Interferon-γ induces expression of interleukin-18 binding protein in fibroblast-like synoviocytes

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Objective. To investigate expression of the endogenous antagonist of interleukin 18 (IL-18) bioactivity, IL-18 binding protein isoform a (IL-18BPa), in fibroblast-like synoviocytes (FLS).

Methods. Long-term cultured FLS from rheumatoid arthritis (RA), osteoarthritis (OA) and spondylarthropathy patients were analysed for spontaneous and cytokine-induced IL-18BPa expression. Messenger RNA and release of IL-18BPa were assessed by semi-quantitative and quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) as well as immunoblot analysis, respectively.

Results. All investigated FLS cultures expressed low amounts of IL-18BPa transcripts. However, there was no detectable release of IL-18BPa from unstimulated synoviocytes. Of the investigated cytokines, only interferon (IFN)-γ markedly up-regulated IL-18BPa mRNA levels. Induction was accompanied by release of IL-18BPa immunoreactivity from FLS. Conditioned media from IFN-γ-stimulated FLS cultures reduced IL-12/IL-18-dependent IFN-γ production by peripheral blood mononuclear cells.

Conclusion. The present data imply that IFN-γ-activated synoviocytes mediate a negative feedback loop via IL-18BPa, which may limit IL-18 biological activity in arthritis.

Key words: IFN-γ, IL-18BP, Synoviocyte, Fibroblast, Rheumatoid arthritis.

The proinflammatory cytokine interleukin 18 (IL-18) is one current focus of research on rheumatoid arthritis (RA) pathogenesis [1, 2]. IL-18 was first identified as an interferon (IFN)-γ-inducing factor [1], but IL-18 is also capable of directly inducing expression of tumour necrosis factor-α (TNF-α) [1, 2]. Although IFN-γ levels are often low, it is detectable in all histological variants of RA synovitis [3]. Data concerning the role of IFN-γ in RA synovitis are conflicting. Addition of IFN-γ to T cell/monocyte co-cultures enhances TNF-α production [4]. In contrast, IFN-γ can down-regulate parameters of joint destruction such as IL-1 [5], matrix metalloproteinases and proliferation of fibroblast-like synoviocytes (FLS) [6]. Contradicting results were also obtained in clinical trials evaluating the therapeutic potential of IFN-γ in RA [7, 8].

Here we investigated expression of IL-18 binding protein (IL-18BP), a decoy receptor of IL-18 [9]. Serum levels of IL-18BP are augmented in septic patients [10], and injection of human IL-18BP inhibits lipopolysaccharide-induced IFN-γ in mice by 90% [9]. In the present study on FLS cultures, we evaluated expression of IL-18BP, the most abundant splice variant of human IL-18BP, which exhibits the highest affinity for IL-18 [11].

Materials and methods

Patients

IL-18BPa expression was analysed in long-term cultured FLS from 20 RA [12], three OA, and four spondylarthropathy patients [13]. All patient materials were obtained after receiving informed consent.

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Submitted 9 November 2001; revised version accepted 4 September 2002.

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**FLS cultures**

FLS were isolated either from dissected synovial membrane tissues or from synovial fluids [14]. A total of 5 × 10^5 FLS from five RA patients was cultured for 24 h in duplicate (culture A and B, Fig 1), as unstimulated control, or exposed to IL-1/β (1 ng/ml), IL-2 (100 ng/ml), IL-12 (1 ng/ml), IL-15 (100 ng/ml), IL-18 (200 ng/ml), IFN-γ (5 ng/ml) or TNF-α (10 ng/ml) (Pepro Tech, London). Cells were also stimulated with the combinations IL-12+IL-18 and IL-1/β+TNF-α+IFN-γ.

**IL-18BP reverse transcriptase-polymerase chain reaction (RT-PCR)**

IL-18BP primers were appropriate for amplification of IL-18BP and c; forward: 5’-tgccagctgcttgaagc-3’; reverse: 5’-tcgagcacgggcatcgtcaccaact-3’; annealing temperature 56°C; 3 cycles of PCR as indicated; length of IL-18BP amplicon: 342 bp. β-Actin gene: forward: 5’-caccagccgggaaactggga-3’; reverse: 5’-acgcattcaactgttgattgga-3’; annealing temperature: 60°C; 30 cycles of PCR; length of the amplicon 552 bases.

**Analysis of IL-18BP expression by quantitative real-time PCR analysis**

Primers and probe for IL-18BP were: forward 5’-acccctccagcgactg-3’; reverse 5’-ccttcagctgcttgaagc-3’; probe 5’-caccagcgggcgaactggga-3’. For GAPDH, we used a pre-developed assay (Applied Biosystems, Weiterstadt). PCR was performed on the ABI Prism 7700 Sequence Detector (Applied Biosystems) as follows: one initial step at 50°C for 2 min and 95°C for 10 min was followed by 40 cycles at 95°C for 15 s and 60°C for 1 min.

**Detection of IL-18BP by immunoblotting**

A total of 2 × 10^6 FLS from four RA patients was cultured in serum-free supplemented Ham’s F10 medium (Gibco, Karlsruhe, Germany), as unstimulated control or stimulated with IFN-γ (5 ng/ml). After 48 h, cell-free supernatants were precipitated with trichloroacetic acid, separated by 10% SDS-PAGE (sodium dodecyl sulphate, polyacrylamide gel electrophoresis) and IL-18BP was detected using a rabbit polyclonal antiserum [15].

