Expression of IL-19 and its receptors in RA: potential role for synovial hyperplasia formation

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Objective. IL-19 is a novel cytokine of the IL-10 family. In this study, we sought to examine whether IL-19 plays a role in the pathogenesis of RA.

Methods. Expression of IL-19, IL-20 receptor 1 (IL-20R1) and IL-20R2 was examined by RT-PCR and immunohistochemical analysis in rheumatoid synovium. The effects of IL-19 on synovial cells established from rheumatoid synovium (RASCs), with regard to IL-6 production and signal transducers and activators of transcription3 (STAT3) activation, were examined by ELISA and western blot analysis, respectively. The effect of IL-19 on RASC apoptosis was examined by Hoechst staining, flow cytometry analysis of annexin V binding and caspase-3 activity.

Results. IL-19, IL-20R1 and IL-20R2 mRNA were detected by RT-PCR in synovial tissues from RA patients. Immunohistochemical analysis showed IL-19 was predominantly expressed in the hyperplastic lining layers of RA synovial tissues. The majority of IL-19-positive cells were vimentin-positive and CD68-positive synovial cells, serving as markers of fibroblasts and macrophages, respectively. IL-20R1 and IL-20R2 (IL-20Rs) were expressed in both the lining and sublining layers of RA synovium. In RASC, IL-19 was induced by lipopolysaccharide stimulation and constitutive expression of IL-20Rs was observed, suggesting IL-19 has an autocrine action. In terms of this function, IL-19 induced STAT3 activation and increased IL-6 production by RASC above the medium control. Moreover, IL-19 significantly reduced RASC apoptosis induced by serum starvation.

Conclusions. These data suggest that IL-19, produced by synovial cells, promotes joint inflammation in RA by inducing IL-6 production and decreasing synovial cell apoptosis.

Key words: Rheumatoid arthritis, Interleukin-10 family, Interleukin-19, Synovial hyperplasia, CD68, Vimentin, Synovial cells, STAT3, Interleukin-6, anti-apoptosis.

Introduction

RA is an immune-mediated, inflammatory joint disease characterized by leucocyte invasion and synovial cell activation followed by cartilage and bone destruction [1]. It is now well established that pro-inflammatory cytokines, such as TNF-α and IL-1, are involved in the inflammatory process which is partly counterbalanced by the anti-inflammatory mediators, such as IL-10, soluble TNF receptors and the IL-1 receptor antagonist (IL-1Ra) [2, 3]. Today, biologic agents such as anti-TNF-α antibodies and anti-TNF-α receptor antagonists are widely used for RA therapy. However, many patients do not respond to such anti-cytokine therapy, indicating disease heterogeneity. Different cytokines may play a dominant role in these patients. Therefore, continued efforts should be made to search for any novel cytokines that are critically involved in RA pathogenesis.

IL-19 is a novel cytokine from the IL-10 family, which includes IL-10, IL-19, IL-20, IL-22, melanoma differentiation-associated gene (MDA)-7, IL-24) and AK155 (IL-26). IL-19 was originally cloned by searching EST databases for IL-10 homologues and its mRNA expression was principally detected in human monocytes under basal conditions [4]. Low levels of IL-19 mRNA were detected in B cells, but not in T cells [5]. Activation of monocytes with lipopolysaccharide (LPS) or GM-CSF resulted in induction of IL-19 [4]. Later, Hsing et al. [6] confirmed that IL-6, TNF-α and IFN-γ also induced IL-19 in human monocytes.

The functional receptor for IL-19 has been identified as a heterodimer of IL-20 receptor 1 (IL-20R1) and IL-20R2, through which IL-19 induces the activation of signal transducers and activators of transcription3 (STAT3) [7]. A previous study demonstrated that both IL-20R1 and IL-20R2 genes are highly expressed in normal skin, testis and lung [8]. IL-20R1 and IL-20R2 were immunohistochemically stained on basal and suprabasal keratinocytes in healthy skin and up-regulated in psoriatic skin [9].

