IL-18 binding protein increases spontaneous and IL-1-induced prostaglandin production via inhibition of IFN-γ

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IL-18 shares with IL-1 the same family of receptors and several identical signal transduction pathways. Because of these similarities, IL-18 was investigated for its ability to induce prostaglandin E_2 (PGE_2) synthesis in human peripheral blood mononuclear cells (PBMC), a prominent, proinflammatory property of IL-1. IL-18 was highly active in PBMC by inducing the synthesis of the chemokine IL-8; however, no induction of PGE_2 synthesis nor cyclooxygenase type-2 gene expression was observed in PBMC stimulated with IL-18. In the same cultures, IL-1β induced a 12-fold increase in PGE_2. Although IL-1β-induced IL-8 synthesis was augmented 3-fold by IL-18, IL-18 suppressed IL-1β-induced PGE_2 production by 40%. The suppressive effect of IL-18 on PGE_2 production was mediated by interferon (IFN)-γ because anti-human IFN-γ-antibody prevented IL-18-induced reduction in PGE_2. Consistent with these observations, IL-12, a known inducer of IFN-γ, augmented IL-1β-induced IFN-γ but suppressed IL-1β-induced PGE_2 by 75%. IL-18 binding protein (IL-18BP) is a naturally occurring and specific inhibitor of IL-18. When recombinant IL-18BP was added to PBMC cultures, unexpectedly, spontaneous PGE_2 production increased. PGE_2 production was also increased by the addition of IL-18BP to PBMC stimulated with either IL-1β or IL-12 and also in whole blood cultures stimulated with *Staphylococcus epidermidis*. These studies demonstrate that IL-18BP decreases endogenous IL-18 activity by reducing IFN-γ-mediated responses.

IL-18, initially described in 1989 as interferon (IFN) γ inducing factor (1), shares with IL-1β its signature amino acid sequences, three-dimensional structural similarities, and caspase-1 processing of the precursor form (reviewed in ref. 2). Both chains of IL-18 receptor (IL-18R) are members of the IL-1 receptor (IL-1R) family. The ligand binding IL-18R chain (3), now termed IL-18Rα, was initially described as the IL-1 receptor-related protein (4). It shares chromosomal location with IL-1R types I and II (5) and is similar to the IL-1R type I (3, 6, 7). The IL-18 receptor accessory protein-like molecule (8), also known as IL-18Rβ chain, is similar to the IL-1R accessory protein (9) and is required for signaling (8). IL-12, a known IFN-γ inducer, increases the surface expression of the IL-18Rα (10) and is thought to account for the synergism of IFN-γ by IL-18 and IL-12.

IL-1 and IL-18 signaling involves activation of identical cytoplasmic messengers: MyD88, IL-1 receptor-associated kinase, tumor necrosis factor (TNF) receptor-associated factor-6, p38 mitogen-activated protein kinase, jun kinase, and β-casein kinase TNF/IL-1-activated protein. Although previously considered as specifically activated by IL-1, these second messengers are also activated by IL-18 (6, 11, 12). The ability of IL-18 to induce the synthesis of TNFα, IL-1β, IL-8, and other chemokines (13) as well as to activate nuclear factor-κB (14) places IL-18 together with other pro-inflammatory cytokines. Therefore, it was expected that IL-18 would share other pro-inflammatory activities with IL-1. IL-1 consistently induces prostaglandin (PG) synthesis (15–17) and cyclooxygenase type-2 (COX-2) gene expression (reviewed in ref. 18). Olee and coworkers reported that IL-18 is produced by articular chondrocytes and induces collagenases and COX-2 gene expression (19).

In contrast to IL-18, IL-18 binding protein (IL-18BP) is a novel, constitutively expressed and secreted protein that resembles the extracellular domains of Ig-like receptors but has no transmembrane form (20). IL-18BP binds IL-18 with a high affinity (dissociation constant of 400 pM) and blocks its biological activities (20, 21). Because IL-18 is one of the early signals leading to IFN-γ production by T-helper (Th) type-1 cells, blocking IL-18 activity by IL-18BP may be involved in down-modulation of the early phases of immune responses. However, PGE_2 also down-modulates immune responses, particularly of T-cells. Because of signaling similarities of IL-18 with those of IL-1, we studied the role for IL-18 and IL-18BP in PGE_2 production by human peripheral blood mononuclear cells (PBMC).