**Interferon-γ production by IL-12/IL-18-stimulated peripheral blood mononuclear cells (PBMC) cultivated in FLS-derived conditioned medium**

To investigate whether conditioned media from IFN-γ-stimulated FLS may contain IL-18BP activity, the following protocol was performed [15]: 10^6 FLS/ml culture medium from four different RA patients were kept as control or stimulated with IFN-γ (20 ng/ml, 16 h). Thereafter, FLS were washed with phosphate-buffered saline (3 ×) and incubated in control medium (48 h, serum free). Cell-free supernatants were concentrated 5-fold using Ultra-free-4 Biomax 10K centrifugal filters (Millipore, Bedford). Concentrated conditioned media were pre-incubated for 30 min without stimuli or with IL-12/IL-18 (20 ng/ml each), PBMC were isolated as described, resuspended in conditioned media and production of IFN-γ was determined after 24 h by enzyme-linked immunosorbent assay (ELISA) (Pharmingen, Hamburg). Experiments were performed in duplicate, statistics were done by Wilcoxon test of two-tied groups.

**Results**

Constitutive IL-18BP mRNA expression was detected by RT-PCR (35 cycles) in all 27 unstimulated FLS cultures investigated (data not shown). Only one amplicon with the length representing IL-18BP was detected. IFN-γ strongly up-regulated IL-18BP mRNA (Fig. 1A). In unstimulated FLS, more than 30 cycles were needed to detect IL-18BP amplicons, whereas the IL-18BP mRNA detection limit was reduced below 25 cycles in cultures challenged with IFN-γ. TNF-α, IL-1β, IL-2 (data not shown), IL-12, IL-15 (data not shown), IL-18, IL-12 + IL-18 showed no IL-18BP-inducing effect, and
IL-1β + TNF-α did not significantly enhance IFN-γ-induced IL-18BPα mRNA levels (Fig. 1B). Quantitative real-time PCR confirmed induction of IL-18BPα by IFN-γ in FLS (Fig. 1C). Similar results were obtained using human dermal fibroblasts (data not shown).

IFN-γ-induced mRNA in RA-FLS was accompanied by secretion of IL-18BPα immunoreactivity (Fig. 2A). Immunoreactivity appeared with a molecular mass of about 45 kDa, which is in keeping with previous reports [11, 15]. As shown in Fig. 2B, compared with conditioned media obtained from unstimulated cells, conditioned media from IFN-γ-stimulated FLS reduced production of IL-18BPα in PBMC exposed to IL-12/IL-18.

Discussion

IL-18 can activate both T cells and macrophages in the microenvironmnet of RA synovitis. IFN-γ, IL-1β and TNF-α are supposed to be induced in RA joints [1–3] and production of TNF-α and IL-1β is of paramount relevance, as highlighted by successful anti-cytokine therapies in RA [16, 17]. IL-18 bioactivity in RA might be controlled by IL-18BPα and this was addressed in this investigation. Expression of IL-18BPα mRNA in FLS and in dermal fibroblasts was markedly augmented by IFN-γ. Gene induction was accompanied by release of IL-18BPα and coincided with release of an activity from FLS that reduced IL-12/IL-18-induced IFN-γ in PBMC. IFN-γ-induced IL-18BPα has been recognized in several different cell types [15, 18, 19]. Here we present the first data on induction in resident synoviocytes. Up-regulation of IL-18BPα underscores the importance of these cells in the control of IL-18 bioactivity in synovitis. The present data suggest that FLS contribute to the complex network of immunoregulation that ensures control of primarily macrophage-derived IL-18 during synovitis. Induction of IL-18BPα was characteristic for IFN-γ. A murine model of septic antigen-induced arthritis illustrates that IFN-γ can display proinflammatory properties when given in the initiation phase. In contrast, IFN-γ ameliorates joint inflammation when given later on [20]. Detrimental functions of IFN-γ may include a more effective antigen presentation at the onset of disease, whilst inhibition of IL-18 through IFN-γ-induced IL-18BPα could represent a negative feedback mechanism upon established inflammation. Such a feedback principle concurs with reported overexpression of IFN-γ in IFN-γ receptor-deficient mice evaluated in collagen-induced arthritis [21]. In patients with adult-onset Still’s disease [22], in Crohn’s disease [19] and in patients with sepsis [10], levels of IFN-γ-inducing IL-18 appear to correlate with expression of IL-18BPα. These observations suggest a close association of IL-18 with expression of its decoy receptor in inflammatory diseases.

Induction of IL-18BPα by IFN-γ could prove to be a crucial endogenous corrective that counterregulates IL-18-mediated leucocyte activation in rheumatoid arthritis. Although this feedback loop is functional in RA materials, there is still IL-18 bioactivity demonstrable in RA synovial fluids, despite the presence of an IL-18 inhibitory activity [23]. Therefore, therapeutic administration of IL-18BPα in patients may modulate the cytokine balance, thereby ameliorating established arthritis, as recently observed in a RA animal model [24].

Acknowledgements

This work was supported by the Riese- and the Klein-Foundation. We thank S. Rehart for providing synovial material. We thank J. Bauer, S. Garkisch and S. Höfler for technical assistance.

References


