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Galectin-1 induces 12/15-lipoxygenase expression in murine macrophages and favors their conversion toward a pro-resolving phenotype

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ABSTRACT

During the resolution of inflammation macrophages undergo functional changes upon exposure to pro-resolving agents in their microenvironment. Primarily, engulfment of apoptotic polymorphonuclear (PMN) cells promotes conversion of macrophages toward a pro-resolving phenotype characterized by reduced CD11b expression. These macrophages are not phagocytic, do not respond to TLR ligands, and express relatively high levels of the pro-resolving enzyme 12/15-lipoxygenase (LO). Here, we report that the immuno-regulatory lectin galectin-1 is selectively expressed by $CD11b^{high}$, but not $CD11b^{low}$ macrophages. Upon exposure *in vivo* and *ex vivo*, galectin-1 directly promoted macrophage conversion from a $CD11b^{high}$ to a $CD11b^{low}$ phenotype and up-regulated the expression and activity of 12/15-LO. Moreover, galectin-1 treatment *in vivo* promoted the loss of phagocytic capacity (efferocytic satiation) in peritoneal macrophages and down-regulated secretion of TNF-α, IL-1β, and IL-10 upon LPS exposure. Our results suggest that galectin-1 could be an essential mediator in the control of macrophage function during the resolution of inflammation.

1. Introduction

During the active resolution of inflammation [1,2] several components of the immune response are eliminated [3]. Polymorphonuclear (PMN) cells undergoing apoptosis are cleared by macrophages and other phagocytic cells in a non-phlogistic fashion [4–8]. Apoptotic cell engulfment by phagocytes (also termed efferocytosis [4]) is mediated by signals that are expressed on the surface of apoptotic cells and their corresponding receptors (reviewed in [5,6]). Apoptotic cells also serve as resolution cues for macrophages, as their recognition evokes distinct signaling events that block the release of pro-inflammatory mediators and allow further engulfment of apoptotic cells [7,11]. Release of pro-inflammatory mediators is activated by bacterial moieties, and its down-regulation is termed immune-silencing/reprogramming [8,9]. Macrophage reprogramming is accompanied by the production of TGF-β and, in some cases, IL-10 [10–13], cytokines that can promote resolution and wound repair. The engulfment of apoptotic leukocytes by macrophages also leads to inhibition of inducible nitric oxide (NO) synthase (iNOS) expression and stimulates the expression of arginase-1 in the RAW 264 macrophage cell line [14], thereby preventing reactive NO production. In addition, the expression of 15-lipoxygenase (LO)-1, which is involved in the generation of pro-resolving lipid mediators [14–17], as well as the production of angiogenic growth factors [18] by macrophages are consequent to the uptake of apoptotic cells. Of note, molecular entities that are selectively expressed on apoptotic PMN cooperate with soluble bridging molecules in binding distinct receptors on the surface of macrophages. These receptors mediate both apoptotic cell clearance and immune-silencing in resolution-phase macrophages and other phagocytes [13,19–21].

Macrophages are highly diverse cells that adopt various functional phenotypes upon receiving differentiation cues from their surrounding environment [22–24]. Recent reports indicate that macrophages acquire distinct phenotypes during the resolution of acute inflammation [15,25]. A typical phenotype, which distinguishes resolution-promoting macrophages, is characterized

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by low expression of CD11b (CD11b^{low}) and is generated upon engulfment of apoptotic PMN [15]. CD11b^{low} macrophages are distinct from either pro-inflammatory, classically-activated (M1) or anti-inflammatory, alternatively-activated (M2) macrophages, since they do not express iNOS or arginase-1 [15]. Macrophage conversion to the CD11b^{low} phenotype results in significant functional changes in addition to the reduction in surface expression of CD11b and F4/80 [15]. Specifically, CD11b^{low} macrophages stop producing TNF-α and IL-1β, do not engulf apoptotic PMN (efferocytic satiation), increase the production of TGF-β and the expression of 12/15-LO, and emigrate to lymphatic vessels [15].

Galectin-1 (Gal-1; encoded by *Lgals1*) belongs to the galectin family of proteins. Members of this lectin family typically act in the extracellular milieu through binding to a myriad of glycosylated receptors on the surface of immune cells [26]. Gal-1 is involved in the regulation of both innate and adaptive immunity. It suppresses antigen presentation and NO production by macrophages [27,28], and de-activates classically-activated microglia during autoimmune neuroinflammation [33]. It also promotes the termination of adaptive immunity by inhibiting pro-inflammatory cytokine production, dampening cell adhesion and trafficking, selectively deleting T helper (Th)1 and Th-17 cells [29–31], and promoting IL-10 secretion [32–34]. Gal-1 also induces the differentiation of tolerogenic dendritic cells and regulatory T cells [31,35]. Importantly, Gal-1 is highly expressed in macrophages during peritonitis and its expression is associated with successful resolution of inflammation [36,37].

The present study reveals that Gal-1 is expressed by a distinct macrophage population and contributes to a pro-resolving macrophage phenotype both *in vivo* and *ex vivo*. Exposure to Gal-1 promotes macrophage conversion from a CD11b^{high} to a CD11b^{low} phenotype characterized by enhanced 12/15-LO expression and activity, reduced efferocytic scores *in vivo*, and diminished cytokine secretion *ex vivo*.