With regards to function, Liao et al. [10] were the first to report that IL-19 up-regulated the production of IL-6 and TNF-α in monocytes, indicating the pro-inflammatory property of this cytokine. In contrast, some studies have reported that IL-19 is anti-inflammatory. For example, IL-19 increased IL-10 production in peripheral blood mononuclear cells (PBMCs) [11], while long-term exposure of T cells to IL-19 down-regulated IFN-γ and up-regulated IL-4 and IL-13 [12]. These observations suggest that IL-19 plays an important role in the immune system.

Recently, we and others have reported that IL-10 family cytokines, IL-20 and IL-22, were involved in the pathogenesis of RA [13, 14]. However, the pathophysiological function of IL-19 in autoimmune arthritis is not clarified. In this study, we sought to examine whether IL-19 plays a role in the pathogenesis of RA. Therefore, our aim is to analyse the expression of IL-19 using synovial samples derived from RA patients. We further examined the effects of recombinant IL-19 (rIL-19) on synovial cells established from rheumatoid synovium (RASCs). Our data suggest that IL-19, as produced by synovial cells, promotes joint inflammation in RA by inducing IL-6 production and decreasing synovial cell apoptosis.

Patients and methods

Antibodies and cytokines

Mouse anti-human IL-19 mAb and goat anti-human IL-19 polyclonal antibodies (pAbs), mouse anti-human IL-20R1 mAb,
goat anti-human IL-20R2 pAb, recombinant human IL-19, TNF-α and IL-6 were purchased from R&D Systems (Minneapolis, MN, USA). Mouse anti-vimentin mAb (clone V9) was from Neomarkers (Fremont, CA, USA). Mouse anti-CD3 mAb and Histofine simple stain MAX PO (MULTI and G), were from Nichirei (Tokyo, Japan). Mouse anti-CD20 and anti-CD68 mAb were from Dako (Glostrup, Denmark). Rabbit anti-phospho-STAT3 (Tyr705) and anti-STAT3 antibodies were purchased from Cell Signaling Technology (Frankfurt, Germany). ALP-conjugated anti-rabbit IgG was from Promega (Madison, WI, USA). Alexa Fluor® 488-conjugated rabbit anti-goat IgG, 594 rabbit anti-goat IgG and 488 goat anti-mouse IgG were from Molecular Probes (Eugene, OR, USA). 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) was from Roche Diagnostics (Indianapolis, IN, USA).

Patient sampling
Synovial tissues from either RA (n = 7) or OA patients (n = 3) were obtained after joint replacement surgery. Synovial tissues that had been obtained from patients who were injured in traffic accidents and had received surgical treatments were used as normal control tissues (n = 2). All synovial tissues were fixed in 4% paraformaldehyde overnight and then paraffinized. PBMCs from normal donors were isolated by Ficoll-Hypaque density-gradient centrifugation. This study was approved by the Gunma University ethical committee, and all samples were obtained after the patients had given their informed consent.

Immunohistochemical analysis
Paraffinized synovial tissues of 3 μm thickness were deparaffinized, placed in 0.01 M sodium citrate buffer (pH 6.0), and heated two times for 5 min in a microwave oven. After inactivation of endogenous peroxidase with 0.5% metaperiodic acid in phosphate-buffered saline (PBS) for 10 min, sections were blocked for 10 min with serum-free protein block (Dako Cytomation, Carpinteria, CA, USA). Sections were incubated at 4°C overnight with optimally diluted primary antibodies (mouse anti-IL-19, IL-20R1, vimentin, CD3, CD20, CD68 mAb and goat anti-IL-20R2 pAb). After washing with PBS, sections were incubated with Histofine simple stain MAX PO (MULTI or G) as secondary antibodies for 1 h at room temperature. After washing with PBS, the signals were developed with diaminobenzidine (Nichirei). For dual-labeling immunofluorescence, tissue sections were deparaffinized and blocked as described above. Sections were then incubated with a mixture of primary antibodies (goat anti-human IL-19 pAb and mouse anti-CD68 or vimentin mAb) at 4°C overnight. After washing in PBS, slides were next reacted with a mixture of Alexa Fluor® 488 goat anti-mouse IgG, 594 rabbit anti-goat IgG and DAPI for 1 h. Images were then acquired and digitally processed.

