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Lectin KM^+-induced neutrophil haptotaxis involves binding to laminin

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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 the extracellular matrix (ECM) and neutrophils depend on the lectin ability to recognize mannose-containing glycans. Here, we report the binding of KM^+ to laminin and demonstrate that this interaction potentiates the KM^+-induced neutrophil migration. Labeling of lung tissue by KM^+ located its ligands on the endothelial cells, in the basement membrane, in the alveolus, and in the interstitial connective tissue. Such labeling was inhibited by 400 mM D-mannose, 10 mM Manα1-3[Manα1-6]Man or 10 μM peroxidase (a glycoprotein-containing mannosyl heptasaccharide). Laminin is a tissue ligand for KM^+, since both KM^+ and anti-laminin antibodies not only reacted with the same high molecular mass components of a lung extract, but also determined colocalized labeling in basement membranes of the lung tissue. The relevance of the KM^+-laminin interaction to the KM^+ property of inducing neutrophil migration was evaluated. The inability of low concentrations of soluble KM^+ to induce human neutrophil migration was reversed by coating the microchamber filter with laminin. So, the interaction of KM^+ with laminin promotes the formation of a substrate-bound KM^+ gradient that is able to induce neutrophil haptotaxis.

Keywords: Lectin; *Artocarpus integrifolia*; Neutrophil haptotaxis; Extracellular matrix; Laminin; Haptotactic gradient

1. Introduction

Cell migration is crucial in a wide range of biological phenomena. This process is particularly important in inflammatory response since the leukocyte movement toward an injured tissue is essential for the host defence against infection. Leukocyte migration is a multistep process dependent on a sequence of ligand-receptor interactions. The early events of leukocyte rolling and its firm adhesion to the endothelial surface are well understood. They are mediated by selectins, integrins and their counter-ligands, i.e., mucin-like glycoproteins and intercellular adhesion molecule-1, -2 (ICAM-1, -2), respectively [1–4]. Homophilic interactions established by platelet–endothelial cell adhesion molecule-1 (PECAM-1) expressed on leuko-

Abbreviations: Ab, antibody; ANOVA, analysis of variance; BSA, bovine serum albumin; CRD, carbohydrate recognition domain; DIC, differential interference contrast; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; EHS, Elgelbreth–Holm–Swarm; fMLP, formyl-methionyl-leucyl-phenylalanine; ICAM, intercellular adhesion molecule; Ig, immunoglobulin; IL-8, interleukin-8; HRP, horseradish peroxidase; MCP-1, monocyte chemoattractant protein-1; 2-ME, 2-mercaptoethanol; MIP-1α, macrophage inflammatory protein-1α; MNCF, macrophage-derived neutrophil chemotactic factor; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PECAM-1, platelet–endothelial cell adhesion molecule-1; PMSF, phenylmethylsulfonyl fluoride; PVP, polyvinylpyrrolidone; S.E., standard error of the mean; SDS, sodium dodecyl sulfate

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cytes and endothelium cells are critically involved in leukocyte migration through the blood vessel wall [5]. Once the endothelial barrier is crossed, leukocytes are faced with the basement membrane and the underlying connective tissue, and they need to get in contact with the extracellular matrix (ECM) in order to keep on moving. Basement membrane is formed by a network of extracellular polymers predominantly consisting of laminin isoforms and type IV collagen [6]. The interactions responsible for the vectorial progression of leukocytes into the extravascular environment are poorly understood.

Leukocyte movement is driven by a positive gradient of cell attractant. While the attractant is soluble in chemotaxis, in haptotaxis it is bound to a substrate such as ECM components on the surface of endothelial cells, in the basement membrane, and in the connective tissue [7,8]. The demonstration of heparan sulfate-binding sites on some chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1) molecules has allowed one to infer that heparan sulfate proteoglycans on endothelial cells and in the ECM can capture chemokines and present them to leukocytes [9–14]. Despite some contradictory observations regarding this effect of proteoglycans on in vivo IL-8-induced neutrophil migration [15,16], it is thought that chemokines associated with ECM components modulate the movement of leukocytes through the perivascular tissue [14]. In the case of galectin-3, a β-galactoside-binding animal lectin that promotes migration of inflammatory cells [17] and adhesion of neutrophils to laminin [18], its interaction with laminin could be involved in the leukocyte migration through the ECM [19]. However, tissue ligands for attractants involved in neutrophil haptotaxis have not yet been clearly identified.

