Interleukin (IL)-23 p19 expression induced by IL-1β in human fibroblast-like synoviocytes with rheumatoid arthritis via active nuclear factor-κB and AP-1 dependent pathway


Objectives. To explore the source of the p19 subunit of interleukin-23 (IL-23) in joints with rheumatoid arthritis (RA), the effects of IL-1β and tumour necrosis factor (TNF)-α on the IL-23 gene expression in RA fibroblast-like synoviocytes and the effect of IL-23 on proinflammatory cytokines.

Methods. Expression of IL-23 p19 in joints was examined by immunohistochemical analysis of patients with RA and osteoarthritis (OA). The effects of IL-1β and TNF-α on the expression of IL-23 p19 and IL-12 p35 subunits in human fibroblast-like synoviocytes from RA patients (HFLS-RA) were determined by reverse transcriptase polymerase chain reaction (RT-PCR), quantitative PCR and western blotting assay. Blockade of nuclear factor kappaB (NF-κB) or AP-1 activation was used to verify the involvement of intracellular signal pathways of the induction of p19. IL-23-induced IL-8 and IL-6 productions were determined in HFLS-RA by RT-PCR and enzyme-linked immunosorbent assay.

Results. IL-23 p19 was expressed in the synovium from RA, but not from OA patients. Similar to the protein expression, IL-23 p19 mRNA could be detected by RT-PCR in four of five RA synovial fluid mononuclear cells (SFMC). IL-1β and TNF-α could induce RA fibroblast-like synoviocytes to produce the IL-23 p19 subunit. The effects of IL-1β were much stronger than TNF-α. These responses were observed in both a dose-responsive and time-dependent manner. IL-1β produced weakly enhanced gene expression of the p35 subunits of IL-12. IL-1β also promotes the p35 expression, a subunit of IL-12, but weakly. In addition, the NF-κB and the AP-1 inhibitors down-regulated the expression of IL-23 p19 mRNA induced by IL-1β. IL-23 receptor (IL-23R) was constitutive expression in HFLS-RA. Moreover, IL-23 up-regulated the IL-8 and IL-6 mRNA and protein levels in a dose-dependent manner in HFLS-RA.

Conclusions. Our results demonstrate that IL-23, produced by mononuclear cells in synovial fluid with RA and HFLS-RA, promotes inflammatory responses in RA by inducing IL-8 and IL-6 production from HFLS. IL-1β regulates IL-23 p19 expression via NF-κB and AP-1 pathways. This report also demonstrates that IL-23 could promote inflammatory responses in HFLS-RA by stimulating IL-8 and IL-6 production.

Keywords: IL-23, Rheumatoid arthritis, HFLS-RA, IL-1β.

Introduction

Rheumatoid arthritis (RA) is a chronic immune disorder that affects the joints and promotes joint destruction. The characteristics of RA are synovial fibroblast proliferation and macrophage infiltration, which are induced by chemokines and cytokines. Cytokines, such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α, are important mediators of inflammation and tissue destruction in RA [1].

IL-23, a recently discovered cytokine, is a heterodimer consisting of a p40 subunit of IL-12 and a unique p19 subunit. IL-23 p19 and IL-12 p35 are encoded by separate genes [2]. IL-12 is an immunoregulatory cytokine that plays a major role in the generation of Th1 cell-mediated immunity [3]. IL-23 has some biological functions similar to, but distinct from, IL-12. Similar to IL-12, IL-23 induces interferon (IFN)-γ production from T cells. The difference is that IL-23 can promote CD4⁺ CD45RB<sup>low</sup> memory T cells (Th<sub>17</sub>) to secrete IL-17. Macrophages, dendritic cells, haemopoietic cells and Th1 cells have been identified as sources of IL-23 [2]. In a recent study, IL-23 rather than IL-12 was seen as a critical cytokine involved in the pathogenesis of experimental autoimmune encephalomyelitis [4]. Similar results were also seen in animal models of RA [5]. Absence of IL-23 is protected from collagen-induced arthritis, whereas loss of IL-12 is exacerbated. IL-23 gene-targeted mice did not develop clinical signs of disease and were completely resistant to the development of joint and bone pathology. Therefore, IL-23 may be an essential promoter of end-stage joint autoimmune inflammation [5]. In this study, we examined the mechanism of IL-23 production in fibroblast-like synoviocyte and the role of IL-23 in the pathogenesis of RA. To this aim, we analysed the mRNA and protein levels of IL-23 p19 and IL-12 p35 in the samples derived from RA patients. In addition, we examined the effects of recombinant IL-23 (rIL-23) on cultured fibroblast-like synoviocytes derived from RA patients (HFLS-RA). Our results demonstrate that IL-23 p19 and IL-12 p35 are expressed in RA synovial fluids, and that the chemokine-induction actions of IL-23 may play an important role in the pathogenesis of RA.

