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The dual regulation of substance P-mediated inflammation via human synovial mast cells in rheumatoid arthritis

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Abbreviations:
Ab, antibody; EIA, enzyme immnoassay; IL, interleukin; IMDM, Iscove's modified Dulbecco's medium; IQR, inter-quartile range; LSM, lymphocyte separation medium; m, monoclonal; MCs, mast cells; MrgX2, Mas-related gene X2; NK-1R, neurokinin receptor 1; NP, neuropeptide; OA, osteoarthritis; PG, prostaglandin; RA, rheumatoid arthritis; rh, recombinant human; SCF, stem cell factor; SP, substance P; TAC, tachykinin; TLCK, N-α-Tosyl-L-lysine chloromethyl ketone; TNF, tumor necrosis factor

ABSTRACT

Background: Neural pathways are thought to be directly involved in the pathogenesis of rheumatoid arthritis (RA). Although synovial mast cells (MCs) are activated by substance P (SP), the role of MCs in neural pathways in RA remains unknown. The aims of this study were to investigate 1) whether tachykinins are produced by synovial MCs and whether production differs in RA and osteoarthritis (OA) patients, and 2) what is the responsible receptor for SP in synovial MCs.
Methods: Synovial tissues were obtained from patients with RA or OA undergoing joint replacement surgery. Cultured synovium-derived MCs were generated by culturing dispersed synovial cells with stem cell factor. SP expression was investigated using immunofluorescence and enzyme immunoassays. Mas-related gene X2 (MrgX2) expression was reduced in human MCs using a lentiviral shRNA silencing technique.
Results: SP expression was localized around the cell membrane in 41% (median) of the MCs in synovium from RA but in only 7% of that from OA, suggesting the activation of MCs. Synovial MCs expressed tachykinin (TAC) 1 mRNA, the expression of which was upregulated by the aggregation of FcεRI or the addition of aggregated IgG. However, the released SP appeared to be rapidly degraded by MC chymase. Synovial MCs were activated with SP through MrgX2 to release histamine without producing proinflammatory cytokines.
Conclusions: Activated synovial MCs may rapidly degrade SP, which may downregulate the SP-mediated activation of synoviocytes in RA. On the other hand, SP activates MCs to induce inflammatory mediators, suggesting the dual regulation of SP-mediated inflammation by MCs in RA.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by the immune cell-mediated destruction of the joint architecture. Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-17A, have been assigned pivotal roles in the destruction of the joint architecture in patients with RA.[1] In addition, neural pathways are thought to be directly involved in the pathogenesis of RA. Substance P (SP), the transcript of which is *tachykinin* (*TAC*) *1*, is a

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peptide neurotransmitter of some unmyelinated primary afferent nociceptors that is released from the peripheral terminals of primary afferent fibers.[2–4] SP may play an important role not only in pain, but also in inflammation. SP has a number of pro-inflammatory effects that are mediated by neurokinin-1 receptor (NK-1R) in peripheral tissues.[5–7]

Human TAC peptide genes consist of 3 genes: TAC1, TAC3, and TAC4.[4] SP, NKA, neuropeptide (NP) K, and NPγ are encoded by the TAC1 gene. The TAC1 gene generates a pre-mRNA that is spliced, giving rise to four different mRNA isoforms (α, β, γ, and δ). The SP precursor sequence is synthesized from all four isoforms, whereas the NKA sequence is only present in β and γTAC1 mRNAs, the NP-γ sequence is only present in γTAC1 mRNA, and the NPK sequence is only encoded by the βTAC1 isoform. The transcripts of NKB and hemokinin-1 are *TAC3* and *TAC4*, respectively.[4]

Evidence of the involvement of SP in experimental arthritis models has been obtained: (I) SP-positive$^{(+)}$ nerve fibers are found in the lining cell layer with some fibers branching towards the synovial space in rats[8]; (II) an increased level of SP is observed in rat adjuvant arthritis[9]; (III) exogenous SP increases the severity of adjuvant-induced arthritis in rats[10,11]; and (IV) the severity of joint inflammation or pain is reduced by NK-1R antagonists.[10–13] In human RA, some evidence of a role of SP also exists: (I) an increased level of SP has been observed in synovial fluid from patients with RA[14–19]; (II) SP^{+} nerve fibers are found in the synovium[20–22]; and (III) SP stimulates collagenase release and prostaglandin (PG) E_2, IL-6, and IL-8 production by synoviocytes from patients with RA, and SP enhances synoviocyte proliferation[5,7] and induces the expression of vascular cell adhesion molecule-1 on synoviocytes from RA patients.[6]

