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Galectin-1 and galectin-3 expression profiles in classically and alternatively activated human macrophages ☆

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ABSTRACT

Background: Galectins have been identified as modulators of many monocyte/macrophage functions. In the response to a wide range of environmental cues macrophages may exhibit different biochemical and biological characteristics, but two main subtypes, classically (M1) and alternatively (M2) activated macrophages have been recognized. To contribute to elucidation of role and regulation of galectin-1 and galectin-3 in differently activated macrophages we explored their expression profiles in these cells.
Methods: Human monocytes obtained from blood donors were differentiated into classically (M1) and alternatively (M2a/M2c) activated macrophages. Gene and protein expression levels of intra- and extracellular galectins were investigated by qRT-PCR, Western-blot, flow cytometry, and ELISA while cytokine and surface receptor expression profiling was performed by flow cytometry.
Results: Differentiation/polarization of human monocytes into classically (M1) and alternatively (M2a/M2c) activated macrophages was followed by profound changes of galectin-3 expression and its proteolytic cleavage. Expression and secretion of Gal-3 was tightly regulated and significantly differed among classically (M1) and alternatively (M2a/M2c) activated macrophages, while the differences of galectin-1 expression profiles were not as pronounced. Human monocytes exhibited high amount of free galectin-3 receptors, while on both types of activated macrophages were fully saturated.
Conclusions: Galectin-3 is more distinctive descriptor of macrophages differentiation/activation than galectin-1. Its specific expression and secretion pattern in M1 *vs.* M2a/M2c macrophages contributes to better understanding of its role and regulation in these cells.
General significance: Recognition of distinct galectin-1 and galectin-3 expression profiles in differently activated macrophages provides a new insight on biological characteristics of these cells and sheds a new light of galectin-3 as a modulator of individual macrophage subset. This article is part of a Special Issue entitled Glycoproteomics.

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1. Introduction

Macrophages are crucial regulatory cells of host defense and tissue homeostasis. Characterized by a high level of plasticity, macrophages are able to modulate their biological functions in the response to a wide range of microenvironmental stimuli. In general, two main subtypes of macrophages have been recognized according to the cytokine production profile and the expression of surface receptors; macrophages differentiated from monocytes by the exposure to granulocyte macrophage colony-stimulating factor (GM-CSF) named M1 and those exposed to macrophage colony-stimulating factor (M-CSF), named M2. M1 macrophages could be additionally activated by the exposure to IFN-γ and/or LPS yielding so-called classically activated macrophages, while activated M2 macrophages were consequently named alternatively activated macrophages [1–3]. While M1 macrophages mediate resistance to intracellular pathogens, tissue destruction, and anti-tumor resistance, M2 macrophages are generally oriented to tissue remodeling and repair, resistance to parasites, immunoregulation, and tumor promotion [4]. By more precise distinction, based on the biochemical characteristics and biological functions, three different subtypes of activated M2 macrophages have been recognized: (i) M2a or alternatively activated macrophages by IL-4 or IL-13, (ii) M2b or Type II, activated by glucocorticoids or immune complexes and (iii) M2c activated by IL-10, also called true deactivated macrophages [5].

Galectin-1 (Gal-1) (~14.5 kDa) and galectin-3 (Gal-3) (~32 kDa), most studied members of the β-galactoside binding lectins, have been implicated in numerous biological processes and play a significant role in many aspects of monocyte-macrophage cell biology. Though structurally distinct (Gal-1 has one carbohydrate recognition domain (CRD), while Gal-3 besides CRD possesses additional, N-terminal non-lectin domain), both galectins can be present intracellularly, secreted

Abbreviations: Gal-1, galectin-1; Gal-3, galectin-3

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by an unknown mechanism outside of the cells or bound on cell-surface receptors or extracellular matrix ligands. Inside the cells they bind their ligands presumably through protein–protein interaction, while binding of cell-surface ligands initiates a dimerization of Gal-1 and oligomerization of Gal-3 and triggering a cascade of, so far mostly known, transmembrane signaling events [6,7].

