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The expression of IL-20 and IL-24 and their shared receptors are increased in rheumatoid arthritis and spondyloarthropathy

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Abstract

The purpose of this study was to analyze the expression of the two proinflammatory cytokines IL-20 and IL-24 and their shared receptors in rheumatoid arthritis and spondyloarthropathy. IL-20 was increased in plasma of rheumatoid arthritis patients compared with osteoarthritis patients and IL-24 was increased in synovial fluid and plasma of rheumatoid arthritis and spondyloarthropathy patients compared with osteoarthritis patients. IL-20 and IL-24 mRNA was only present at low levels in the synovium. In the synovial membrane, IL-20 protein was present in mononuclear cells and neutrophil granulocytes whereas IL-24 protein was observed in endothelial cells and mononuclear cells. IL-20 receptor type 1 and IL-22 receptor were expressed by granulocytes in the synovial fluid. In synovial fluid mononuclear cell cultures, stimulation with recombinant human IL-20 or recombinant human IL-24 induced monocyte chemoattractant protein 1 (CCL2/MCP-1) secretion, but not tumour necrosis factor α mRNA synthesis or IL-6 secretion. Both IL-20 and IL-24 showed correlations to CCL2/MCP-1 in plasma from rheumatoid arthritis and spondyloarthropathy patients. This study associates IL-20 and IL-24 to the synovium of rheumatoid arthritis and spondyloarthropathy and results indicate that the two cytokines contribute to disease pathogenesis through recruitment of neutrophil granulocytes and induction of CCL2/MCP-1.

Keywords: IL-20; IL-24; Arthritis

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory disease that causes progressive joint damage and disability. In this inflammatory process, cytokines including IL-1, IL-6, IL-8, IL-10, monocyte chemoattractant protein 1 (CCL2/MCP-1), and tumour necrosis factor (TNFα) play a prominent role [1–3]. Spondyloarthropathy (SpA) comprises a group of related diseases characterized by spinal inflammation and peripheral joint oligoarthritis often including cutaneous manifestations. The role of cytokines is better characterized in RA than in SpA. However, TNFα blockers have been shown to be beneficial in the treatment of both psoriatic arthritis and ankylosing spondylitis suggesting a role for cytokines in the pathogenesis of these diseases [4–7].

IL-20 and IL-24 are two recently identified members of the IL-10 family of cytokines [8]. IL-20 expression has predominantly been described in monocytes [9], and keratinocytes [10], whereas IL-24 expression has primarily been found in macrophages [11,12], monocytes, T cells [9], and keratinocytes [10]. IL-20 and IL-24 signal through the receptor complexes IL-20 receptor type 1 (IL-20R1)/IL-20 receptor type 2 (IL-20R2) and IL-22 receptor (IL-22R)/IL-20R2 [13,14].

IL-20 has primarily been associated with psoriasis, as IL-20 expression in transgenic mice has been shown to

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cause skin abnormalities very similar to those observed in psoriasis [15]. Furthermore, the mRNA of IL-20 and of its three receptor subunits have been found in lesional psoriatic skin [16–18]. IL-24, also known as melanoma differentiation-associated gene 7, was discovered due to its ability to induce apoptosis in melanoma cells [19]. Furthermore, IL-24 has been shown to be expressed at increased levels in psoriasis [16].

The involvement of IL-20 and IL-24 in rheumatic diseases is not clarified. IL-20 and its three receptor subunits have been found in the synovial membrane of patients with RA [20,21]. IL-20 has also been shown to induce cytokine secretion in synovial fibroblasts, neutrophil chemotaxis, synovial fibroblast migration, and endothelial cell proliferation. Interestingly, the soluble IL-20R1 has been found to decrease the severity of collagen induced arthritis in rats suggesting that cytokines, which bind this receptor, are important in the pathogenesis of arthritis [20].