Cell culture of RASC and human keratinocytes
RASCs were isolated and cultured as previously described [14]. Briefly, synovial tissues were minced into 2–3 mm pieces and treated for 2 h with 2 mg/ml of type I collagenase in DMEM (Sigma, St Louis, MO, USA) at 37°C under continuous shaking. After removal of tissue debris through a nylon mesh, the cells were washed with DMEM and then seeded in 75-cm² flasks in DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS) (Sigma). All in vitro experiments were carried out using primary RASCs between passages 4 and 8. Normal adult primary human epidermal keratinocytes (NHEKs; Cambrex, Walkersville, MD, USA) were cultured in keratinocyte basal medium-2 (KGM-2) (Cambrex).

Immunofluorescence staining of RASCs
For immunofluorescence, RASCs were seeded in an eight-well Lab-Tek Chamber Slide System (Nunc, New York, NY, USA) and cultured until 80% confluence, then fixed with 100% methanol at −20°C for 15 min. Slides were incubated with protein block for 10 min and then incubated at 4°C overnight with optimally diluted primary antibodies (mouse anti-human IL-20R1 mAb or goat anti-human IL-20R2 pAb). After washing with PBS, slides were incubated with secondary antibodies (Alexa Fluor® 488 goat anti-mouse IgG or 488 rabbit anti-goat IgG) and DAPI for 1 h at room temperature.

RT-PCR for IL-19, IL-20R1, IL-20R2 expression
Total RNA was obtained with an RNasey mini kit (Qiagen, Chatsworth, CA, USA) from homogenized synovial tissues, PBMCs, RASCs and NHEKs. Single-stranded cDNA was synthesized by reverse transcription of 2 μg total RNA, using oligo(dT) primer (Invitrogen, San Diego, CA, USA) and the Omniscript RT kit (Qiagen), to a final volume of 40 μl. Amplification of the cDNA template was performed in 50 μl PCR mix containing 1 μl cDNA template, 200 μM dNTP, 5 μl PCR Gold Buffer (Applied Biosystems, Foster City, CA, USA), 2.5 mM MgCl₂, 1.25 U AmpliTaq Gold DNA polymerase (Roche Laboratories, Nutley, NJ, USA), and 0.5 μM of each primer. Amplification conditions for IL-19, IL-20R2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows: 10 min at 94°C followed by 35 cycles, each consisting of 30 s at 94°C, 30 s at 60°C and 45 s at 72°C and finally 7 min at 72°C. The conditions for IL-20R1 were the same except for an annealing temperature of 62°C. The sequences of oligonucleotide primers were as follows: for IL-19 (322 bp), 5’-TGTCAG GAAAGAGGAGTTG-3’ and 5’-AGCGCCTAATAACAAGC CGTA-3’; for IL-20R1 (646 bp), 5’-CAAGAGAAACACCACCTCA GCAA-3’ and 5’-GGAGACCGAAAGTACACACA-3’; for IL-20R2 (302 bp), 5’-GCAGGAGAACATGTCAAAAAT-3’ and 5’-TCTCAAGGTCTGGGAGGAC-3’; for GAPDH (452 bp), 5’-ACCACAGTCATGCCCATAC-3’ and 5’-TCCAACCACCT GTTGCTGTA-3’.

ELISA
The concentration of IL-6 in cell culture supernatants was determined by ELISA obtained from R&D Systems.

