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IL-18BPa:Fc cooperates with immunosuppressive drugs in human whole blood

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Abstract

The proinflammatory cytokine interleukin (IL)-18 appears to be involved in the pathogenesis of diseases associated with immunoactivation and inflammation. Consequently, blockage of IL-18 bioactivity by use of IL-18 binding protein (IL-18BP) is likely a promising therapeutic concept. In the present study, we investigated immunomodulatory activities of IL-18BPa:Fc in human whole blood cultures. We report that IL-18BPa:Fc (200 ng/mL) significantly inhibited lipopolysaccharide (LPS, 10 ng/mL)/IL-12 (5 ng/mL)-induced release of interferon-γ (IFNγ) and matrix metalloproteinase-9 (MMP-9) from whole blood cultures of healthy donors. Notably, IL-18BPa:Fc (200 ng/mL) further reinforced dexamethasone (5 nM)- or mycophenolic acid (2 μM)-mediated reduction of LPS/IL-12-induced IFNγ production by an additional 50.5 or 49.9%, respectively. To investigate effects of IL-18BP:Fc in the context of autoimmune diseases, experiments were performed with whole blood obtained from patients with systemic lupus erythematosus or Wegener's granulomatosis undergoing immunosuppressive therapy. After *ex vivo* stimulation with LPS (10 ng/mL), production of IFNγ and MMP-9 was determined. Both mediators likely contribute to renal inflammation frequently seen in these diseases. In accord with the aforementioned data, LPS (10 ng/mL)-induced IFNγ was significantly reduced by coincubation with IL-18BPa:Fc at 200 ng/mL. IL-18BPa:Fc also inhibited production of MMP-9. The present data demonstrate that IL-18BPa:Fc has the potential to amplify anti-inflammatory actions of immunosuppressive drugs, and thus may prove to be a valuable novel pharmacological component in the treatment of human autoimmune diseases.

Keywords: Interleukin-18; Interferon-γ; Inflammation; Immunosuppressive agents; Autoimmune diseases

1. Introduction

IL-18 is a proinflammatory cytokine that is of pivotal importance as a costimulus for production of IFNγ. IL-18 is also able to mediate IFNγ-independent functions like expression of tumor necrosis factor-α, IL-1β, IL-8 [1], and MMP-9 [2]. Enhanced production of IL-18 has been linked to the pathogenesis of diseases such as rheumatoid arthritis [3], nephrotic syndrome [4], and SLE [5]. Blockage of IL-18 bioactivity by use of its secreted decoy receptor IL-18BPa therefore is a promising novel therapeutic strategy [6]. Upregulation of IFNγ has been associated with autoimmune diseases such as SLE [7] and WG [8] and levels of MMP-9 are augmented in SLE [9] which likely contributes to the inflammatory character of this syndrome [2].

Current treatment of autoimmune diseases by immunosuppressive agents aim at undermining lymphocyte proliferation and chronic inflammation. However, treatment

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Abbreviations: Dex, dexamethasone; IL, interleukin; IL-18BPa, IL-18 binding protein a; IL-18BP:Fc, IL-18BPa as Fc-chimeric molecule; IFNγ, interferon-γ; LPS, lipopolysaccharide; MMF, mycophenol mofetil; MPA, mycophenolic acid; MMP-9, matrix metalloproteinase-9; PBMC, peripheral blood mononuclear cells; SLE, systemic lupus erythematosus; WG, Wegener's granulomatosis; WBC, whole blood cultures.

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with immunosuppressants and anti-inflammatory drugs is unspecific and accompanied by side-effects that may cause serious clinical complications. Thus, an important goal of pharmacological research is to minimize concentrations of these immunosuppressive agents, possibly by use of combination therapy. Here, we investigated immunopharmacological effects of IL-18BPa:Fc in the human whole blood system.

2. Materials and methods

Dex and MPA were from Calbiochem-Novabiochem and Sigma, respectively. LPS (026B6) was from Sigma. IL-12 and IL-18BPa:Fc were from R&D systems. IL-18BPa:Fc is a recombinant chimeric protein consisting of IL-18BPa and the Fc domain of human IgG1.

2.1. Human whole blood (WBC) and peripheral blood mononuclear cell cultivation (PBMC)

Heparinized peripheral blood was obtained from healthy donors and patients after informed consent and was mixed with an equal volume of culture medium (RPMI 1640 supplemented with 25 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin). One milliliter aliquots were transferred into round-bottom polypropylene tubes (Greiner). Sealed tubes were incubated at 37° and 5% CO_2. Plasma was prepared and was stored at −70°. PBMC were freshly isolated and cultivated in the aforementioned medium plus 1% (v/v) heat-inactivated human AB serum (Sigma) as described [2].