Materials and methods

Patients’ sample

Synovial tissues from either RA or osteoarthritis (OA) patients were obtained after joint replacement surgery. Then tissues were fixed in 4% paraformaldehyde overnight and paraffinized. All patients met the American College of Rheumatology (formerly, American Rheumatism Association) 1987 revised criteria for RA [6] and OA [7]. None of the patients were receiving corticosteroids at the time of surgery. Informed consent was obtained from each patient and the experimental protocol was approved by the Tri-Service General Hospital Institutional Review Board.

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Immunohistochemistry analysis

Three-micrometer-thick paraffinized mice joint sections were deparaffinized, placed in 0.01 M sodium citrate buffer (pH 6.0) and heated twice 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 incubated with 10% horse serum in PBS for 1 h. Sections were incubated at 4°C overnight with 100× diluted primary goat antimouse IL-23 p19 antibody (R&D Systems, Inc.). Sections were incubated with the Dako Envision Plus system to amplify the signal after washing with PBS. The signals were finally developed with diaminobenzidine (Nichirei, Minneapolis, MN, USA).

Isolation of synovial fluid-derived monocytes

Synovial fluid was collected from the knee joints of five patients with RA (mean duration of RA: 11.8 ± 3.3, from range 8–16 yrs; mean titre of RF: 207.7 ± 178.7 IU/ml, from range 0–436 IU/ml) for 6 h at 37°C. Primary HFLS isolated from patients with RA were maintained in 10% FBS, 2 mM L-glutamine, 3 mM NaHCO₃, 100 units/ml of penicillin and 100 μg/ml of streptomycin at 37°C (5% CO₂, 95% air) in a humidified air atmosphere. In the experiments, we plated 1 x 10⁶ RA synoviocyte cells (3rd to the 8th passage) in a 100 mm Petri dish. Then the cells were washed with DMEM containing 0.2% LAH and treated with IL-1β (1 ng/ml) (R&D Systems Inc.,) in the fresh DMEM and 0.2% LAH medium for 18–24 h.

Dose response and time course of IL-1β and TNF-α effect on IL-23 p19 mRNA expression

To examine the effect of IL-1β and TNF-α, the major cytokines in the pathogenesis of RA, on IL-23 production, HFLS cells (1 x 10⁶ in 100 mm Petri dish in serum-free modified Eagle’s medium) were cultured for 18 h with various concentrations of IL-1β (0, 0.01, 1 and 10 ng/ml) and TNF-α (1 and 10 ng/ml). Total RNAs of HFLS-RA were collected and the expressions of IL-23 p19 and IL-12 p35 were analysed by semiquantitative and real-time reverse transcriptase polymerase chain reaction (RT-PCR). In addition, 1 ng/ml IL-1β was used to stimulate HFLS-RA for 3, 6, 18 and 24 h to determine the time-dependent expression of IL-23 p19 protein by western blotting.