Gal-1 inhibits arachidonic acid release [8], blocks nitric oxide synthesis and increases arginase activity, suggesting a role for this protein in triggering a state of alternative activation in cells of the monocyte/macrophage lineage [9]. Accordingly, Gal-1 treatment inhibits IFN-γ-induced FcγRI-dependent phagocytosis and major histocompatibility complex (MHC) II expression in human monocytes and macrophages [10] and blocks IL-12 secretion from parasite-infected macrophages [11]. It was also suggested that Gal-1-glycan lattices may have evolved to negatively regulate the antigen-presenting function and activation of monocytes/macrophages [12].

On the other hand, Gal-3 was shown to modulate the production of some cytokines, such as IL-1 [13], IL-5 [14] and IL-8 [15] in the cells of monocyte-macrophage lineage, as well as in some other cell types [16–18]. Gal-3 also induces Ca^{2+} influx in monocytes [19], acts as a chemoattractant for monocytes, macrophages and endothelial cells [20], enhances macrophage clearance of apoptotic neutrophils [21], regulates alternative macrophage activation [22] and participates in many other processes (reviewed in [23–25]).

However, the role and regulation of Gal-1 and Gal-3 expression during differentiation of human monocytes into particular macrophage subsets and their activation is mainly unknown. To provide better insight into biology of differently activated macrophages on one hand, and Gal-1 and Gal-3 on the other. In this study we determined the intra- and extracellular expression profiles of Gal-1 and Gal-3 in classically (M1) and alternatively (M2a/M2c) activated macrophages obtained by differentiation/activation of human blood monocytes *in vitro*.

2. Material and methods

2.1. Material

All chemicals were of analytical grade, and if not stated otherwise were purchased from Sigma (St. Louis, MO, USA). Isopropyl-β-D-thiogalactoside (IPTG) was from Fermentas (St. Leon-Rot, Germany), complete EDTA-free Protease Inhibitor Cocktail Tablets from Roche (Basel, Switzerland), Immobilon PVDF-membranes from Millipore Corp. (Bedford, MA, USA), Ficoll-Paque Plus and enhanced chemiluminescence (ECL) Western blot detection kit from Amersham Biosciences (Cardiff, UK), while RPMI 1640 medium, fetal bovine serum (FBS) and Antibiotic-Antimicotic were from Gibco (Grand Island, NY, USA). Wizard Genomic DNA Purification Kit was purchased from Promega (Madison, WI, USA), recombinant human (rh)-M-CSF, rh-GM-CSF, rh-IFN-γ, rh-IL-10 and Alexa Fluor® 488-labeled annexin V and 7-aminoactinomycin D (7-AAD) from Invitrogen (Carlsbad, CA, USA). Goat anti-human Gal-1, rh-IL-4 and Quantikine® Human Gal-3 Immunoassay were from R&D Systems (Minneapolis, MN, USA), and horseradish peroxidase (HRP)-labeled goat anti-rat IgG were purchased from Calbiochem (St. Louis, MO, USA) and goat anti-histone H2B, HRP-labeled donkey anti-goat IgG and fluorescein isothiocyanate (FITC)-labeled rabbit anti-goat IgG from Santa Cruz Biotechnology (Columbia, SC, USA). Phycoerythrin (PE)-labeled mouse anti-human CD14, PE-labeled mouse anti-human CD163, FITC-labeled mouse anti-human CD206 and PE-labeled mouse anti-rat IgG were from BD (Franklin Lakes, NJ, USA), human Th1/Th2 11plex FlowCytomix Kit and Accutase enzyme cell detachment medium from eBioscience (San Diego, CA, USA), RNeasy® Mini Kit was from Qiagen (GmbH, Hilden, Germany). Hybridoma cell line TIB-166 that produces rat monoclonal anti-human Gal-3 antibodies (M3/38) and human acute T-cell leukemia cell line Jurkat were purchased from ATCC (Manassas, VA, USA), EndoTrap Red from Hyglos GmbH (Bernried am Starnberger See, Germany) and Limulus Amebocyte Lysate (LAL) Pyrogent Plus Single Test Kit was purchased from Lonza (Walkersville, MD, USA). All chemicals and labware used in the experiments were endotoxin free.