In this study, synovial fluid, plasma, and synovial membranes from RA and SpA patients were studied. Osteoarthritis (OA) patients served as non-inflammatory disease controls while healthy volunteers were included as normal controls. IL-20 and IL-24 levels were measured, the cellular sources and targets of IL-20 and IL-24 were studied and the effect of IL-20 and IL-24 on cytokine production was analyzed. This study provides new insight into the role of IL-20 in rheumatic diseases and presents the first association between IL-24 and these diseases.

2. Materials and methods

2.1. Patients and samples

Synovial fluid and plasma were collected from patients with RA ($n = 24$) and SpA ($n = 22$). Subgroups included under the category SpA were psoriatic arthritis ($n = 11$), reactive arthritis ($n = 2$), enteropathic arthritis associated with inflammatory bowel disease ($n = 2$), and undifferentiated spondyloarthritis ($n = 7$). The inclusion criteria were the requirement of therapeutic arthrocenthesis and the absence of anti-TNFα treatment. Patients with RA and OA were diagnosed in accordance with the criteria established by the American College of Rheumatology (ACR) [22,23], patients with SpA were classified according to the European Spondyloarthropathy Study Group (ESSG) criteria [24], and psoriatic arthritis patients were diagnosed according to the CASPAR criteria [25]. OA patients ($n = 15$) were included to serve as non-inflammatory disease controls while normal healthy volunteers ($n = 22$) were also included to serve as controls. Synovial fluid and plasma were collected from patients with OA while only plasma was collected from normal healthy volunteers. Patient characteristics are listed in Table 1. Synovial fluid was collected during therapeutic arthrocenthesis, transferred to tubes containing EDTA, centrifuged and frozen. EDTA blood samples were collected in continuation of the therapeutic arthrocenthesis and plasma was harvested following centrifugation and frozen.

Table 1
Characteristics of patients with RA, SpA, and OA and normal healthy volunteers (NHV) included for synovial fluid and/or plasma analysis

Characteristic	Patients with RA ($n = 24$)	Patients with SpA ($n = 22$)	Patients with OA ($n = 15$)	NHV ($n = 22$)
Age, mean years	55.3	44.6	71.1	41.1
Sex, no. of females	21	18	8	11
CRP, mean concentration (nmol/l)	278	128	<75	ND
Rheumatoid factor positive, no. of patients	14	0	0	ND
Medications received, no. of patients				
None	9	8	15	22
DMARD	15	14	0	0
Methotrexate	14	7	—	—
Salazopyrine	6	6	—	—
Cloroquine	3	0	—	—
Cyclosporine A	1	1	—	—
Penicillamine	0	1	—	—

ND, not determined.

Synovial membranes were collected from a group of patients in connection with arthroplasty. Immunohistochemical staining was performed on samples from eight patients with RA (age, 62.9 mean years; sex, 7 females) and five patients with OA (age, 68.2 mean years; sex, 3 females).

All samples were obtained after informed consent according to the Danish Data Protection Agency, the Local Ethical Committee (Project Nos. 20050046 and 20060012) and the Declaration of Helsinki.

2.2. ELISA

To quantify the concentration of IL-20 the Human IL-20 ELISA Development Kit from PeproTech EC (UK) was used according to the manufacturer's recommendations. To quantify the concentration of IL-24 Maxisorp 96 well flat-bottom plates (NUNC) were coated with 5 μg/ml mouse IgG2b anti-IL-24 (R&D, USA, MAB1965). Plates were then blocked with 1% BSA. Serial dilutions of recombinant human IL-24 (R&D, 1965-IL-025) and samples were added in duplicates followed by the addition of 1 μg/ml goat IgG anti-IL-24 (R&D, AF1965). For detection, mouse IgG anti-goat HRP (Jackson, USA, 205-035-108) was added. The reaction was developed by incubating with ABTS substrate (Sigma–Aldrich, USA). The OD was read at 405 nm with a reference reading at 630 nm. The minimum detectable concentration of human IL-24 for this ELISA system was 0.4 ng/ml. Rheumatoid factor in patient samples did not seem to cross link capture and detection antibodies in the ELISA systems as low levels of cytokines were detected