The role of NF-κB in IL-1β induced IL-23 p19 gene expression. IL-1β is a key activator of AP-1 and NF-κB. To establish a cause and effect relationship between IL-1β induction of IL-23 p19 and NF-κB and AP-1, we used pyrrolidine dithiocarbamate (PDTC) (Sigma) as a general inhibitor of NF-κB [9] and curcumin (Sigma) as an inhibitor of AP-1 [10, 11] to prevent NF-κB-mediated or AP-1-mediated transcriptional activation. HFLS cells (1 x 10⁶ in 100 mm Petri dish in serum-free modified Eagle’s medium) were pre-treated with various concentrations of PDTC or curcumin (0, 6.25, 12.5, 25 and 50 μM) for 1 h, and then treated with IL-1β for 18 h. IL-23 p19 gene expression was determined by semiquantitative RT-PCR.

Total RNA extraction, semiquantitative RT-PCR and quantitative real-time PCR (qPCR)

Total cellular RNA was extracted from SFMC of RA patients or from cells using the Qiagen kit (Qiagen, Hilden, Germany). For RT-PCR analyses, 1 μg of total RNA was converted into cDNA using oligo(dT) and reverse transcriptase (Clontech, Palo Alto, CA, USA). The following PCR primers were used in HFLS-RA cells: human IL-23, 5′-TGCAAAAGATTCACCCAGGGTCTGA-3′ (sense, 302–320 bp) and 5′-TAGGGTCCACCTTGTAGGCT TGC-3′ (anti-sense, 723–743 bp). Primers for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was from Clontech (Palo Alto, CA, USA). The amplification was performed at 94°C for 45 s, 58–60°C for 45 s and 72°C for 60 s. PCR products were linearly produced between 25 and 28 cycles for IL-12 p35 and GAPDH. The amplified PCR products were analysed with 2% agarose gel and visualized with ethidium bromide staining. Real-time RT-PCR was performed on an ABI-Prism 7000 PCR cycler (Applied Biosystems, Foster City, CA, USA). The following validated PCR primers and TaqMan MGB probes (6FAM-labelled) were used: IL-23p19 (HS00373234_m1). As endogenous controls, two independent primer sets with TaqMan probe were used: GAPDH (HS99999905_m1). PCR mix was prepared according to the manufacturer’s instructions (Assay on demand; Applied Biosystems) and thermal cycler conditions were as follows: 1 x 10 min 95°C, 40 cycles denaturation (15 s, 95°C) and combined annealing/extension (1 min, 60°C). Relative quantification was performed by comparison of threshold cycle values of samples with serially diluted standards. Running program was performed according to the ABI PRISM 7000 Sequence Detection System default temperature settings (2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 1 min at 60°C) in a volume of 25 μl with 1 x TaqMan Universal Master Mix (Applied Biosystems).

Effect of IL-23 on the production of IL-8, IL-6 and IL-17

HFLS-RA cells were used to investigate the effect of IL-23 on the protein levels of IL-8 and matrix metalloproteinase (MMP)-3. Cells were plated into a 24-well culture dish at 3.5 x 10⁶ cells per well in 300 μl of DMEM containing 10% FBS. After 24 h, the medium was replaced with serum-free medium containing various doses of IL-23 (0, 1, 10, 100 and 1000 ng/ml). After 18 h, the supernatants were collected and stored at −20°C until analysed. IL-8, IL-6 and IL-17 were analysed by a sandwich enzyme-linked immunosorbent assay (ELISA) using R&D assay kits according to the manufacturer’s protocol.

Western blot analysis

HFLS-RA cells were plated into a 100 mm Petri dish (1 x 10⁶ cells/dish) with DMEM containing 10% FBS and were cultured for 24 h. After washing with PBS, the cells were incubated with 0.1 and 1 ng/ml IL-1β for 24 h. The cell extracts (50 μg of protein per lane) were separated for IL-23 p19 on a 15% SDS-PAGE and for IL-23 receptor on a 10% SDS-PAGE with the Laemmli buffer system. Protein separated on gels was transferred onto polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia Biotech) with an isophor electrotransfer apparatus (Hoefer Scientific Instruments, San Francisco, CA, USA) at 200 mA for 3 h until analysed. The membranes were blocked with PBS containing 5% non-fat milk. The blots were stained with anti-p19Ab (R&D) of anti-IL-23RAb (R&D) for 1 h at room temperature, followed by a secondary staining with peroxidase-conjugated anti-goat

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IgG (Sigma). The protein bands on the filter were visualized by the ECL system (Amersham, Little Chalfont, UK).