2.2. Detection of IFNγ, IL-18 and MMP-9 in human serum

Levels of IFNγ (Pharmingen) and IL-18 (MBL) were assessed by enzyme-linked immunosorbent assay (ELISA) as instructed by the manufacturers. Determination of gelatinolytic activity caused by MMP-9 was performed by SDS–PAGE zymography as described in [2].

2.3. Statistical analysis

Data are shown as means ± SEM, pg/mL, or (percent of LPS/IL-12). Unless otherwise indicated, data were analyzed by Wilcoxon test on raw data using Sigma Stat (Jandel Scientific).

3. Results

Suppression of IL-12/IL-18-induced IFNγ production by PBMC confirmed bioactivity of IL-18BPa:Fc (Fig. 1A). To further investigate immunomodulatory effects of IL-18BPa:Fc, WBC derived from healthy donors were broadly activated by the combination LPS/IL-12. As shown in Fig. 1B, induction of IFNγ was significantly inhibited by IL-18BPa:Fc. A dose–response curve revealed that IL-18BPa:Fc as low as 12.5 ng/mL was able to significantly modulate IFNγ production. In some experiments, concentrations of IL-18BPa:Fc below 50 ng/mL were unexpectedly more effective than higher concentrations (Fig. 1D). In addition to IFNγ, secretion of MMP-9 was inhibited by IL-18BPa:Fc in all experiments performed (Fig. 1C). IL-18 was detected in WBC and was significantly increased by LPS/IL-12 (Fig. 1E).

Next, we sought to elucidate whether IL-18BPa:Fc is able to reinforce anti-inflammatory properties of two immunosuppressive drugs, namely glucocorticoids [10] and mycophenolate mofetil [11]. For *in vitro* studies, the glucocorticoid dexamethasone and the active metabolite of MMF, MPA were used. Dexamethasone (5 nM) and MPA (2 μM) alone decreased LPS/IL-12-induced IFNγ by 44.3 and 32.2%. Coincubation with IL-18BPa:Fc further enhanced inhibition by dexamethasone or MPA significantly by another 50.5 and 49.9% (Fig. 2A). Furthermore, actions of IL-18BPa:Fc on WBC obtained from patients with SLE and WG were investigated. These patients were under immunosuppressive therapy with glucocorticoids, cyclophosphamide, or MMF. Augmented levels of IL-12 have been reported for both autoimmune diseases [5,8]. Thus, WBC were stimulated with LPS (10 ng/mL) alone. Under these conditions in 63% of experiments ($n = 19$) only weak (below 200 pg/mL) or undetectable induction of IFNγ by LPS was observed, which should be due to effective immunosuppression during therapy. In seven independent WBC experiments obtained from four different patients, a robust induction of IFNγ was detected. The clinical data of these patients are shown in Table 1. In all these seven experiments, release of IFNγ could be reduced by coincubation with

Table 1

Patient	Age gender	Autoimmune disease	Organ manifestation symptoms	Immunosuppressive therapy
M	35, female	SLE	Skin, kidney proteinuria (0.2 g per day), leukopenia ANA, anti-DNA positive C3 low	Prednisone (7.5 mg per day) MMF (1.5 g per day p.o.)
G	32, female	SLE	Lung, kidney increasing proteinuria (1.3 g per day) ANA, anti-DNA positive C3 low	Prednisone (10 mg per day)
P	22, male	WG	Lung, kidney proteinuria (5.7 g per day) cANCA positive	Prednisone (2.5 mg per day), cyclophosphamide (100 mg per day p.o.)
B	40, male	WG	Kidney proteinuria (0.2 g per day) cANCA positive	Cyclophosphamide (75 mg per day p.o.)

