaling pathways, IgM-to-IgA class switching and mast cell degranulation. We proposed that galectin-3 is a key element to drive immune responses against *S.mansoni* and consequently suggested this protein as potential pharmacological target.

ABBREVIATIONS

IL: Interleukin; TNF-α: Tumor Necrosis Factor – alpha; WT: Wild type; Gal-3-/- galectin-3 deficient mice; MLNs: Mesenteric lymph nodes

INTRODUCTION

Schistosomiasis is a neglected-tropical disease affecting about 240 million people around the world. In Latin America, *Schistosoma mansoni* is the major infecting form when the aquatic-larval *cercariae* contact body superficies of individuals swimming in lagoons and dams [1]. After skin penetration, *cercariae* induce immune responses that drive schistosome maturation. The adult worms live in the mesenteric venous system in constant sexual reproduction and egg deposition. Generally, the eggs are expelled to intestinal lumen or trapped in the mesenteric vessels and hepatic portal zone [2].

The pathogenesis of the schistosomiasis is established by macrophage responses against *S.mansoni*-eggs accumulated in the liver resulting in portal inflammation and hypertension that cause liver fibrosis and failure in patients and experimental models, including mice [3]. Regarding to murine models, migrating immature worms (schistosomules) evoke a T_H1 immune response 3 weeks after the infection that persist for approximately 20 days [4]. On day 45-50 post-infection, high levels of IL-4 and IL-5 drive the acute phase of schistosomiasis hallmarked by typical T_H2 responses. On day 85-90 post-infection, the chronic phase is characterized by down-regulation of both cytokines and liver inflammation shifting from exudative response to fibrotic pattern [5]. Independently of the stage of the disease, lymphoid and myeloid cells are recruited to the liver or lymphoid organs in response to local and circulating antigens, respectively.

SCHISTOSOMIASIS: ACUTE AND CHRONIC PHASES

T_H1 response is characterized by mobilization of bone marrow monocytes to mesenteric venous system reacting against schistosomules. Liver-resident macrophages (Kupffer cells) respond immediately to egg-deposition secreting pro-inflammatory cytokines IL-1 and TNF-α [6]. These cells modulate the formation of exsudative granulomas, hepatocyte functions and hepatic stellate cell activation [7]. In accordance, serum levels of TNF-α is significantly increased in humans with similar stage of the disease [8]. Pro-inflammatory $Ly6C^{low}CD11b^{+}CCR2^{+}CX3CR1^{-}$ and pro-fibrotic $Ly6C^{high}CD11b^{+}CCR2^{-}CX3CR1^{+}$ monocytes are involved with initial response in definitive hosts [9].

T_H2 response is established by the presence of egg-antigens with local and systemic effects in the course of the acute

phase. Although *S.mansoni*-eggs are continuously delivered in the mesenteric system and drained to the liver, the hepatic parenchyma remains functional because, at least in part, the concentric fibrogranulomatous reaction isolates the eggs and antigens [2]. However, soluble egg-antigens give the bloodstream and amplify the polyclonal B cell activation in secondary lymphoid tissues [5]. After day 40 post-infection, it is possible to observe germinal centers and enhancement of antibody production [10,11].

GALECTIN-3: POSSIBLE IMMUNOLOGICAL TARGET TO CONTROL SCHISTOSOMIASIS

S.mansoni synthesizes GalNAc_1-4(Fuc_1-3)GlcNAc (Lac-DiNAc) structures (*N*-acetylgalactosamine _1-4 *N*-acetylglucosamine), glycoconjugates which interact with galectin-3 (Gal-3) produced by host-immune cells eliciting a robust immune response towards the parasite [12,13]. Gal-3 is a β-galactoside-binding protein that plays crucial roles in cell-cell and cell-matrix interactions during inflammation, tumor progression and homeostasis. It is localized in extra or intracellular compartments suggesting complex multifunctionality, such as pro-inflammatory functions and cellular proliferation, respectively [14].