**Results**

**IL-23 p19 expression in synovial tissue from RA patients**

IL-23 is a recently identified cytokine of the IL-12 family and its pathophysiological function is not well-known. To determine whether IL-23 p19 is involved in the pathogenesis of RA, we examined the localization of IL-23 p19 in synovium of RA and OA using immunohistochemical analysis with a specific antibody against IL-23 p19. As shown in Fig. 1, IL-23 was expressed in the synovial lining with RA, but not in OA synovium and negative control.

**Dose response and time course of IL-1β effect on IL-23 p19 expression**

Immunohistochemical analysis showed that synovial fibroblast expresses IL-23 p19 proteins in RA samples. To examine the production of IL-23, we hypothesized that the stimulator is IL-1β, which is the major cytokine in the pathogenesis of RA. We cultured HFLS from patients with RA and treated with or without variant concentrations of IL-1β for 18 h. Without IL-1β stimulation, the mRNA expression of IL-23 p19 subunit in HFLS-RA was hardly detected. IL-1β at 0.1 ng/ml induced a 50 000-fold increase in IL-23 p19 mRNA above the value in medium controls. IL-23 p19 mRNA was strongly up-regulated in a dose-responsive manner in response to IL-1β in HFLS-RA (Fig. 2A and B). IL-1β not only induced IL-23 mRNA but also enhanced IL-23 protein expression in HFLS (Fig. 2C).

In a time-dependent manner, IL-23 p19 mRNA expression in HFLS-RA was found to increase at 6 h post-stimulation with 1 ng/ml IL-1β, and reached a maximum at 18 h. IL-1β could induce IL-23 p19 expression from 6 to 24 h after IL-1β treatment. The tendencies of IL-1β-induced IL-23 p19 expression are shown in Fig. 2D. Data were confirmed by real-time RT-PCR (Fig. 2E). mRNA for IL-12 p35 subunits was constitutively expressed in HFLS-RA. TNF-α may suppress gene expression of IL-12 p35 subunits. Over 50% decrease of p35 mRNA basal level was detected after the administration of 100 ng/ml TNF-α (Fig. 3D).

**Inhibition of NF-κB and AP-1 activity suppresses IL-1β effects on IL-23 p19 gene expression in HFLS-RA**

IL-1β is a key activator of AP-1 and NF-κB. To examine the involvement of NF-κB pathway in IL-1β-induced IL-23 expression, pyrrolidine dithiocarbamate (PDTC), an NF-κB inhibitor was used. Results demonstrated that PDTC (12.5–50 μM/ml) significantly suppressed the IL-1β-induced IL-23 p19 and IL-12 p35 mRNA levels (Fig. 4A and B). These findings indicated that the IL-1β-induced IL-23 p19 and IL-12 p35 expressions were mediated by NF-κB activation in HFLS-RA.

Curcumin is known to inhibit the expression of the c-jun gene induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), but not the c-fos gene [12], and to suppress the TPA-induced binding activity of AP-1. The c-jun inhibitory action is mediated by AP-1 suppression. Administration of curcumin suppressed appreciable amounts of IL-1β-induced IL-23 p19 mRNA at doses of 12.5–50 μM/ml (Fig. 4C). Data were confirmed by real time RT-PCR. In the presence of different concentrations of curcumin, IL-23 p19 production reacted in a dose-dependent manner, but IL-12 p35 expression showed no difference (Fig. 4D). These findings indicated that activation of AP-1 is necessary for IL-1β-mediated IL-23 p19 gene expression in HFLS-RA.