Detection of STAT3 activation by western blot
RASCs were seeded in 6-cm² dishes and cultured until 80% confluence. Cells were rested for 24 h in DMEM containing 1% FCS, then stimulated with 100 ng/ml of rIL-19 for the indicated periods. Cells were harvested and western blot was performed. Briefly, cells were washed with cold PBS and homogenized in lysis buffer containing 1.5% Triton X-100, 150 mM Tris–HCl, pH 7.2, 150 mM NaCl, 15 mM EDTA, 3 mM phenylmethylsulphonyl fluoride, 30 μg/ml aprotinin, 30 μg/ml pepstatin A, 3 mM sodium orthovanadate and 15 mM pyrophosphate. After centrifugation at 17,500 g for 10 min, the protein concentration of the supernatants was determined using a micro BCA protein assay kit (Pierce, Rockford, IL, USA). Protein samples containing 10 μg of total protein were separated on a 10% SDS-PAGE and transferred onto a polyvinyl difluoride membrane. Blots were initially incubated in Tris-buffered saline containing 0.1% Tween-20 and 5% non-fat dry milk powder (T-TBS milk) to block non-specific binding. This was followed by further incubation overnight with rabbit anti-phospho-STAT3 (Tyr705) antibodies or rabbit anti-STAT3 antibodies at a dilution of 1:1000 in T-TBS milk at 4°C. After several washes, the membrane was incubated with AP-conjugated anti-rabbit IgG at a dilution of 1:5000 for 1 h at room temperature. The bands were visualized using 5-bromo-4-chloro-3-indoly phosphates/nitroblue tetrazolium (BCIP/NBT Sigma Fast tablets) as the substrate, in accordance with the supplier’s instructions.
Detection of apoptosis by Hoechst staining

Cell apoptosis was determined by Hoechst staining. Briefly, RASCs were cultured to confluence in 10% FCS DMEM medium and then harvested. Subsequently, cells were seeded in DMEM with 0 or 10% FCS at 1 x 10⁶ cells/ml in 24-well plates, in the presence or absence of 100 ng/ml of rIL-19. After 72 h, Hoechst 33342 (Molecular Probes) was added to each well. After 10 min, the cells were observed under UV light (365/380 nm) by fluorescence microscopy at x 200 magnification. Cells containing condensed or fragmented nuclei were identified as apoptotic cells. At least 100 cells were counted and the percentage of apoptotic cells was determined for each sample.

Flow cytometry analysis of annexin V binding

RASCs were cultured to confluence in DMEM containing 10% FCS. Cells were further incubated in DMEM with 0 or 10% FCS, in the presence or absence of 100 ng/ml of IL-19, for 24 h. For detection of early apoptosis, we used the annexin V-FITC apoptosis detection kit I (BD Biosciences Pharmingen, San Diego, CA, USA) with slight modification. Briefly, cells in each dish were washed with cold PBS and then trypsinized. All cells were collected, resuspended in 500 μl of 1 x binding buffer, and incubated in the dark with 5 μl of annexin V-FITC for 15 min at room temperature. Cells were then analysed by flow cytometry (BD FACScalibur; BD Biosciences, San Diego, CA) and 1 x 10⁵ cells per experimental condition were counted.

Active caspase-3 assay

RASCs were prepared as described above. For detection of active caspase-3, we used a Caspase-3 Colorimetric Assay Kit (R&D Systems) according to the manufacturer’s instructions. A recombinant active human caspase-3 (R&D Systems) was used for the standard curve.

Statistical analysis

All experiments were repeated at least three times. Reported results are representative of several experiments, all of which are expressed as the mean ± S.E.M. of three separate experiments. Statistical significance was determined by one-way analysis of variance followed by Scheffé’s F-test. A P-value of <0.05 was considered to represent a statistically significant difference between group means.

Results

Expression of IL-19 in RA synovial tissues

To examine whether IL-19 is involved in the pathogenesis of RA, we first examined the mRNA expression of IL-19 by RT-PCR in synovial tissue samples derived from RA patients (n=7). Significant levels of IL-19 mRNA were detected in all synovial tissues from RA patients who had active synovitis (Fig. 1A). However, its expression was not detected in those obtained from OA patients (n=3) and normal donors (n=2). Representative findings of two RA, one OA and one normal donor are shown in Fig. 3A. PBMCs from the normal donor stimulated with 1 μg/ml of LPS for 24 h were used as a positive control.