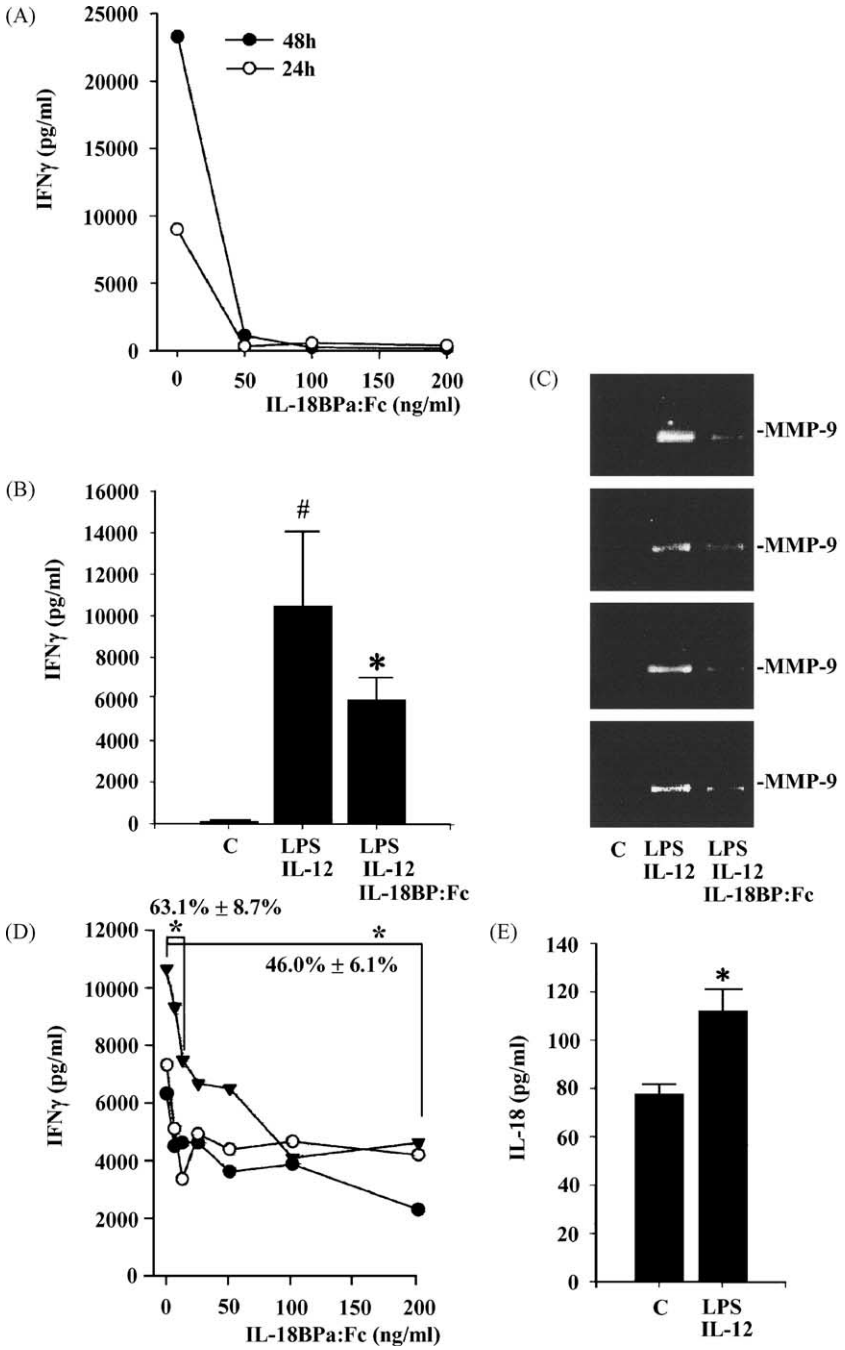

Fig. 1. IL-18BPa:Fc inhibits IFNγ and MMP-9 in WBC from healthy donors exposed to LPS/IL-12. (A) PBMC were stimulated with IL-12 (10 ng/mL)/IL-18 (50 ng/mL) in presence or absence of the indicated concentrations of IL-18BPa:Fc for 24 or 48 hr. Thereafter, IFNγ was determined by ELISA. (B–E) WBC were stimulated with LPS (10 ng/mL)/IL-12 (5 ng/mL) in the presence or absence of IL-18BPa:Fc at 200 ng/mL (B, C) or the indicated concentrations of IL-18BPa:Fc (D). After 24 hr, plasma IFNγ was determined by ELISA (B, D). (B) Mean IFNγ ± SEM are shown (N = 9). $^{\#}P < 0.05$ compared to unstimulated control; $^{*}P < 0.05$ compared to LPS/IL-12 alone. (D) Data of three donors are given as absolute IFNγ concentrations. $^{*}P < 0.05$ compared to LPS/IL-12 alone (paired Student's *t*-test on raw data). The percent values quantify inhibition at 12.5 and 200 ng/mL of IL-18BPa:Fc for these three donors. (C) Release of MMP-9 was assessed by zymography. Four representative gels of five independently performed experiments are shown. (E) WBC were stimulated with LPS (10 ng/mL)/IL-12 (5 ng/mL). After 24 hr, plasma IL-18 was determined by ELISA. Mean IL-18 ± SEM are shown (N = 6). $^{*}P < 0.05$ compared to unstimulated control.