**IL-23 p19 expression by synovial fluid mononuclear cells of patients with RA**

To assess other sources of IL-23 p19 in human RA, we investigated whether IL-23 p19 could be produced by SFMCs isolated from patients with RA. By RT-PCR, we examined mRNA expression of the p19 subunit of IL-23 in SFMC obtained from patients with RA. As shown in Fig. 5, IL-23 p19 mRNA was detected in mononuclear cells from four of five RA synovial fluid. IL-12 p35 mRNA was also expressed in all patients. The source of IL-23 p19 is not only HFLS, but also mononuclear cells from synovial fluid in patients with RA.

**IL-23R expression in HFLS-RA**

IL-23 acts on its own receptor (IL-23R) and induces intracellular signalling subsequently. IL-23R is composed of two subunits termed IL-12β 1 and IL-23R [13]. We examined IL-23R expression in HFLS-RA by western blotting. IL-23R did not respond to IL-1β in HFLS-RA (Fig. 6). The results indicated that IL-23R was constitutive expression in HFLS-RA.
FIG. 2. IL-1β-stimulated IL-23 p19 gene expression in HFLS-RA cells. HFLS cells (1 × 10^6 in a 100 mm Petri dish in serum-free modified Eagle’s medium) were cultured for 18 h with various concentrations of IL-1β (0, 0.01, 0.1, 1 and 10 ng/ml) or were cultured with IL-1β 1 ng/ml for different time course (0, 3, 6, 18 and 24 h). (A, D) IL-23 p19 and IL-12 p35 expressions were determined by semiquantitative RT-PCR. Similar results were obtained in four experiments with cells from four different RA patients. (B, E) Changes in mRNA levels for IL-23 p19 and IL-12 p35 in response to IL-1β treatment were measured by quantitative RT-PCR. Data for each sample is normalized to the GAPDH mRNA level present in each sample and normalized again between samples to the levels of IL-23 p19 mRNA present in unstimulated or time zero condition. (C, F) IL-1β-induced IL-23 p19 protein expression in HFLS-RA. Shown is the western blot analysis using anti-IL-23 p19 antibody. Proteins (50 μg/lane) were separated on 15% SDS–PAGE.
IL-23 induced production of IL-8 and IL-6 but not IL-17 in HFLS-RA

To assess the possible role of IL-23 in human RA, we tested changes in IL-8 and IL-6 mRNA and IL-17 protein expressions in IL-23-stimulated HFLS-RA. IL-8 and IL-6 mRNA were up-regulated in response to IL-23 administration (1–100 ng/ml) for the 18 h treatment. IL-6 was constituent expressed in HFLS-RA. IL-23 can induce IL-6 expression concentration dependently. The minimum dose of IL-23 inducing IL-8 was 1 ng/ml in HFLS-RA (Fig. 7A). As shown in Fig. 7B, IL-6 and IL-8 protein levels were examined by ELISA, data revealed that IL-23 significantly induced IL-6 and IL-8 secretion in HFLS-RA. IL-6 and IL-8 protein were detectable in all the supernatants of the eighth passage HFLS-RA cells. Contrarily, IL-17 and MMP-3 were undetectable in IL-23-conditioned cells supernatants. These observations supported the finding that IL-23 enhanced the syntheses of IL-8 and IL-6 in HFLS-RA. On the other hand, IL-23 was unable to induce IL-17 or MMP-3 production.

Discussion

IL-23 is a recently identified cytokine of the IL-12 family, and it plays an important role in the activation of memory T cells accompanied by IL-17 secretion. IL-23 p19 is mainly produced by activated DCs and macrophages. However, the pathophysiological function of IL-23 is not well-known. In this study, we found that IL-23 p19 was expressed in the synovial lining in synovium membrane of patients with RA. In patients with RA, IL-23 p19 mRNA was expressed by mononuclear cells in the synovial fluid. In vitro, IL-1β induced IL-23 p19 expression in HFLS with RA. Cultured HFLS-RA expressed IL-23R, and the IL-23R was expressed constitutively. In addition, recombinant IL-23 induced expression of IL-8 and IL-6 by HFLS-RA. Based on these findings, we hypothesized that IL-23 p19, produced by synovial fibroblasts and mononuclear, promotes inflammatory responses in RA synovial tissues by inducing production of IL-6 and IL-8.