In the course of murine schistosomiasis, Gal-3 staining is positive within the granulomas, in Kupffer cells, and myelopoietic niches. Using gal-3-/- infected-mice, we observed that acute liver granulomas showed a productive-exudative pattern in contrast to necrotic-exsudative pattern observed in wild type (WT) mice. Moreover, in the absence of Gal-3, the pattern of collagen organization was disturbed with non-concentric fibers dispersed throughout the parenchyma and numerous myeloid cell niches abnormally distributed around the schistosomal eggs [15]. During the last decade, we demonstrated that Gal-3 is involved with pathogens of schistosomiasis regulating leukocyte mobilization from the bone marrow, macrophage functions and B lymphocyte differentiation in the spleen and mesenteric lymph nodes (MLNs), and inflammatory reaction in the peritoneal cavity in mice infected with *S.mansoni* [15-18].

Macrophages were significantly reduced whereas monocytes were significantly increased in the liver of gal-3-/- infected mice. Moreover, these monocytes poorly differentiated into macrophages *in vitro*. Furthermore, the number of $B220^{+/low}CD138^{+}$ plasma cells was substantially increased in the bone marrow [15].

Lymphocytes are frequently activated by adult worm- and egg-antigens drained to MLNs and spleen inducing a significant polyclonal B cell reaction [6]. In both organs of WT chronically-infected-mice, Gal-3 was detected in large non-lymphoid follicular cells and small/rounded extrafollicular cells [16,17]. MLNs of gal-3-/- infected mice presented higher number of clusters of B220+ B lymphocytes in the cortex, paracortex and medulla in comparison with WT mice. Moreover, CD138+ plasma cells were abnormally localized within lymphoid follicles and significantly increased in extrafollicular sites [16]. In the spleen, the absence of Gal-3 during schistosomiasis was correlated with intense white pulp reaction, and disorganized B lymphocyte and CD138+ plasma cell niches [17]. These histological data corroborated with serological aspects, since the high number of plasma cells in gal-3-/- infected-mice was associated with increased serum levels of IgG, IgE and IgA [15,18].

The peritoneal cavity has been considered a reservoir of circulating leukocytes (monocytes, T and B lymphocytes and granulocytes) and resident cells (macrophages, mast cells and B lymphocytes) frequently detected attached in the omentum and mesentery [19,20]. These cells react promptly against the presence of *S.mansoni* antigens resulting in macrophage hyperactivity and B cell activation leading to IgM-to-IgE class switching [11,21,22]. The mesentery and omentum of gal-3-/- chronically-infected mice were enriched by clusters of CD138+ plasma cells and Blimp-1+ immunoglobulin-secreting plasma cells, directly correlated with high levels of serum IgA as well as with peritoneal B1 lymphocyte differentiation into IgA-secreting plasma cells by IL-5 and TGF-β dependent manner [18].

GALECTIN-3 IS ASSOCIATED WITH POTENTIAL MOLECULAR AND CELLULAR TARGETS DURING SCHISTOSOMIASIS

In order to investigate possible molecular mechanisms involving Gal-3 and the pathogenesis of schistosomiasis, IL-5 and TGF-β pathways could be interesting targets. *S.mansoni* expresses SmRK1a (TGF-β receptor) suggesting a possible immunomodulation by hosts [23]. Moreover, TGF-β-mediated myofibroblast activation and matrix production were significantly inhibited in gal-3-/- mice induced to chronic liver fibrosis [24]. IL-5 gene expression is down-regulated by Gal-3 in distinct cell types [25]. In addition, both cytokines are essentials for IgM-to-IgA-class switching [26].