Materials and Methods

Reagents and Cytokines. RPMI 1640 culture medium was purchased from Cellgro (Waukesha, WI) and was prepared as described (13). Lyophilized lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 as well as other chemicals including Histopaque-1077 were purchased from Sigma. Recombinant human IL-18 was expressed and purified by Vertex Pharmaceuticals (Cambridge, MA) as described (13). IL-1β was supplied by Cistron (Pine Brook, NJ). IFN-γ was a gift from Michael Palladino (Genentech). Granulocyte-macrophage colony-stimulating factor was obtained from R & D Systems. IL-12 was a gift from Genetics Institute (Cambridge, MA). Recombinant human IL-18BP (IL-18BPa His6-tag) was purified from COS cells as described (20, 21) or from Chinese hamster ovary cells (21) (Interpharm Laboratories, Nes Ziona, Israel). The monoclonal mouse anti-human IFN-γ antibody used in this study was developed and characterized as reported (22).

Isolation of PBMC and Whole Blood. These studies were approved by the Combined Colorado Investigational Review Board. Residual peripheral blood was obtained from blood lines after plateletpheresis of healthy volunteers. PBMC were isolated from this blood over Histopaque cushions. The cells were aspirated

Abbreviations: BP, binding protein; Th, T-helper cell; IL-1R, IL-1 receptor; PGE_2, prostaglandin E_2; TNF, tumor necrosis factor; PBMC, peripheral blood mononuclear cells; COX-2, cyclooxygenase type-2; LPS, lipopolysaccharide; RT-PCR, reverse-transcribed PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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from the interface, were washed three times in pyrogen-free saline, were resuspended in RPMI 1640, were cultured in flat-bottomed 24-well plates (Becton Dickinson) unless otherwise stated, and were incubated at 37°C in humidified air with 5% CO_2. Whole blood was collected in heparinized tubes as described (23). Whole blood was stimulated with heat-killed *Staphylococcus epidermidis* at a ratio of 10 organisms/white blood cell (24). After 24 hr, the whole blood cell culture was lysed in 0.5% Triton X-100 and was assayed for PGE_2.

Macrophage Preparation. To study purified macrophages, PBMC were allowed to adhere to flat-bottomed six-well plates (Becton Dickinson) at 37°C for 6 hr in serum-free RPMI 1640 medium. Plastic-adhered cells were washed three times with saline and were cultured in RPMI 1640 supplemented with 10% of fetal bovine serum and 10 ng/ml of granulocyte-macrophage colony stimulating factor. The medium in macrophage cultures was replaced every 3 days. Cells were used in experiments on day 6 after isolation.

Analysis of Cytokines. The liquid-phase electrochemiluminescence method was employed to measure IFN-γ (23) and IL-8 (13) concentrations in cell culture media. The amount of chemiluminescence was determined by using an Origen Analyzer (Igen, Gaithersburg, MD). The limit of detection of IFN-γ and IL-8 was 62 pg/ml and 40 pg/ml, respectively.

Analysis of PGE_2. An enzyme-linked immunoassay using acetylcholinesterase-conjugated tracer was used for quantification of PGE_2 levels in culture media as described (25, 26). Precoated plates with polyclonal goat anti-mouse antibody, tracers, standards, and mouse monoclonal anti-PGE_2 antibody were from Cayman Chemicals (Ann Arbor, MI). PBMC supernatants were separated from residual cells by centrifugation in a microfuge at $5{,}000 \times g$ for 5 min at 4°C and were assayed for PGE_2 in duplicate at dilutions of 1:2 to 1:200 without purification. Standard curves and samples were analyzed by using the four-parameter curve fit option on DELTA SOFT 3 software (Biometallics, Princeton). The sensitivity of the assay was 25 pg/ml.