2. Materials and methods

2.1. Reagents

ELISA kits for mouse TNF-α (catalog no. DY210), IL-1β (DY201), and IL-10 (DY417) were obtained from R&D systems. FITC-conjugated anti-mouse Gr-1 (108406), PE-conjugated anti-mouse F4/80 (12216) and PerCP-conjugated anti-mouse CD11b (101230) antibodies were obtained from Biolegend. Goat anti-mouse arginase-1 (ab60176) was from Abcam, rabbit anti-mouse 15-lipoxygenase-1 (160707) was purchased from Cayman chemical, goat anti-mouse MMP-9 (AF909) was from R&D systems, goat anti-mouse CD11b (sc6614), rabbit anti-mouse superoxide dismutase (SOD) 1 (sc11407), goat anti-mouse β-actin (sc1008), goat anti-mouse tubulin (sc9104) and rabbit anti-mouse GAPDH (sc25778) were from Santa Cruz Biotechnology. Recombinant Gal-1 was obtained from Drs. Rabinovich and Lichtenstein and purified according to the protocol described [31]. Rabbit anti-Gal-1 polyclonal antibody was obtained from Dr. Rabinovich's laboratory and used as described [33]. Anti-goat (82462) and anti-rabbit horseradish peroxidase-conjugated IgG (A31573) were obtained from Jackson ImmunResearch laboratories. Zymosan A (Z4250-IG), LPS (L2654), staurosporine (s4400), Carboxyfluorescein succinimidyl ester (CFSE) and PKH2-PCL green fluorescence linker kit were purchased from Sigma–Aldrich. RPMI 1640 (685991) was obtained from GIBCO. Docosahexaenoic acid (DHA) and Resolvin D1 (RvD1) EIA kit were obtained from Cayman chemicals.

2.2. Mouse peritonitis

Male C57BL/6 mice (7–8 weeks; protocol approved by the Ethics Committee, The Technion, Israel, authorization no. IL-009-01-2010) were purchased from Harlan Biotech, Israel, and maintained under SPF conditions at the animal facility of the Faculty of Medicine, The Technion, Haifa, Israel. All mice were injected intraperitoneally (i.p.) with freshly-prepared zymosan A (1 mg/ml, 1 mg/25 g body weight) in sterile phosphate-buffered saline (PBS). At 66 h post zymosan A injection, mice were sacrificed, their peritoneal cavity was lavaged with 5 ml of PBS and peritoneal exudates were collected. Exudate cells and supernatants were obtained by centrifugation for further analysis and experimentation. In other experiments resident peritoneal cells were collected from unchallenged mice. In some experiments, recombinant Gal-1 (4–8 μg in 1 ml of PBS) were injected i.p. after 48 h and peritoneal cells were collected at 66 h for analysis. In other experiments PKH2-PCL green (0.25 μM, 1 ml) was injected i.p. alongside Gal-1 (4 μg in one ml of PBS) 48 h post peritonitis initiation and peritoneal or spleen cells were recovered 18 h later, immuno-stained for F4/80 and analyzed by flow cytometry.

2.3. Determination of protein expression by Western blotting

Peritoneal exudates were collected 66 h post zymosan A injection, and exudate cells were immuno-stained with FITC-conjugated rat anti-Ly-6G, PE-conjugated rat anti-F4/80, and PerCP-conjugated rat anti-mouse CD11b (Biolegend). Macrophages were sorted to CD11b^{high} and CD11b^{low} populations (>95% purity) using FACSAria (Beckton-Dickinson) as in [15]. The sorted populations of CD11b^{high} and CD11b^{low} macrophages were washed with PBS and lysed in RIPA buffer containing Protease Inhibitors Cocktail (PIC, Roche). Cell lysates were run by SDS-PAGE followed by Western blotting for Gal-1 or GAPDH as a loading control.

Peritoneal macrophages from Gal-1 or vehicle-treated mice were isolated 66 h post zymosan A injection and lysed. In *ex vivo* experiments, lysates were prepared after incubation of macrophages for 24 h with recombinant Gal-1 (1 μg/ml), AC (1:5 M/AC ratio) or vehicle control. Lysates were run by SDS-PAGE and membranes were immuno-blotted for CD11b, arginase-1, 15-lipoxygenase-1, SOD-1, and MMP-9, or β-actin, tubulin, and GAPDH as loading control (used interchangeably).

2.4. Isolation of mouse peritoneal macrophages

Cells were recovered from peritoneal exudates 66 h after zymosan A challenge. Macrophages were labeled with PE-conjugated rat anti-F4/80 and isolated using EasySep PE selection magnetic beads following the manufacturer's instructions (StemCell Technologies).

2.5. Apoptotic cell preparation

Apoptosis was induced in Jurkat T cells using staurosporine (1 μg/ml; 4 h). Cells were washed twice with PBS, re-suspended in culture medium and added to macrophages plated as described in Sections 2.6 and 2.8.

2.6. FACS analysis

To evaluate leukocyte subsets according to CD11b expression, resident peritoneal cells or exudate cells from Gal-1 (4–8 μg/mouse)- or vehicle-treated mice were blocked with anti-CD16 and anti-CD32 mAb, and then stained (20 min, 4 °C) with FITC-conjugated anti-mouse Gr-1 (0.5 μg/1 × 10^6 cells),

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