Cellular mechanisms are also interesting strategies to understand and control the evolution of schistosomiasis and other parasitic diseases. Regarding to Gal-3, our data indicated monocyte-macrophage and B lymphocyte-plasma cell differentiation as possible cellular targets. However, growing evidence pointed to mast cells as potential cell target involving Gal-3 and the pathogenesis of schistosomiasis. Considering that mast cells enhance B cell expansion and differentiation into IgA-producing plasma cells [27], we investigated a possible correlation between these cells, Gal-3 and IgA levels found in gal-3-/- *S.mansoni*-infected mice. In these mice, peritoneal mast cells were frequently degranulated indicating an enhanced stage of activation. In fact, the *in vitro* and *in vivo* treatment with IL-5+TGF-β1 induced an increase in mast cell number and degranulation status, both associated with elevated number of peritoneal IgA+ B cells in the absence of Gal-3 [18].

The synergistic IL-5/TGF-β1 effects can be studied in the near future, in order to test therapeutic strategies based on cytokines against *S. mansoni*. In contrast, each cytokine alone does not offer an effective therapy. Helminth-infected mice over expressing IL-5 presented intense eosinophilia, but inflammatory and antibody responses were similar to wild type mice [28]. Eosinophil-ablated mice and control littermates were equivalent on egg deposition, liver fibrosis and serum-hepatic enzymes [29]. Moreover, IL-5 deficient mice presented smaller granulomas completely devoid of eosinophils and approximately 40% of reduction in hepatic fibrosis [30].

TGF-β1 is considered a fibrogenic mediator in schistosomiasis synthesized by granuloma cells [31]. However, studies with TGF-$\beta1^{-/-}$ mice indicated the existence of TGF-β1 independent liver fibrosis induced by infection [32]. The possible use of TGF- β1 as therapeutic strategy was firstly discussed after the identification of this cytokine controlling embryogenic events of *S. mansoni* [33]. On the other hand, serum and hepatic TGF- β1 levels are significantly reduced after treatment with anti-parasite drugs [34,35]. In the liver granulomas, TGF- β1 activates hepatic stellate cells (HSCs) which secrete inflammatory metabolites, such as cystenil leukotrienes and prostaglandin D2 [36,37]. Recently, it was demonstrated that hepatic granuloma derived myofibroblasts of S. mansoni-infected mice were able to secrete IL-5 and eotaxin after TGF-β1 and IL-13 stimulation [38].

In this context, Gal-3 would be analyzed as mediator of pathways that control hepatic stallate cells/myofibroblast activation, and possible interference with IL-5 and TGF-β1 secretion. Gal-3 is up-regulated in HSCs during their differentiation into myofibroblasts [24] and recombinant Gal-3 induced hepatic stellate cell proliferation [39]. Furthermore, Gal-3 favors phagocytosis by HSCs and liver fibrosis *in vivo* [40]. Recently, we suggested that myofibroblasts are potential cellular targets to control liver fibrosis in the course of schistosomiasis by linking multiple/complex signaling mechanisms: Gal-3, epigenetic factors (such as histone deacetylases) and sonic hedgehog [41].

DISCUSSION AND CONCLUSION

Schistosomiasis is a complex helminthic disease that induces a robust systemic immune response and fibrogranulomatous reaction in the liver. Currently, Gal-3 has been described as potent modulator of cell-cell and cell-extracellular matrix interactions, critical events to establishment of pathogenesis of schistosomiasis. Data obtained during the last decade pointed to macrophages, plasma cells and mast cells as possible cellular targets of Gal-3. The differentiation and functions of these cell types were severely disturbed during schistosomiasis evolution in the absence of Gal-3. Moreover, Gal-3 is involved with IL-5 and TGF-β expression, indicating that both cytokines can be added to the list of possible molecular targets of Gal-3 during schistosomiasis. Together, data discussed here suggested that Gal-3 negatively regulates B cell differentiation into plasma cells, induces monocyte differentiation into macrophages and controls mast cell activation by IL-5/TGF-β dependent manner during schistosomiasis. We concluded that Gal-3 is a key element to drive cell fate decision during *S.mansoni*-infection, consequently, a potential pharmacological target to future studies and treatment of schistosomiasis.

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The authors contributed equally to this work.

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