Reverse-Transcribed (RT)–PCR. Total RNA was isolated from PBMC by using Tri-Reagent (Molecular Research Center Inc., Cincinnati). In brief, cells were pelleted and lysed in Tri-Reagent, and the RNA was sequentially isolated after chloroform extraction and isopropanol precipitation. The RNA was dissolved in diethyl pyrocarbonate-treated water and was quantitated by using GeneQuant (Amersham Pharmacia). To prepare cDNA, 1 μg of total RNA was reverse transcribed by using random hexamers as a template. The reaction took place in a total volume of 20 μl containing 5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris·HCl (pH 8.3), 1 mM of each dNTP, 20 units of RNase inhibitor, and 50 units of Moloney leukemia virus reverse transcriptase (Perkin–Elmer, Branchburg, NJ). The reaction was incubated at 42°C for 30 min and was terminated by 95°C for 5 min. For PCR, 2 μl of the RT product was used with primers (20 mM each): 5′-TTG TTC CAG ACA AGC AGG C-3′ (sense) and 5′-CAT TCC TAC CAC CAG CAA CC-3′ (antisense) for COX-2 (19) or 5′-ACC ACA GTC CAT GCC ATC AC-3′ (sense) and 5′-TCC ACC ACC CTG TTG CTG TA-3′ (antisense) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (obtained from CLONTECH). The reaction took place in a total volume of 50 μl of PCR SuperMix (Life Technologies, Gaithersburg, MD) containing 22 mM Tris·HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 mM dGTP, mM dATP, mM dTTP, mM dCTP, and 22 units of recombinant *Taq* DNA polymerase/microliter. The following sequence was performed in a thermocycler (Perkin–Elmer) for each PCR reaction: 90°C for 5 min and 55°C for 5 min (1 cycle), followed immediately by 72°C for 1 min, 90°C for 1 min, and 55°C for 1 min (with variable number of cycles) and a final extension phase at 72°C for 10 min. The variable number of cycles was to ensure that amplification occurred in the linear phase and that differences between control and experimental conditions were maintained by adopting a limited number of cycles. The PCR amplification using GAPDH as the internal control was performed on each sample to insure that differences between tubes were not the result of unequal concentrations of RNA.

The PCR products were separated on 1.6% agarose gels containing 1× Tris-borate-EDTA (50 mM Tris/45 mM boric acid/0.5 mM EDTA, pH 8.3) with 0.5 mg/ml ethidium bromide, were visualized by UV illumination, and were photographed. The predicted sizes of the PCR products were 337 bp and 452 bp for COX-2 and GAPDH, respectively.

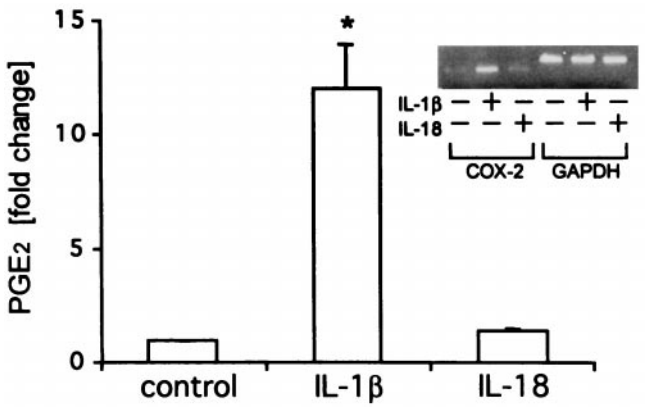

Fig. 1. Production of PGE_2 by PBMC in response to IL-1β or IL-18. PBMC were cultured at 5×10^6 cells/ml in 1 ml of RPMI containing IL-12 (5 ng/ml) and were incubated for 48 hr in the presence or absence of IL-1β (100 ng/ml) or IL-18 (5 nM). The data represent the fold-change ±SEM over the level of spontaneous PGE_2 production in unstimulated cells (set at 1). $n = 3$ donors (*, $P < 0.01$). *Inset* depicts the RT-PCR-amplified mRNA of COX-2 and GAPDH from PBMC stimulated with IL-1β (100 ng/ml) or IL-18 (5 nM) and rotated at 7 rpm (Rollerdrum, New Brunswick Scientific) for 6 hr at 37°C. RT-PCR data represent one of three similar experiments.

