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Neutrophil activation induced by the lectin KM+ involves binding to CXCR2

Gabriela Pereira-da-Silva [a], Andréa N. Moreno [a,b], Fabiana Marques [c], Constance Oliver [a], Maria Célia Jamur [a], Ademilson Panunto-Castelo [a], Maria Cristina Roque-Barreira [a,*]

[a] *Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, 14049-900, Ribeirão Preto, SP, Brazil*
[b] *Centro de Ciências Biológicas e da Saúde, Pontifícia Universidade Católica do Paraná, R. Imaculada Conceição, 1155, 80215-901, Curitiba, PR, Brazil*
[c] *Departamento de Clínica Médica, Divisão de Cardiologia, Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14048-900, Ribeirão Preto, SP, Brazil*

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Abstract

The lectin KM+ from *Artocarpus integrifolia*, also known as artocarpin, induces neutrophil migration by haptotaxis. The interactions of KM+ with both neutrophils and the extracellular matrix depend on the lectin's ability to recognize mannose-containing glycans. In the present study, we characterized the binding of KM+ to human neutrophils and the responses stimulated by this binding. Exposure to KM+ results in cell polarization, formation of a lamellipodium, and induction of deep ruffles on the cell surface. By fluorescence microscopy, we observed that KM+ is distributed homogeneously over the cell surface. KM+/ligand complexes are rapidly internalized, reaching maximum intracellular concentrations at 120 min, and decreasing thereafter. Furthermore, KM+ binding to the surface of human neutrophils is inhibited by the specific sugars, D-mannose or mannotriose. KM+-induced neutrophil migration is inhibited by pertussis toxin as well as by inhibition of CXCR2 activity. These results suggest that the KM+ ligand on the neutrophil surface is a G protein-coupled receptor (GPCR). The results also suggest that neutrophil migration induced by KM+ involves binding to CXCR2.

Keywords: Lectin; *Artocarpus integrifolia*; Neutrophil; Cell migration; CXCR2 receptor; Inflammation

1. Introduction

Neutrophils play a crucial role in inflammation and their accumulation within injured tissue is a hallmark of the acute inflammatory response. The movement of neutrophils from the circulation to the sites of injury is initiated and directed by numerous exogenous and endogenous attractants that bind and activate seven-transmembrane G-protein-coupled receptors (GPCR) [1–4]. Neutrophils stimulated by GPCR-binding attractants such as chemokines, C5a and N-formyl peptide, rapidly change their morphology from rounded cells covered with microvilli to elongated cells with ruffles. The activated cells also have pseudopodia that form broad lamellepodia that are extended anteriorly in the direction of the increasing concentration of attractant, whereas a contractile uropod is formed posteriorly. The result is a morphologically polarized cell that is efficient at vectorial migration [5–8].

Lectins are included among the multiple attractants that stimulate the neutrophil migration. Lectins are ubiquitous proteins that exhibit specific and reversible carbohydrate-binding activities [9]. Since lectins have a great versatility and diversity in binding to carbohydrates, they function as cell recognition mediators in a wide range of biological systems [10]. Studies have shown that many plant lectins can induce an inflammatory response characterized by dose-dependent plasma exudation and neutrophil migration in rats or mice [11–19].

Abbreviations: CRD, carbohydrate recognition domain; CXCR-1, CXC-chemokine receptor type 1; CXCR-2, CXC-chemokine receptor type 2; ECM, extracellular matrix; FACS, fluorescence activated cell sorter; FITC, fluorescein isothiocyanate; fMLP, formyl-methionyl-leucyl-phenylalanine; GCP-2, granulocyte chemotactic protein-2; GPCR, G protein-coupled receptor; IL-8, interleukin-8; MAPK, mitogen-activated protein kinase; MNCF, macrophage-derived neutrophil chemotactic factor; PAGE, polyacrylamide gel electrophoresis; PLC, phospholipase C; PTx, pertussis toxin; PBS, phosphate-buffered saline; S.E.M., standard error of the mean; SDS, sodium dodecyl sulfate; WGA, wheat germ agglutinin

* Corresponding author. Tel.: +55 16 3602 3062; fax: +55 16 3633 6631.
E-mail address: mcrbarre@fmrp.usp.br (M.C. Roque-Barreira).

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Indeed, lectin–carbohydrate interactions may trigger many neutrophil functions. Lectins with different carbohydrate specificities such as concanavalin A (mannose-specific), wheat germ agglutinin (WGA-specific for N-acetylglucosamine and sialic acid), selectins (specific for sialic acid containing structures, especially sialyl-Lewis^x), and some members of the galectin family (lactose-specific) have been shown to induce neutrophil responses [20–25]. Selectins and their counter-receptors were extensively studied as mediators of the initial tethering and rolling of neutrophils along the endothelium of the vessel wall [26] and the function of these molecules was thought to be restricted to regulating of cell–cell adhesive interactions. More recent studies demonstrate their role in intracellular signaling and leukocyte activation. Binding of selectin molecules on neutrophils initiates transmembrane signals that modulate specific functional roles, such as adhesion-dependent leukocyte functions, β_2-integrin activation, O_2^- production. Protein kinase-dependent pathway plays a key role in selectin-dependent leukocyte activation, which is illustrated by the fact that binding of L-selectin to sulfatides on the surface of neutrophils induces tyrosine phosphorylation of a variety of cellular proteins including MAP kinase, and PLCγ [27]. Other studies have reported that some cytotoxic functions also are initiated by lectins such as WGA [28], galectin-3 [24], and galectin-1 [25]. For members 1 and 3 of the galectin family, their increased binding to the neutrophil surface is associated with an enhancement of neutrophil response, which involves a mechanism dependent on granule mobilization [24,25]. Recently, it had been shown that galectin-3 increases the phagocytic activity of neutrophils, modulates neutrophil degranulation and influences neutrophil survival, probably through a mechanism involving the stimulation of p38 MAPK [29]. A plant lectin, *Sambucus nigra* agglutinin, is also reported as able to induce neutrophil degranulation, through a mechanism that apparently involves signaling systems dependent on G proteins and lipid metabolism [30].