IL-18BPa:Fc. A significant 33.6% reduction was detected for IL-18BPa:Fc at 200 ng/mL. Interestingly, in two donors (B and M) lower concentrations of IL-18BPa:Fc (<50 ng/mL) mediated better inhibition compared with 200 ng/mL (Fig. 2B). Figure 2C separately displays data from one of these patients (M) with WBC experiments performed after three consecutive hospital visits (M1: flare up; M2, M3: earlier visits). As shown in Fig. 2D, release of MMP-9 in response to LPS also was suppressed by IL-18BPa:Fc.

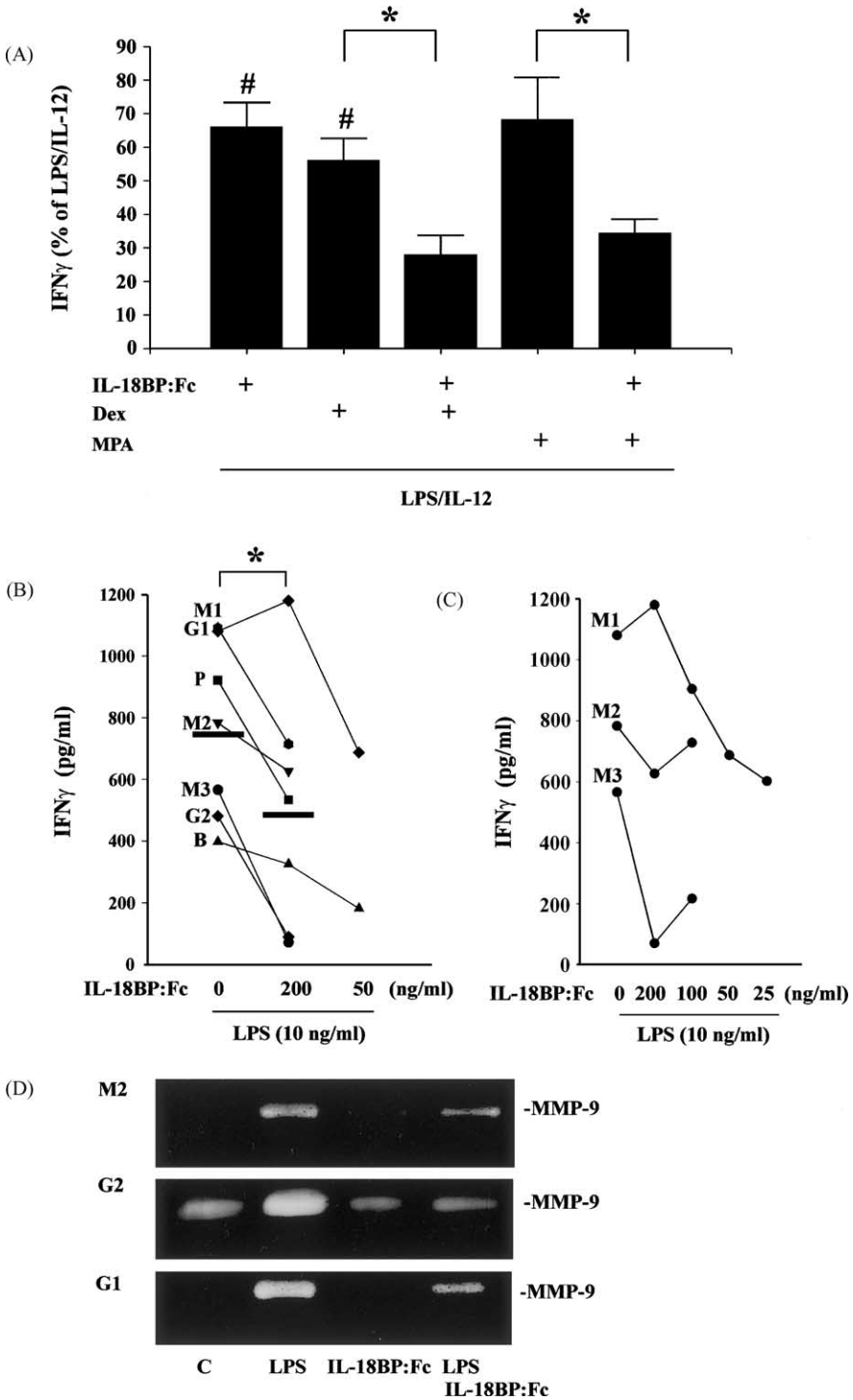

Fig. 2. IL-18BPa:Fc cooperates with immunosuppressive drugs in WBC. (A) WBC were stimulated with LPS (10 ng/mL)/IL-12 (5 ng/mL), with LPS/IL-12 plus IL-18BPa:Fc (200 ng/mL), with LPS/IL-12 plus Dex (5 nM), with LPS/IL-12 plus Dex plus IL-18BPa:Fc, with LPS/IL-12 plus MPA (2 μM), or with LPS/IL-12 plus MPA plus IL-18BPa:Fc. After 24 hr, plasma IFNγ was determined by ELISA. Data are shown as percent of LPS/IL-12 ± SEM (N = 6). $^{\#}P < 0.05$ compared to LPS/IL-12 alone. $^{*}P < 0.05$ compared to either LPS/IL-12 plus Dex or LPS/IL-12 plus MPA. (B) Seven independent WBC experiments were performed using blood from four patients (Table 1). WBC were stimulated with LPS (10 ng/mL) or with LPS plus the indicated concentrations of IL-18BPa:Fc. After 24 hr, plasma IFNγ was determined by ELISA. Absolute IFNγ concentrations are shown. $^{*}P < 0.05$ compared to LPS alone. (C) Data from three consecutive hospital visits of patient M are shown. WBC were treated as in (B). (D) WBC were kept as unstimulated control or stimulated with LPS (10 ng/mL) or with LPS plus IL-18BPa:Fc (200 ng/mL). After 24 hr, release of MMP-9 was assessed by zymography. Three independent experiments with WBC from two different patients (M and G) are shown.

4. Discussion

Current treatment of autoimmune diseases is based on immunosuppressive and anti-inflammatory regimens that utilize drugs like glucocorticoids, cyclophosphamide, or MMF. As serious side effects often accompany chronic treatment, reduction of drug dosage and steroid-sparing regimen are desired and may be achieved by combination therapy. In this context, anticytokine strategies are of particular interest. This is illustrated by the success of arthritis therapies based on methotrexate in