Statistical Analysis. Cytokine and PGE_2 were measured in duplicate, and the average of the two measurements was used in the statistical analysis. Because the responses of individual blood donors vary, control values were set at 1, and the mean as fold-change over control (± standard error of the mean SEM) was calculated. Group means were compared by analysis of variance using Fisher's least significant difference and paired comparison *t* test. Analysis was performed with the statistical package STATVIEW 512+ (BrainPower, Calabasas, CA).

Results

IL-18 does not induce PGE_2 production or COX-2 gene expression in PBMC. To evaluate the effect of IL-18 on COX-2 gene expression, PBMC were stimulated with either IL-1β or IL-18 for 6 hr. To avoid expression of the COX-2 gene secondary to monocyte adherence to plastic surfaces, PBMC were rotated during the incubation at 7 rpm (Rollerdrum, New Brunswick Scientific). Unlike chondrocytes (19), the level of COX-2 gene expression by IL-18 in PBMC did not differ from the control level (Fig. 1 *Inset*). In the same cells and under the same conditions, IL-1β markedly increased steady state levels of COX-2 mRNA. In each of these experiments, under the same conditions employed to induce PGE_2 production or COX-2 gene expression, IL-18 increased IL-8 production 12.3 ± 1.6-fold over spontaneous synthesis ($n = 3$, $P < 0.01$) as previously reported (13).

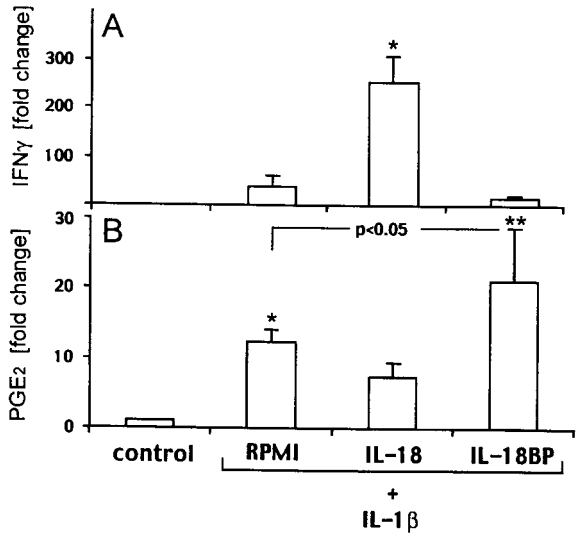

Fig. 2. The effect of IL-18 and IL-18BP on IL-1β-induced PGE_2 and IFN-γ production in PBMC. PBMC were cultured in flat bottom wells at 5×10^6 cells/ml in RPMI containing IL-12 (5 ng/ml) in the presence or absence of IL-1β (100 ng/ml). In addition, PBMC were simultaneously stimulated with either IL-18 (5 nM) or IL-18BP (100 ng/ml). After 48 hr, levels of IFN-γ (*A*) and PGE_2 (*B*) were measured. The data represent the fold-increase ±SEM over that of the spontaneous production in unstimulated cells (set at 1). $n = 8$ donors. (*A*) *, $P <$ 0.05 compared with IL-1β only. (*B*) *, $P < 0.05$ compared with control; **, $P <$ 0.01 compared with the combination of IL-1β plus IL-18.