In a recent study, IL-23 rather than IL-12 was thought to be the critical cytokine in autoimmune inflammation of the brain [4]. Using gene-targeted mice lacking IL-23 (p19<sup>−/−</sup>), Cua et al. [4] showed that the specific absence of IL-23 is protective, whereas loss of IL-12 exacerbates collagen-induced arthritis. Absence of IL-23 is protected from collagen-induced arthritis, whereas loss of IL-12 is exacerbated. IL-23 gene-targeted mice did not develop clinical signs of disease and were completely resistant to the development of joint and bone pathology. It seems that IL-23 is an essential promoter of end-stage joint autoimmune...
inflammation [5]. Recent observations from Sato et al. [14] demonstrated that the mRNA of the p19 subunit of IL-23 was expressed in synovium derived from RA patients. Similar results were also shown in our study; IL-23 p19 was expressed in synovium membrane of patients with RA detected by immunohistochemical analysis.

Many cytokines participate in the pathogenesis of inflammatory diseases, including RA. For example, TNF-α, IL-1β and IL-6 have been detected in joint fluid during the development of joint inflammation [15]. IFN-γ, transforming growth factor (TGF)-β, IL-10 and IL-12 (p40) mRNA were detected in SFMC of patients with early disease (<1 yr duration) as well as in patients with long-standing arthritis [16]. IL-1β and TNF-α up-regulate IL-23 subunit p19 gene expression in human colonic subepithelial myofibroblasts [17]. IL-12 is considered to play a critical role in inducing Th1 cell-mediated organ-specific autoimmune
levels of serum IL-1 found to result in systemic inflammation accompanied by elevated IL-12, IL-6, and colony-stimulating factor (G-CSF) [22].

RA synovial cells have been shown to spontaneously produce low levels of IL-12 p40 and IL-12 p70 proteins [19]. IL-23 was discovered as an IFN-γ-inducing cytokine. mRNA for the p19 component of IL-23 is expressed in invariant cell types, including endothelial, haematopoietic cells, B cells, T cells, macrophages and dendritic cells [2]. In this study, we found IL-12 p35 and IL-23 p19 mRNA expressions in SFMC of patients with RA. This is the first report demonstrating IL-23 p19 mRNA expression in SFMC of patients with RA. Furthermore, the expression of p19 subunit was found in fresh isolated SFMC from patients, but weakly in the primary fibroblast-like synoviocytes of RA (HFLS-RA) cultures. However, HFLS-RA had increased expression of p19 after stimulation by IL-1β and TNF-α. This phenomenon is similar to the observation of IL-23 expression increase during mucosal inflammation in Crohn's disease [20] and inflammatory bowel disease [21]. These results suggest that the synovium has not been the only source of IL-23 p19 in RA. Infiltrated mononuclear cells, including T cell, may play a role. Our data supported evidence that SFMCs are sources of IL-23 p19, and the production of IL-23 p19 may contribute to the pathogenesis of RA.

Previous reports have demonstrated that overexpression of p19 in vivo induces overexpression of the structurally related cytokines IL-12, IL-6, and colony-stimulating factor (G-CSF) [22]. Recently, the transgenic expression of the IL-23 subunit p19 was found to result in systemic inflammation accompanied by elevated levels of serum IL-1β and TNF-α [23].

The major pathology in RA occurs in the synovium through the synthesis of various cytokines, proteases, superoxide and chemokines. Synovial fluid is more readily accessible; the production of cytokines, such as IL-1, was documented in this compartment [24]. In this study, we showed that IL-23 p19 mRNA expression in SFMC of RA, and IL-1β or TNF-α can induce IL-23 p19 expression in HFLS of RA, and other inflammatory cytokines were induced by p19 subsequently. This suggests that the secretion of IL-23 p19 plays an important role in amplifying the inflammatory response.