In patients with RA, the number of degranulated mast cells (MCs) is increased in synovial tissue and is correlated with the disease activity.[23–26] The amounts of MC mediators, such as histamine and tryptase, in synovial fluids are also increased in these patients, suggesting that the activation of MCs may be involved in the pathogenesis of RA.[24,26–28] We recently reported that aggregated IgG activated human synovial MCs through FcγRI and FcγRII[29] and that SP has a stimulatory effect on human synovial MCs, causing degranulation.[29] Recently, mouse bone marrow-derived MCs (BMMCs) were reported to exhibit an upregulation of TAC4 mRNA expression following the aggregation of FcεRI and to produce hemokinin-1 without affecting TAC1 mRNA expression and SP production.[30] Mas-related gene X2 (MrgX2) is reportedly a G-protein-coupled receptor for basic molecules, including SP, cortistatin, and somatostatin, on human cord blood-derived cultured MCs[31] and human skin MCs.[32] However, whether synovial MCs are potential sources of tachykinins remains unknown. Therefore, we hypothesized that (I) in addition to nerve fibers, MCs may produce tachykinins in synovia from patients with RA; and (II) some SP receptor other than NK-1R may play an important role in the pathogenesis of RA.

Methods

Ethical considerations

This study was approved by the Ethics Committee of the Nihon University School of Medicine, and all the subjects provided written informed consent in accordance with the Helsinki Declaration of the World Medical Association.

Reagents

SP, a NK-1R antagonist (PC-96345), and calcium ionophore A23187 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Human brain cDNA was purchased from BioChain Institute (Newark, CA, USA). The following antibodies (Abs) were purchased from the indicated sources: anti-human SP mAb (clone 266815; R&D Systems, Minneapolis, MN, USA), anti-human MrgX2 mAb (clone 477533; R&D Systems); anti-human MrgX2 polyclonal Ab (Abcam, Cambridge, UK); biotinylated anti-FcεRIα mAb (clone CRA1; eBioscience, San Diego, CA, USA), and anti-human tryptase mAb (clone AA1; DakoCytomation, Carpinteria, CA, USA).

Generation of cultured synovium-derived MCs

Human cultured synovium-derived MCs were generated as described previously.[29] Briefly, fresh samples of synovial tissues were obtained after total knee arthroplasty at the Nihon University, after obtaining informed consent. The synovial cells were enzymatically dispersed and centrifuged using a density-gradient consisting of 22.5% HistoDenz solution (Sigma–Aldrich) and lymphocyte separation medium (LSM: Organon Teknika, Durham, NC, USA). The cells at the LSM interface and in the pellet fraction were collected and washed. The cells were then cultured in serum-free Iscove's methylcellulose medium (Stem Cell Technologies, Vancouver, BC, Canada) and Iscove's Modified Dulbecco's Medium (IMDM; Invitrogen, Grand Island, NY, USA) supplemented with 100 ng/mL of recombinant human stem cell factor (rhSCF) (PeproTech, Rocky Hill, NJ, USA) and 50 ng/mL of rhIL-6 (PeproTech). On day 42, methylcellulose was dissolved in PBS, and the cells were resuspended and cultured in IMDM containing 0.1% BSA, 100 ng/mL of rhSCF, and 50 ng/mL of rhIL-6.

RNA isolation and RT-PCR

Total RNA was isolated from the cultured synovium-derived MCs, and real-time quantitative RT-PCR was performed as described previously.[29] Human gene-specific primers and probe sets for TAC1, TAC3, TAC4, MrgX2, IL-6, IL-8, osteopontin, and GAPDH were designed using the Assay-on-Demand service (Applied Biosystems, Tokyo, Japan). The primers used for RT-PCR were as follows: TAC1, sense primer (5′-CGACAGCGACCAGATCA AGGAGG-3′) and antisense primer (5′-TGCATTGCACTCCTTTCAT-3′); TAC3, sense primer (5′-AGCTTTGGGGCTGTCTGTAA-3′) and antisense primer (5′-CCCATAAGTCCCACAAAGAAG-3′); and TAC4, sense primer (5′-TTCTCCTGATGGAGCTGTCC-3′) and antisense primer (5′-CCCAGGTCTCTGCTTCAGTG-3′). The TAC1 primer pair allowed the α, γ, and β transcripts of the TAC1 gene (which give rise to PCR products of 210, 219, and 264 bp, respectively) to be discriminated.[33] The TAC1 gene comprises seven exons and is transcripted into three different mRNAs as a result of alternative splicing.

Preparation of aggregated IgG

Aggregated IgG was prepared as described previously.[29] Briefly, aggregated IgG was prepared by heating human IgG (Jackson Immune Laboratory, West Grove, PA, USA) at 63 °C for 1 h. Large aggregates were removed by centrifugation at 10,000 ×*g* for 30 min. The aggregated IgG that was used for the experiments was present in the supernatant.

MC activation

For the aggregation of FcεRI, MCs were sensitized with 0.5 μg/mL of human IgE polyclonal Ab (Calbiochem, San Diego, CA, USA) for 30 min at 37 °C. The cells were washed once and resuspended in IMDM containing 0.1% BSA, 100 ng/mL of rhSCF, and 50 ng/mL of rhIL-6. The IgE-sensitized MCs were stimulated with anti-human IgE (DakoCytomation) for the indicated time periods for total RNA