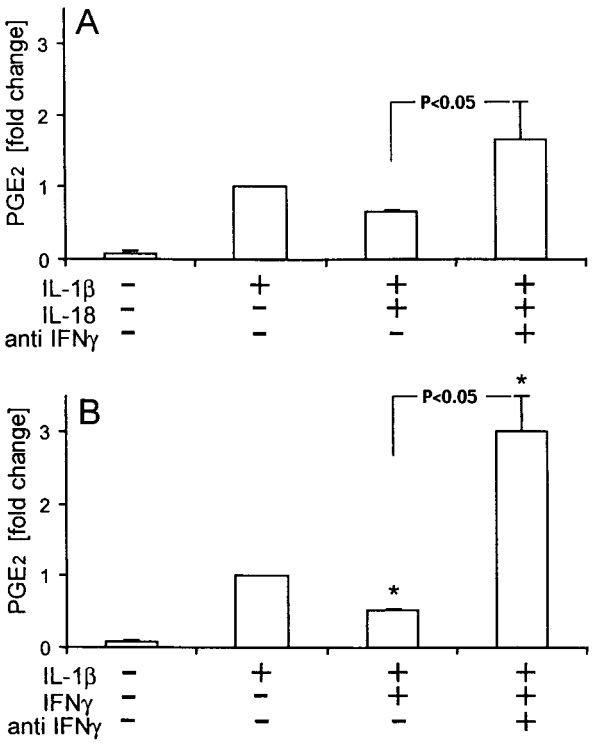

Fig. 3. Effect of anti-IFN-γ antibody on suppression of IL-1β-induced PGE_2 production by IL-18. (*A*) PBMC from three donors were cultured in flat bottom wells at 5×10^6 cells/ml in RPMI containing IL-12 (5 ng/ml) in the absence or presence of IL-1β (100 ng/ml), IL-18 (5 nM), or the combination of these plus anti-IFN-γ antibody (1:1,000). (*B*) PBMC from the same donors were incubated with IL-1β (100 ng/ml), IFN-γ (30 units/ml), or the combination of these plus anti-IFN-γ antibody (1:1,000). PGE_2 data represent the fold-change ±SEM over that of the production in IL-1β-stimulated cells (set at 1). *, $P < 0.05$ as calculated by paired *t* test compared with levels from unstimulated cells.

As shown in Fig. 1, the mean increase in IL-1β-induced PGE_2 was 12-fold after 48 hr of incubation compared with unstimulated cells (set at 1, $P < 0.01$). In contrast, PGE_2 synthesis in these same PBMC cultures stimulated with IL-18 was not detected. Overall, there was a positive response to IL-1β-induced PGE_2 in each of 16 human blood donors examined, but the level of induction varied over a broad range. In unstimulated PBMC cultures, 48-hr production of PGE_2 ranged from 0.1 to 2 ng/5×10^6 PBMC. In IL-1β-stimulated cultures, PGE_2 increased 3- to 100-fold (range 1.5–60.0 ng/5×10^6 PBMC) over control cultures. IL-8, but not PGE_2, production in response to IL-18 was observed in the PBMC of these donors.

IL-18 Does Not Induce PGE_2 Production in Macrophages. The possibility that IL-18 induces PGE_2 in macrophages in the absence of lymphocytes was next investigated. Adherent blood monocytes were cultured for 6 days in the presence of fetal bovine serum and granulocyte-macrophage colony stimulating factor. Macrophage cultures stimulated with 10 ng/ml of LPS served as a positive control. LPS stimulation resulted in 83 ± 31-fold increase of PGE_2 in macrophages ($n = 4$, $P < 0.01$) whereas 5 nM concentrations of IL-18 did not result in PGE_2 synthesis. In the same macrophages that exhibited a failure to produce PGE_2 in response to IL-18, synthesis of IL-8 was 3 ± 0.6-fold increased by IL-18 ($P < 0.05$). No IFN-γ was detected in these cultures.

The Effect of IL-18 and IL-18BP on IL-1β-Induced IL-8 and PGE_2 Synthesis in PBMC. In PBMC, IL-1β-induced IL-8 production was augmented 3-fold by the presence of IL-18 (data not shown). However, IL-1β-induced IFN-γ production was greatly enhanced by IL-18 (Fig. 2*A*) and differed significantly from control levels ($P < 0.05$). In contrast, we observed a consistent reduction of ≈40% of IL-1β-induced PGE_2 synthesis in PBMC in the same IL-18-stimulated cultures (Fig. 2*B*).