2.2. Cell culturing and treatment

Buffy coats were obtained from adult healthy donors who all signed informed consent based on the Helsinki declaration. This study was approved by the Ethic Committees of the Croatian Institute for Transfusion Medicine in Zagreb, where all samples were collected, and by the Ethic Committee of the Faculty of Pharmacy and Biochemistry, University of Zagreb. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-paque gradient centrifugation. To obtain monocytes, PBMCs were plated for 2 h in culture medium (RPMI 1640, supplemented with 10% FBS, 100 U/mL penicillin G, 100 μg/mL streptomycin-sulfate, 250 ng/mL amphotericin B and 2.5 μg/mL plasmocin) under standard conditions. Non-adherent cells were rinsed away with phosphate buffered saline (PBS). Adherent cells were ≥90% pure viable monocytes as determined by light scatter profile, CD14 expression and viability dye exclusion (as described in Section 2.4).

Monocytes were plated at a density of 5×10^5 cells/mL and cultivated for 7 days in culture medium supplemented with 1 ng/mL of rh-GM-CSF or 10 ng/mL of rh-M-CSF to induce differentiation into M1 and M2 subtypes, respectively [26]. On the fourth day of cultivation, medium was replaced with fresh medium of the same content. To obtain classically activated macrophages (M1), on the sixth day culture medium was supplemented with 20 ng/mL of rh-IFN-γ and 100 ng/mL (200 U/mL) of LPS from *Escherichia coli* [27]. Similarly, to obtain alternatively activated macrophages, M2a and M2c, 20 ng/mL of rh-IL-4 or 10 ng/mL of rh-IL-10 were used, respectively [28]. To confirm the differentiation/activation of monocytes into M1 and M2a/M2c macrophage subtypes, cytokine secretion and surface marker (CD163 and CD206) expression profiling, as well as assessment of cell viability was performed (as described in Section 2.4).

Differentiated and activated macrophages were incubated for 24 h in fresh culture medium supplemented with 1 μM rh-Gal-3 and their cytokine secretion profiles were determined. Jurkat acute T-cell leukemia cell line was used to assure the biological activity of purified rh-Gal-3. The cells were asynchronously grown in culture medium and sub-cultured every 2–3 days to maintain cell concentrations between 0.1 and 1×10^6 cells/mL. Aliquots containing 5×10^5 of Jurkat cells/mL were treated for 6 h in culture medium supplemented with 15 μM rh-Gal-3 [29] and cell viability was assessed (as described in Section 2.4).

Following total DNA isolation using Wizard Genomic DNA Purification Kit, absence of mycoplasma in test cells was confirmed by PCR-analysis using MMB Mycoplasma PCR Kit (Merck) according to the manufacturer's instructions.

2.3. rh-Gal-3 production and purification

The *E. coli* strain BL21(DE3) star transformed with plasmid pET-3c containing Gal-3 coding sequence and ampicillin resistance, was a kind gift from Dr Hakon Leffler. Expression of Gal-3 in these bacteria was induced by incubation (3 h) with 0.5 mM IPTG. rh-Gal-3 was isolated from the lysate of induced bacteria on lactosyl-Sepharose column as described previously [30]. Solutions of rh-Gal-3 were additionally purified from residual bacterial endotoxins using EndoTrap Red resin, according to the manufacturer's instructions. The absence of endotoxins in the purified rh-Gal-3 was confirmed by the Limulus amebocyte lysate (LAL) test sensitive to 0.125 EU/mL.

2.4. Flow cytometry

The expression level of CD14 on monocytes, CD163 and CD206 on macrophages and the level of membrane-bound Gal-1 and Gal-3 on

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