combination with IL-1 receptor antagonist or soluble tumor necrosis factor-α receptors [12]. IL-18BP is a novel candidate for anticytokine therapy that proved to be effective in collagen-induced arthritis [3]. Significant upregulation in septic shock patients implies an important role of IL-18BP as a modulator of immune responses [13]. IL-18BP is induced by IFNγ, thus establishing a negative feedback loop that can be observed *in vitro* and *in vivo* [14,15]. Previously, it has been shown that IL-18BP inhibits IFNγ production in IL-12-activated PBMC [16] and in WBC exposed to *Staphylococcus epidermidis* [17]. The present observation that IL-18BPa:Fc significantly decreased LPS/IL-12-induced IFNγ in WBC is in accord with these data. It is of particular relevance that IL-18BPa:Fc was still active at low concentrations (<12.5 ng/mL), a fact that underscores its therapeutic potential. We also report that MMP-9, another mediator of inflammation, was suppressed by IL-18BPa:Fc. Average levels of IL-18 in the sera of healthy donors are 64 pg/mL [13]. Here, we detected 77.3 pg/mL in diluted plasma obtained from unstimulated WBC (1:2 dilution of human whole blood). LPS/IL-12 activation increased IL-18 significantly to 145.4% of control. These data suggest that IL-18 in the WBC system was generated partially *in vivo* and particularly under the influence of LPS/IL-12 in culture.

Using LPS/IL-12-activated WBC from healthy donors, we show that IL-18BPa:Fc further amplified dexamethasone or MPA inhibition of IFNγ release by 50%. A similar effect was observed when WBC from patients with clinically active SLE or WG (Table 1) undergoing immunosuppressive therapy were stimulated *ex vivo* with LPS. Reduction of IFNγ and MMP-9 by IL-18BPa:Fc was detectable in all experiments performed. Control of IFNγ production appears to be an important therapeutic goal in SLE as suggested by murine models of the disease. Moreover, administration of IFNγ can mediate a lupus-like disease in humans [18,19]. Taken together, the present data demonstrate for the first time the potential of IL-18BP as part of combination therapy together with immunosuppressive drugs and further underscore the prospective of IL-18 antagonism in immunopharmacology.

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