Pharmacological interference cytokines of the IL-12 family have received widespread attention in diseases such as multiple sclerosis and RA. To date, little is known about the regulation of IL-23 expression on human DCs. Expression of IL-23 has been reported on monocyte-derived DCs (MoDCs) activated with combinations of cytokines, CD40L, TLR ligands or intact bacteria [2, 25–27]. In RA, IL-1 and TNF-α are cytokines of major importance in joint inflammation and cartilage damage [28]. These cytokines are key activators of the AP-1 and NF-κB pathways [29]. On treatment with curcumin, an AP-1 inhibitor, and PDTC, an NF-κB inhibitor in stimulated IL-1β condition, IL-23 p19 mRNA was reduced markedly. We have demonstrated that IL-23 p19 expression is induced by IL-1β in HFLS from patients with RA via an active NF-κB and AP-1-dependent pathway. The IL-1β-induced IL-23 p19 expression was markedly suppressed by curcumin and PDTC, which are AP-1 and NF-κB inhibitor, individually. These results showed that the secretion of IL-23 p19 induced by IL-1β is AP-1 and NF-κB dependent.

IL-23 binds a receptor composed of IL-12Rβ1 and a second subunit designated IL-23R. IL-23R expression on T cells, NK cells, monocytes and DCs [13] corresponds with the ability of those cells to respond to IL-23. In our results, we are the first to find that IL-23R was expressed in HFLS-RA, and the expression of IL-23R was not affected by IL-1β.

The IL-12 receptor (IL-12R) is composed of two subunits termed IL-12Rβ1 and IL-12Rβ2 [30, 31]. Previous studies have shown that IL-12 could induce RA fibroblast-like synoviocytes to produce IL-6 [22]; HFLS-RA may express IL-12Rβ1 and IL-12Rβ2. It is conceivable that HFLS could respond to IL-23 in the presence of IL-23R.

Indeed, we found that rIL-23 induced production of IL-8 and IL-6 in HFLS-RA. HFLS in RA joints probably account for a source of cytokines such as IL-6 and IL-8 [33, 34]. Although the amount of IL-23-induced IL-6 and IL-8 was not as high as IL-1 and TNF did (1/2 to 1/20, data not shown), the cascade and synergistic effect may have physiological importance. IL-6 levels in the synovial fluid correlates with the presence of local IgM rheumatoid factor and the production of systemic acute-phase proteins [35]. Using an IL-6 monoclonal antibody against its receptor is well tolerated and results in significant improvement in clinical and laboratory measures in RA patients [35, 36]. IL-8 expression in rheumatoid synovial tissue is associated with disease activity [23, 32]. IL-8 is a strong inducer of neutrophil recruitment and is recognized as an important factor that contributes to neutrophil infiltration into synovial fluids in RA [37]. IL-8 exhibits pro-angiogenic activity and induces blood vessel formation and angiogenesis in RA [38]. These findings suggest that IL-23 may mediate IL-6 and IL-8 involved in RA pro-angiogenic. Most reports indicated that IL-23 was a strong inducer of IL-17 [39, 40]. However in our study, IL-17 is undetected in HFLS-RA after IL-23 stimulation. One possibility is that the HFLS we used was unable to express IL-17.

In our results, IL-1β-induced IL-23 p19 expression in HFLS-RA is much more than IL-12 subunit of p35. In addition, IL-23 may promote inflammatory responses in RA by inducing IL-8 and IL-6 production from HFLS. Nevertheless, the actions of IL-23 seem important in the pathogenesis of RA. The role of IL-23 must be more complicated in vivo; the expression and function in different stages of RA animal models, and synergistic effects, requires further study.

Conclusion
Our results demonstrate that IL-23, produced by mononuclear cells in synovial fluid with RA and HFLS-RA, promotes
inflammatory responses in RA by inducing IL-8 and IL-6 production from HFLS. IL-1β regulates IL-23 p19 expression via NF-κB and AP-1 pathways. Our results demonstrate that IL-23 was expressed in mononuclear cells from synovial fluid and IL-1β-conditioned HFLS, and the IL-1β-induced IL-23 production is NF-κB and AP-1-dependent. The elevation of IL-23 may contribute to the promotion of inflammatory responses via IL-6 and IL-8 production.

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