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Lysophosphatidic acid-induced IL-8 secretion involves MSK1 and MSK2 mediated activation of CREB1 in human fibroblast-like synoviocytes

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ABSTRACT

Lysophosphatidic acid (LPA) is a pleiotropic lipid mediator that promotes motility, survival, and the synthesis of chemokines/cytokines such as interleukin-8 (IL-8) and interleukin-6 by human fibroblast-like synoviocytes from patients with rheumatoid arthritis (RAFLS). In those cells LPA was reported to induce IL-8 secretion through activation of various signaling pathways including p38 mitogen-activated protein kinase (p38 MAPK), p42/44 MAPK, and Rho kinase. In addition to those pathways we report that mitogen- and stress-activated protein kinases (MSKs) known to be activated downstream of the ERK1/2 and p38 MAPK cascades and CREB are phosphorylated in response to LPA. The silencing of MSKs with small-interfering RNAs and the pharmacological inhibitor of MSKs SB747651A shows a role for both MSK1 and MSK2 in LPA-mediated phosphorylation of CREB at Ser-133 and secretion of IL-8 and MCP-1. Whereas CREB inhibitors have off target effects and increased LPA-mediated IL-8 secretion, the silencing of CREB1 with short hairpin RNA significantly reduced LPA-induced chemokine production in RAFLS. Taken together the data clearly suggest that MSK1 and MSK2 are the major CREB kinases in RAFLS stimulated with LPA and that phosphorylation of CREB1 at Ser-133 downstream of MSKs plays a significant role in chemokine production.

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1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that is characterized by chronic inflammation and severe destruction of joints leading to functional disability. The etiology of RA remains largely unknown, but includes the activation of synoviocytes and the recruitment of polymorphonuclear neutrophils. Once arrived at the inflamed synovial compartment, activated neutrophils release a variety of proteases, myeloperoxidase, lipid mediators such as prostaglandins and leukotrienes [1], and cytokines/chemokines including IL-1β [2], TNF-α [3], and IL-8 [4]. Ultimately, the inflammatory and hyperplastic synovial tissue invades and destroys the adjoining cartilage and bone [5].

Fibroblast-like synoviocytes (FLS) are prominent in RA pathogenesis. In the healthy joint, FLS are responsible for the synthesis of extracellular matrix proteins including collagen, fibronectin, hyaluronic acid, and other molecules that facilitate the lubrication and function of cartilage surfaces [6]. Under long-standing inflammatory conditions, activated FLS produce a large variety of cytokines, chemokines, and other inflammatory mediators that help to recruit and retain leukocytes, leading to ongoing inflammation and tissue destruction [7–9]. One such chemokine produced by FLS is CXC chemokine interleukin-8 (IL-8), a major chemoattractant for the migration of neutrophils into the synovium [10]. The expression of IL-8 is markedly upregulated in RA, and is associated with development of clinical signs of synovial inflammation [11]. In early RA enhanced IL-8 expression is observed in synoviocytes of the lining layer [12] whereas in established disease, both synoviocytes and macrophages at the cartilage-pannus junction show increased expression of IL-8 [13]. In an animal model of arthritis, neutralizing IL-8 antibodies protect

Abbreviations: ATX, autotaxin; CBP, CREB-binding protein; ERK, extracellular-signal regulated kinase; FLS, fibroblast-like synoviocytes; IL-8, interleukin-8; LPA, lysophosphatidic acid; LPA1, lysophosphatidic acid receptor 1; LPA3, lysophosphatidic acid receptor 3; MAPK, mitogen-activated protein kinase; MSK, mitogen- and stress-activated kinase; PI, propidium iodide; RA, rheumatoid arthritis; RAFLS, fibroblast-like synoviocytes from patients with rheumatoid arthritis; siRNA, small-interfering RNA; shRNA, short hairpin RNA.

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against leukocyte infiltration and tissue damage in the early phase of joint inflammation [10]. Moreover, in RA patients, treatments inhibiting IL-8 production resulted in decreased migration of neutrophils into the joint and diminished arthritis activity [14–16].

The bioactive lipid lysophosphatidic acid (LPA) has been reported to induce cell proliferation, migration, cytokine production, survival, and angiogenesis in many cell types [17]. LPA binds to specific G protein-coupled receptors (LPA1-6) to influence cell behaviour [17]. The majority of extracellular LPA is produced from lysophosphatidylcholine by autotaxin (ATX), a secreted lysophospholipase-D initially identified from melanoma cell lines [18]. Elevated levels of both ATX [19] and LPA [20] have been detected in synovial fluids from RA patients. Studies from our laboratory revealed that human RAFLS express three LPA receptors, LPA1-3 [19]. LPA stimulates significant production and secretion of IL-8 from human RAFLS [19]. In the mouse air pouch model, administration of LPA into the air pouch enhances the infiltration of neutrophils due to the production of KC [21], a murine homologue of IL-8. Although the signaling pathways implicated in LPA-induced IL-8 production have been reported in other cells types [22–25], a thorough understanding of the signaling pathways involved in LPA-induced IL-8 secretion in human FLS is still incomplete.