Next, we sought to examine the localization of IL-19 in RA synovial tissues and performed immunohistochemical analysis with a specific antibody against IL-19. As shown in Fig. 1B, IL-19 was expressed in the hyperplastic lining layers of RA synovium. Positive staining observed in the small vessels was thought to be non-specific, because at higher magnifications staining was observed in the vascular lumen, but not vascular cells such as endothelial cells and smooth muscle cells. In contrast, the expression of IL-19 was not observed in the synovium of OA patients. In the negative control, no significant signals were detected with non-specific immunoglobulins.

To further examine cell types expressing IL-19, CD68, CD20, CD3 and vimentin were stained in sequential slices as markers of macrophages, B cells, T cells and fibroblast-lineage cells, respectively (Fig. 2A). Substantial CD68 or vimentin-positive cells were detected in lining layers and its distribution was similar to that of IL-19, suggesting that macrophages and fibroblasts expressed IL-19. Conversely, CD20-positive cells were observed mainly in lymphocyte aggregates, and CD3-positive cells were more diffuse in the sublining layers. Collectively, these observations suggest that the majority of the IL-19-positive cells were synovial cells of either fibroblast or macrophage lineage, but not infiltrating lymphocytes.

Fig. 1. IL-19 expression in RA synovial tissues. (A) RT-PCR for IL-19 or GAPDH was performed using mRNA extracted from synovial tissues from two RA patients (RA1, 2), one OA patient and one normal donor. PBMCs from the normal donor stimulated with LPS were used as positive control (PC). (B) Immunohistological analysis of RA synovium was performed using specific antibodies against IL-19 or control IgG. Synovial tissue from OA patients was also stained with anti-IL-19 antibodies. Sections were counterstained with haematoxylin. (Original magnification x 100).

Fig. 2. Cellular distribution of IL-19 in RA synovial tissues. (A) Immunohistological analysis of RA synovium was performed using specific antibodies against IL-19, CD68, CD20, CD3, vimentin or control IgG. Sections were counterstained with haematoxylin. (Original magnification x 400).
expressed in RASCs after LPS stimulation at 1 h. It can express IL-19 by RT-PCR. A high level of IL-19 mRNA was expressed IL-19. We next examined whether cultured RASCs express IL-19, IL-20R1 and IL-20R2 expression in RASCs. Immunohistochemical analysis showed that synovial cells express IL-20R1 and IL-20R2 mRNA were detected in samples from RA patients (n = 7), but not from the OA patients (n = 3) and normal donors (n = 2). Representative findings of two RA, one OA and one normal donor are shown in Fig. 3A. NHEKs were used as positive controls. In the next experiment, we sought to examine the cellular distribution of IL-20R1 and IL-20R2 in the rheumatoid synovium by immunohistochemical analysis. As shown in Fig. 3B, high levels of IL-20R1 and IL-20R2 expression were observed in lining layers, indicating that synovial cells express the IL-20R complex. In addition, these were also expressed in sublining layers including lymphocyte aggregates.

**IL-20R1 and IL-20R2 expression in RA synovial tissues**

A functional IL-19 receptor was recently identified as a specific heterodimer consisting of IL-20R1 and IL-20R2 [7]. We investigated IL-20R1 and IL-20R2 mRNA expression in RA synovial tissues by RT-PCR. IL-20R1 and IL-20R2 mRNA were detected in samples from RA patients (n = 7), but not from the OA patients (n = 3) and normal donors (n = 2). Representative findings of two RA, one OA and one normal donor are shown in Fig. 3A. NHEKs were used as positive controls. In the next experiment, we sought to examine the cellular distribution of IL-20R1 and IL-20R2 in the rheumatoid synovium by immunohistochemical analysis. As shown in Fig. 3B, high levels of IL-20R1 and IL-20R2 expression were observed in lining layers, indicating that synovial cells express the IL-20R complex. In addition, these were also expressed in sublining layers including lymphocyte aggregates.