The migration of neutrophils can be stimulated by multiple attractant signals, such as IL-8, complement C5a, and bacterial *N*-formyl peptides [20–22]. In a previous study, we demonstrated that KM^+, a mannose-binding lectin from *Artocarpus integrifolia* seeds, also known as artocarpin, induces neutrophil migration through its carbohydrate recognition domain (CRD) [23]. In vitro assays showed that the KM^+-induced neutrophil migration occurs by haptotaxis rather than chemotaxis. Subsequent in vivo assays demonstrated a selective binding of subcutaneously injected ^{125}I-KM^+ to the blood vessels and the loose connective tissue of rat skin, which correlated with neutrophils adherence to the vascular endothelium and neutrophil infiltration in the perivascular tissue, respectively [24]. We postulated that KM^+-induced neutrophil haptotaxis occurs by simultaneous interactions of the lectin with a glycoprotein on the neutrophil surface and with ECM components [23,24]. In this way, KM^+ could interact with ECM components and mediate cell movement through the extravascular tissue, as has been suggested for chemokines [14,15]. Since some endogenous leukocyte attractants such as the macrophage-derived neutrophil chemotactic factor (MNCF) and galectin-3 exert this role through their CRD [25,17], the lectin KM^+ may provide us with an appropriate model to identify the molecular interactions responsible for neutrophil haptotaxis and may help us to better understand the mechanisms of neutrophil influx toward alveoli that occur in pulmonary infections. In the present study, we have identified laminin as a ligand for KM^+ in lung tissue, and we have demonstrated that laminin could act as a substrate capable of forming a haptotactic gradient of the attractant, enabling the driving of neutrophil movement through the extravascular tissue.

2. Materials and methods

2.1. Reagents

BSA (A7638), fMLP (F3506), PMSF (P7626), EDTA (E1644) and paraformaldehyde were purchased from Sigma Chemical Company, USA. Benzamidine and aprotinin were obtained from Calbiochem-Novabiochem, USA. Lowicryl K4M resin was purchased from Polysciences Europe GmbH (Eppelheim, Germany), glutaraldehyde from Electron Microscopy Sciences (USA), gelatin from Difco Laboratories, USA, Triton X-100 from New England Nuclear (USA), and nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) from Promega, USA. A chemiluminescent substrate for alkaline phosphatase (Lumi-Phos 530) was obtained from Life Technologies, USA.

2.2. Sugars and glycoproteins

Laminin purified from mouse Elgelbreth–Holm–Swarm tumor (L2020), D-mannose, D-galactose, methyl α-D-mannopyranoside and horseradish peroxidase (P8375) were purchased from Sigma. Sucrose was obtained from Merck (Darmstadt, Germany). Mannotriose (Manα1-3[Manα1-6]Man) was obtained from Dextra Laboratories (Reading, UK).

2.3. Antibodies and conjugates

Rabbit anti-laminin IgG (L9393) was obtained from Sigma, streptavidin–alkaline phosphatase conjugate (9542SA) from Life Technologies, neutravidin–alkaline phosphatase conjugate (31002) and biotinylated goat anti-rabbit IgG (31820) from Pierce Chemical Company, (USA), neutravidin Alexa 488 conjugate (A11232) and Alexa 594 goat anti-rabbit IgG conjugate (A11037) from Molecular Probes (USA), and streptavidin–gold, 10-nm gold particles (25269) from Electron Microscopy Sciences.

2.4. Purification and biotinylation of the lectin KM^+

The lectin KM^+ was purified by affinity chromatography as previously described by Santos-de-Oliveira et al. [23]. The lectin purity was analyzed by sodium dodecyl sulfate-