The nuclear transcription factor CREB is widely expressed and known to be activated by signaling events induced by growth factors [26]. LPA also stimulates CREB phosphorylation at Ser-133 in various cells types [27–32]. In addition to protein kinase A many other kinases, including mitogen- and stress-activated kinases (MSKs), can phosphorylate CREB at Ser-133 [33]. MSKs are activated downstream of MAPKs. The structure of MSKs includes two kinase domains connected with a linker region and a C-terminal docking domain which assures binding to the activated extracellular-signal regulated kinase (ERK) and p38 MAPKs. In the case of human MSK1, Ser-360, Thr-581 and Thr-700 are phosphorylated by these MAPKs [34]. Among the residues autophosphorylated by activated MSK Ser-376 is essential for phosphorylation of MSK1 substrates. The activation of MSK2 seems to be very similar to that of MSK1 [35,36]. Because MSKs can be phosphorylated by both ERK and p38 MAPKs, they are activated by many physiological and pathological stimuli. For instance, MSKs are able to integrate signals induced by growth factors, pro-inflammatory cytokines, and cellular stresses [37], as well as those induced by LPA [32,38].

We previously highlighted in RAFLS a role for p42/44 MAPK and p38 MAPK in LPA-mediated IL-8 secretion [19]. To gain insight into the mechanism by which LPA-triggered signal transduction cascades in RAFLS lead to IL-8 secretion we monitored the activation of MSKs and CREB and assessed the contribution of MSK and CREB protein isoforms to chemokine synthesis. Here, we provide the first evidence that LPA promotes IL-8 and MCP-1 secretion through both MSK1 and MSK2 mediated phosphorylation of CREB in RAFLS.

2. Materials and methods

2.1. Reagents

1-Oleoyl-sn-glycero-3-phosphate (LPA) was purchased from Sigma-Aldrich Canada (Oakville, ON, Canada) and Avanti Polar Lipids (Alabaster, AL, USA). TNF-α was from PeproTech Inc. (Rocky Hill, NJ, USA). The Human Cytokine/Chemokine Luminex Multiplex Immunoassay kit was from Millipore Corporation (St. Charles, MO, USA). The Proteome Profiler™ Human Phospho-Kinase Array kit was purchased from R&D Systems Inc. (Minneapolis, MN, USA). Human IL-8 and MCP-1 ELISA kits were purchased from BioSource International Inc. (Camarillo, CA, USA) and R&D Systems Inc. (Minneapolis, MN, USA), respectively. Antibodies to human MSK1, MSK2, and phospho-MSK1 (Ser-376)/MSK2 (Ser-360) were from R&D Systems Inc. Antibodies to ERK, phospho-ERK, p38 MAPK, phospho-p38 MAPK, CREB, phospho-CREB (Ser-133), and phospho-MSK1 (Ser-360) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies to actin and the p85 subunit of PI 3-kinase were from Sigma-Aldrich Canada (Oakville, ON, Canada) and Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), respectively. MSK inhibitor SB747651A was from Axon Medchem (Groningen, The Netherlands). CREB inhibitors KG-501 and 217505 were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada) and Calbiochem (San Diego, CA, USA), respectively. The jetPRIME siRNA transfection reagent was from Polyplus Transfection Inc. (New York, NY, USA). MSK1/2 siRNAs and CREB1/2 shRNA lentiviral particles were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Cell culture reagents were purchased from Wisent Inc. (St-Bruno, QC, Canada). Propidium iodide (PI) was purchased from Invitrogen Canada (Burlington, ON, Canada). Accutase cell detachment solution was from eBioscience (San Diego, CA, USA). All other chemicals and reagents were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada).

2.2. Cell culture

Human primary FLS at passage 0 or 1 were purchased from Asterand (Detroit, MI, USA). Cells were obtained from RA patients who were diagnosed according to the criteria developed by the American College of Rheumatology (ACR) and were undergoing joint surgery on the knee or hip. Cells were maintained under standard conditions (37 °C and 5% CO_2) and grown in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU), and streptomycin (100 μM). Cells were used up to passage 9.

2.3. Cell treatment and viability

Semi-confluent cells were starved with serum-free medium for 24 h before treatment. At the moment of cell treatment, the culture medium was replaced with fresh serum-free medium containing various concentrations of the tested compounds as indicated in details below. Propidium iodide (PI) was used to evaluate the viability of RAFLS by flow cytometry. Cells were detached using Accutase cell detachment solution and incubated with PI (5 μg/ml). PI negative RAFLS were considered viable.

2.4. Multiplex Immunoassay

After starvation, cells were treated with 5 μM LPA for 24 h. Where indicated starved cells were pre-incubated with 80 ng/ml TNF-α for 8 h and washed three times with serum-free medium prior to stimulation with LPA. Cell culture supernatants were collected and human cytokines/chemokines IL-1α, IL-1β, IL-8, IL-15, Eotaxin, GM-CSF, IP-10, MCP-1, MIP-1α, MIP-1β, and RANTES were monitored using a Luminex Multiplex Immunoassay according to the manufacturer's instructions. The dynamic range of the assay is 3.2-10000 pg/ml.

2.5. Proteome Profiler™ Human Phospho-kinase Array

After starvation for 24 h, cells were treated with 5 μM LPA for the indicated times. Cells were then lysed and the cell lysate was applied to the array kit. Changes in the levels of phosphorylated proteins in response to LPA were assayed using the Proteome Profiler™ Human Phospho-kinase Array following the recommended protocol.

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