**IL-19, IL-20R1 and IL-20R2 expression in RASC**

Immunohistochemical analysis showed that synovial cells expressed IL-19. We next examined whether cultured RASCs can express IL-19 by RT-PCR. A high level of IL-19 mRNA was expressed in RASCs after LPS stimulation at 1 μg/ml for 24 h, but not without stimulation (Fig. 4A). Pro-inflammatory cytokines relevant to pathogenesis of RA, TNF-α and IL-6 (both at 10 ng/ml), induced only faint IL-19 mRNA expression. These results imply that RASCs have the potential to express IL-19.

We also examined whether cultured RASCs express IL-20R1 and IL-20R2 using RT-PCR. As shown in Fig. 4B, IL-20R1 and IL-20R2 mRNA were expressed in RASCs under basal conditions. IL-20R1 and IL-20R2 expression at the protein level was further examined by immunofluorescence analysis. RASCs without stimulations were stained with specific antibodies against IL-20R1 and IL-20R2 (Fig. 4C). These data indicate that RASCs have constitutive expression of IL-20R1 and IL-20R2.

**IL-19 induces STAT3 activation and IL-6 production in RASCs**

A recent study has shown that IL-19, after binding to IL-20R complex, induces STAT3 activation [7]. To explore whether IL-20R on RASC is functional or not, we examined STAT3 activation after rIL-19 stimulation by western blot with specific antibodies against phosphorylated STAT3. As shown in Fig. 5A, IL-19 at 100 ng/ml for 10 min induced a 2.5-fold increase of phosphorylated STAT3 expression above basal conditions. Previous studies have shown that IL-19 can induce IL-6 and TNF-α production in monocytes [10]. We next examined whether IL-19 has such an effect on RASCs. RASCs were incubated with various concentrations of rIL-19 for 72 h and IL-6 production in supernatants was determined by ELISA. As shown in Fig. 5B, rIL-19 at 100 ng/ml induced 1.5-fold increase of IL-6 production above medium control.

**IL-19 decreases cell apoptosis induced by serum starvation in RASCs**

Increasing evidence has demonstrated that STAT3 has an anti-apoptotic effect, and at the same time, promotes cellular proliferation in a variety of cells [15]. Next, we examined whether IL-19 had any influence on apoptosis of RASCs. Apoptosis of RASCs was induced by serum starvation in the presence or absence of IL-19; the percentage of apoptotic cells was counted after Hoechst staining. As shown in Fig. 6A, apoptotic cells were increased in serum free (SF), compared with normal serum.
conditions after a 72-h incubation period. Addition of IL-19 at 100 ng/ml under SF conditions significantly reduced apoptosis (SF: 35+/C6 8.7%, SF + IL-19: 11+/C6 2.7%, P < 0.05).

Cells at the early apoptotic stage externalize phosphatidylserine at their cell surface, which can be detected by annexin V binding [16]. To confirm the anti-apoptotic effect of IL-19, cellular binding of annexin V-FITC was examined by flow cytometry. Representative data are shown in Fig. 6B. Serum starvation induced 1.9-fold increase of annexin V-FITC expression above control, but addition of IL-19 in SF reversed it to the basal level [mean fluorescence intensity (MFI); control: 46.5; SF: 90.3; SF + IL-19: 50.3].

Activation of caspase-3 is one of the final common pathways from a variety of signals leading to cellular apoptosis [17]. Caspase-3 activity can be determined by its ability to cleave molecules containing the amino acid motif DEVD, such as poly ADP-ribose polymerase. We used a commercial assay kit to examine whether IL-19 modulates caspase-3 activity after serum starvation. As shown in Fig. 6C, IL-19 significantly reversed an increase of caspase-3 activity induced by SF (control: 29+/C6 7.5; SF: 118+/C6 21; SF + IL-19: 63+/C6 11). Collectively, these data indicate IL-19 can inhibit cell apoptosis induced by serum starvation in RA synovial cells.