In a previous study, we have demonstrated that a structurally well defined D-mannose-specific lectin from *Artocarpus integrifolia* seeds, KM+, also known as artocarpin, is able to induce neutrophil migration both in vivo and in vitro. Its carbohydrate recognition domain is directly responsible for the effect of inducing neutrophil migration, [12] which occurs by haptotaxis and is mediated by simultaneous interaction of the tetrameric lectin with their glycoligands on the extracellular matrix (ECM) and on the neutrophil surface [12,31]. We have recently identified laminin as being the ligand for KM+ on the ECM and have demonstrated that this interaction potentiates the KM+-induced neutrophil migration. The interaction of KM+ with laminin promotes formation of a substrate-bound gradient of KM+, which is necessary for the haptotatic movement of neutrophils that transmigrate through the vascular wall [32]. Although the tissue ligand for KM+ has been identified, its ligand on the neutrophil surface remains unknown.

In the present work, we demonstrate that KM+ interacts with glycans associated with a G-protein coupled receptor (GPCR), which corresponds to CXCR2. The responses triggered by the interaction of KM+ with its neutrophil ligand are comparable to those observed following the binding of well-known neutrophil attractants, indicating that the induction of neutrophil migration should be considered as one of the multiple activities attributed to lectins.

2. Materials and methods

2.1. Reagents

Biobond, cacodylate, Embed 812, fluormount-G, glutaraldehyde, osmium tetroxide, thiocarbohydrizide, and uranyl acetate were purchased from Electron Microscopy Sciences, Fort Washington, PA, USA. fMLP, paraformaldehyde, RPMI medium, and saponin were obtained from Sigma Chemical Company, St. Louis, MO, USA. Formaldehyde was purchased from Merck, Darmstadt, Germany, pertussis toxin from List Biological Laboratories, Campbell, CA, USA, recombinant human IL-8 from R&D Systems Inc., Minneapolis, MN, USA and sulfo-NHS-LC-biotin (sulfosuccinimidyl-6-(biotinamido) hexanoate) from Pierce Biotechnology, Rockford, IL, USA.

2.2. Sugars

D-Mannose and D-galactose were purchased from Sigma. Mannotriose (Manα1–3[Manα1–6]Man) was obtained from Dextra Laboratories Ltd. (Reading, UK).

2.3. Antibodies and conjugates

Anti-human CXCR-1 IgG_{2A} and anti-human CXCR-2 IgG_{2A} were obtained from R&D Systems. Streptavidin-FITC was purchased from Pierce Biotechnology.

2.4. Neutrophil purification

Heparinized human blood from healthy volunteers (USP Research Ethics Committee, 9618/2004) was layered on a neutrophil isolation medium (Cardinal Associates, Santa Fe, NM) density gradient and centrifuged at 400×*g* for 30 min. Neutrophils were washed by centrifugation and suspended in RPMI medium at a concentration of 10^6 cells/ml. Samples were subjected to hypotonic lysis to eliminate remaining erythrocytes. Resulting preparations were 98% pure, with a >95% viability as measured by trypan blue.

2.5. Purification and biotinylation of the lectin KM+

The lectin KM+ was purified by affinity chromatography as previously described by Santos-de-Oliveira et al. [12]. The lectin purity was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and protein concentration was determined by the method of Lowry et al. [33]. KM+ was biotinylated using sulfo-NHS-LC-biotin according to the manufacturer's recommendations.

2.6. Localization of the KM+–ligand complexes on the surface of human neutrophils

2.6.1. Flow cytometry

Human neutrophils (10^6 cells/ml) were incubated with biotinylated KM+ (0.05 mg/ml in PBS) for 30 min at 4 °C. For some of the assays, the lectin had been previously incubated with 2.5 to 10 mM mannotriose, 25 mM D-mannose or 25 mM D-galactose for 60 min at room temperature. After incubation with streptavidin-FITC for 30 min at 4 °C, cells were fixed with 1% formaldehyde and analyzed by FACS (Fluorescence Activated Cell Sorter, Becton-Dickinson Labware, Belford, MA). Results were analyzed using the software Cell Quest.

2.6.2. Fluorescence microscopy

Human neutrophils were placed on coverslips coated with Biobond, incubated with biotinylated KM+ (0.25 mg/ml in PBS) or with PBS alone at