The suppressive activity of IL-18 on IL-1β-induced PGE_2 production (Fig. 2*B*) was further studied by blocking the activity of endogenous IL-18 with IL-18BP. IL-18 is constitutively expressed in human PBMC (27). The presence of IL-18BP completely reversed the enhancing effect of IL-18 on IL-1β-induced IFN-γ (Fig. 2*A*). On the other hand, IL-18BP augmented IL-1β-induced PGE_2 production (Fig. 2*B*) 1.8-fold compared with levels from IL-1β-treated cells ($P < 0.05$) and more than 2-fold compared with the combination of IL-18 plus IL-1β.

The Effects of IL-18 and IL-18BP on IL-1β-Induced PGE_2 Production Are Mediated by IFN-γ. We were able to confirm that IFN-γ suppresses IL-1β-induced PGE_2 release from PBMC (28). To assess the role of IFN-γ on the suppressive effect of IL-18 on IL-1β-induced PGE_2, a neutralizing monoclonal antibody to IFN-γ was added to PBMC. As shown in Fig. 3*A*, the anti-IFN-γ antibody reversed the suppressive effect of IFN-γ on IL-1β-induced PGE_2 production. In fact, there was a 2-fold ($P < 0.05$) increase over the levels produced from cells stimulated with IL-1β plus IL-18. Similar to the IL-18 effect, PBMC stimulated with IL-1β in the presence of IFN-γ resulted in a reduction in IL-1β-induced PGE_2 by 50% (Fig. 3*B*). Incubation of PBMC with IL-1β plus IFN-γ in presence of anti-IFN-γ antibody augmented PGE_2 production almost 3-fold over IL-1β-induced production level (Fig. 3*B*; $P < 0.05$).

We did not observe an effect of IL-18 or IL-18BP on LPS-induced PGE_2 production. LPS (10 ng/ml) alone or in combination with IL-18BP increased PGE_2 production equally (90.5 ± 7.4- and 84.8 ± 13-fold over the level of spontaneous production). Others have reported that IFN-γ does not suppress