Discussion

IL-19 is a novel cytokine of the IL-10 family, but its pathophysiological function is poorly understood; neither its expression, nor its role in RA, has been explored. In this study, we found IL-19 and the IL-20R complex were expressed in rheumatoid synovium. In vitro, cultured RASCs expressed both IL-20R1 and IL-20R2, and induced IL-19 expression by LPS stimulation. Moreover, rIL-19 induced the production of IL-6, STAT3 activation and reduction of apoptosis. Based on these findings, we propose that IL-19, produced by synovial cells, may promote inflammatory responses in RA synovial tissues by preventing cell apoptosis through STAT3 activation in part and by inducing the production of IL-6. RASCs expressed both IL-19 and IL-20R complex consisting of IL-20R1 and IL-20R2, suggesting that IL-19 works in an autocrine as well as a paracrine manner.

Accumulating data suggest that STAT3 has an anti-apoptotic effect that is linked to up-regulation of Bel-xL or down-regulation of Bax [18], which play important roles in regulating caspase-3 activity. In RASCs, inactivation of STAT3 by the dominant negative mutant-induced apoptosis [19]. Shen et al. [20] have shown that constitutively activated STAT3 protects fibroblasts from serum starvation-induced apoptosis. After identifying STAT3 activation by IL-19 in RASC, we found IL-19 reduced cell apoptosis induced by serum starvation. We also found that this reduction was accompanied by a decrease in caspase-3 activity. Decreased apoptosis of synovial fibroblasts, in addition to increased proliferation, is thought to contribute to synovial hyperplasia [21]. In the current study, IL-19 did not affect the proliferation of RASCs (data not shown). IL-19 may induce...
synovial hyperplasia through suppressing apoptosis rather than increasing proliferation.

A previous study has shown that IL-19 induces IL-6 and TNF-α production in monocytes [10]. Pathogenic roles of IL-6 in RA have been established, and IL-6 targeting therapies are now used in the clinical setting. In this study, IL-19 induced IL-6, but not TNF-α production, in RASCs (data not shown). IL-19, in addition to modulating apoptosis, may contribute to joint destruction indirectly by increasing IL-6 production. Furthermore, IL-19 and IL-6 may cooperatively work in activating STAT3, because IL-6 is shown to induce STAT3 activation.

Several studies have suggested that IL-19 may play pivotal roles in the immunopathology of psoriasis [22]. IL-19 was expressed in basal and suprabasal keratinocyte layers of psoriatic skin and its expression disappeared by either cyclosporin or calcipotriol treatment, correlating with changes in clinical and histological scores [23]. Moreover, IL-19 induced hyperplasia of epidermal keratinocytes and STAT3 activation [22] that is linked to the development of psoriasis [24]. Keratinocytes expressed both IL-20R1 and IL-20R2 [22] and expressed IL-19 after IL-1β stimulation [25], suggesting that IL-19 has an autocrine function. This evidence indicates that IL-19 was expressed in the active phase of psoriatic skin and worked as a pro-inflammatory modulator in the pathogenesis of psoriasis.

In the current study, we found that IL-19 was expressed in the hyperplastic lining layers of RA synovium accompanied with a considerable leucocyte infiltration. These observations led us to hypothesize that IL-19 may play an important role in synovial hyperplasia formation in the active phase of RA, but not in the chronic phase. Based on this finding, that IL-19 expression was well correlated with leucocyte infiltration, we also hypothesized that IL-19 may induce chemokine production. However, we did not detect monocyte chemoattractant protein-1 up-regulation by IL-19 in RASCs (data not shown). Comprehensive analysis, such as gene array or proteomics for chemokine production in IL-19 in RASCs, is an interesting subject requiring further clarification.

Recently, five novel IL-10 family members have been discovered, IL-19, IL-20, IL-22, IL-24 and IL-26. Exploration of how production by IL-19 in RASCs, is an interesting subject requiring further analysis. IL-19, IL-20, IL-22, IL-24 and IL-26 are necessary. In conclusion, our results suggest that IL-19, produced by synovial cells, promotes inflammatory response in RA synovium by inducing IL-6 production and decreasing synovial cell apoptosis. We propose that further studies should be necessary for the clinical application of IL-19 targeting therapy.

### References