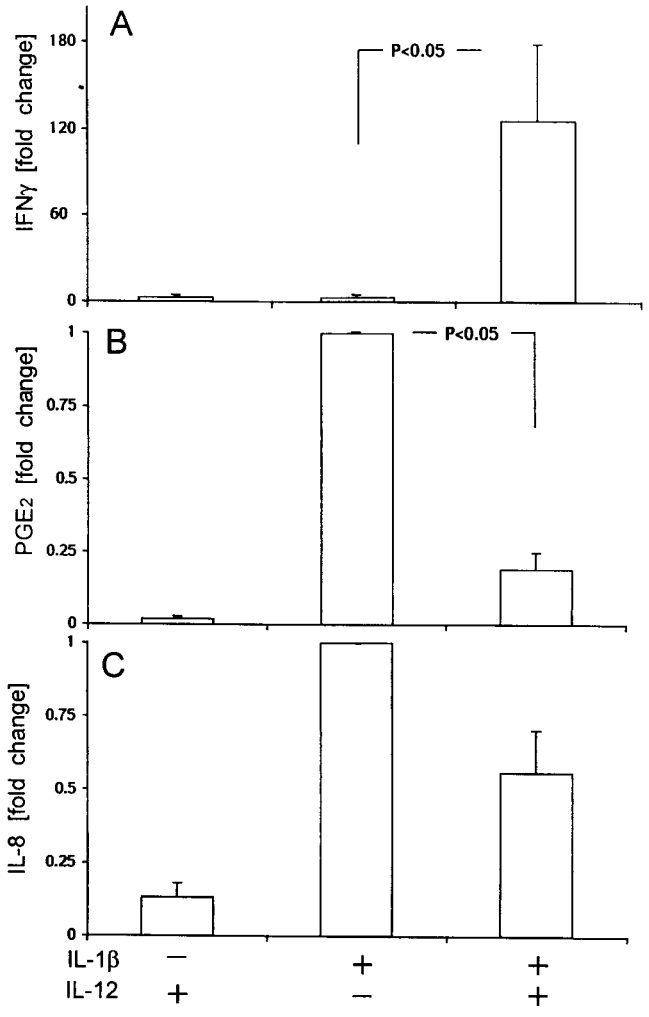

Fig. 4. The effect of IL-12 on IL-1β-induced PGE_2, IL-8, and IFN-γ production in PBMC. PBMC from five donors were cultured in flat bottom wells at 5×10^6 cells/ml in RPMI and were incubated with either IL-1β (100 ng/ml), IL-12 (5 ng/ml), or the combination of these. After 48 hr, IFN-γ (*A*), PGE_2 (*B*), and IL-8 (*C*) were measured. The data represent the fold-change ±SEM over that of the production in IL-1β-stimulated cells (set at 1). Statistical significance was calculated by paired *t* test.

LPS-induced COX-2 expression whereas IL-4, IL-10, or IL-13 do (29). The selective inhibitory effect of IFN-γ on IL-1β-induced COX-2 but not on LPS-induced COX-2 gene transcription has been observed (30), similar to the selective effect of IFN-γ on IL-1β induced by IL-1α (31, 32).

IL-12 Induces IFN-γ Synthesis and Suppresses PGE_2 Production in IL-1β-Treated PBMC. IL-12 is also an IFN-γ-inducing cytokine (33). The addition of IL-12 to IL-1β-stimulated PBMC induced IFN-γ production (Fig. 4*A*). But, as expected with increased IFN-γ production, IL-12 suppressed 75% of IL-1β-induced PGE_2 production (Fig. 4*B*; $P < 0.05$). IL-12 also exhibited a trend to decrease IL-1β-induced IL-8 production (Fig. 4*C*).

The induction of IFN-γ by IL-12 in PBMC likely includes the activity of constitutively produced endogenous IL-18 (27). To reveal a role for endogenous IL-18, resting PBMC were treated with increasing concentrations of IL-12 in the presence or absence of IL-18BP. As depicted in Fig. 5, IL-18BP prevented IL-12-induced IFN-γ production in PBMC at each concentration tested. These data demonstrate that IL-12-induced IFN-γ from

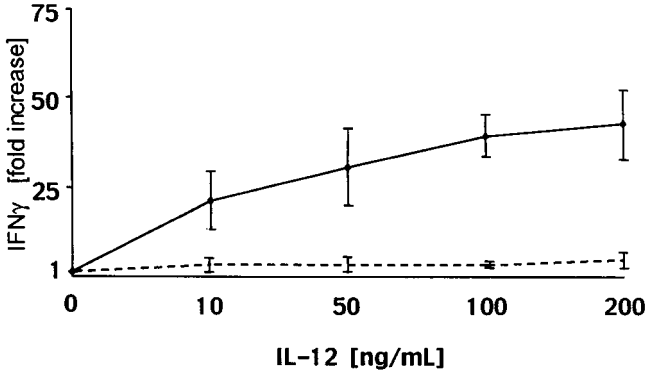

Fig. 5. Effect of IL-18BP on IL-12-induced IFN-γ production in PBMC. PBMC from three donors were cultured in flat bottom wells at 5×10^6 cells/ml in RPMI and were incubated in the absence or presence of 0, 10, 50, 100, or 200 ng/ml of IL-12 (unbroken line). The broken line represents cultures incubated with IL-12 plus 100 ng/ml of IL-18BP. IFN-γ levels represent the fold-change ±SEM over that of the production in unstimulated cells (control, set at 1).

PBMC depends on bioactive IL-18, similar to IL-12-induced IFN-γ in mice (34).

Endogenous IL-18 Controls Basal PGE_2 Production in PBMC. To test whether endogenous IL-18 controls basal PGE_2 production, PBMC were incubated in the presence of IL-12 or IL-18BP. As depicted in Fig. 6, incubation of unstimulated PBMC with IL-12 resulted in a 50% decrease in PGE_2 production compared with basal levels. In the same cells, neutralization of endogenous IL-18 by IL-18BP resulted in a 2.3-fold increase in PGE_2 production over the spontaneous level ($P < 0.05$).

IL18BP Augments PGE_2 Production in Whole Human Blood Cultures Stimulated with *S. epidermidis*. As shown in Fig. 7, whole human blood production of PGE_2 increased 10-fold after stimulation with *S. epidermidis* during a 24-hr culture. However, similar to the augmentation of PGE_2 production in PBMC stimulated with IL-12, there was increased production of PGE_2 in these whole blood cultures when IL-18BP was added (Fig. 7). These data support the concept that endogenous IL-18 suppresses PGE_2 induced by an exogenous, pro-inflammatory microbial agent via IFN-γ (24).

Discussion

Because of its similarities to IL-1 signal transduction, IL-18 was initially considered to induce PGE_2 production in PBMC as does

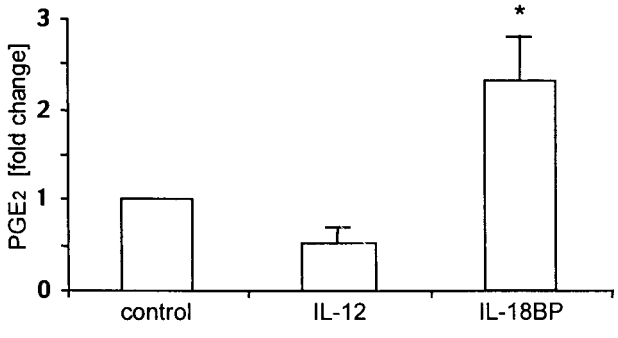

Fig. 6. Effect of IL-12 and IL-18BP on spontaneous production of PGE_2. PBMC from 4 donors were cultured in flat bottom wells at 5×10^6 cells/ml in RPMI and were incubated in the absence or presence of IL-12 (5 ng/ml) or IL-18BP (100 ng/ml). PGE_2 production represents the fold-change ±SEM over that in unstimulated cells (set at 1). *, $P < 0.05$ as calculated by paired *t* test.

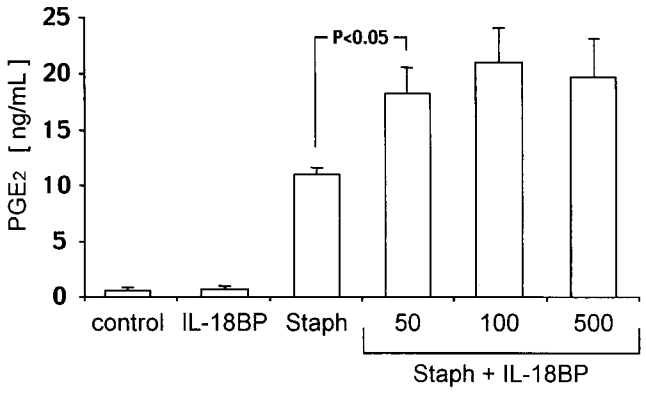

Fig. 7. Effect of IL-18BP on PGE_2 production in whole blood cultures. Whole blood was obtained from three donors, and 0.5 ml was aliquoted into 75-mm polypropylene tubes to which was added a suspension of *S. epidermidis* (0.5 ml of RPMI). After 24 hr at 37°C, the cultures were lysed in Triton X-100 (0.5%) and were assayed for PGE_2. IL-18BPa His6-tag was added at the time of stimulation with *S. epidermidis*, and the concentrations (ng/ml) are indicated under the horizontal axis.

IL-1-triggered PGE_2 synthesis in blood monocytes (35) and synovial fibroblasts (15). It was anticipated that IL-18, primarily acting on T and natural killer cells, would induce TNFα production with subsequent IL-1β and PGE_2 synthesis in monocytes. This would be analogous to IL-18 induction of IL-8 in PBMC (13). Nevertheless, despite this parallel with IL-18 induction of IL-8, PGE_2 synthesis was not induced by IL-18 in PBMC or in cultured macrophages. We also demonstrate that, in PBMC, IL-18, unlike IL-1, did not induce COX-2 gene expression. The induction of COX-2 by IL-1 is mediated by nuclear factor κB (36), and IL-18 also induces nuclear factor κB in T-cell clones (3, 14). Nevertheless, COX-2 gene expression in PBMC was not induced by IL-18.

This study unexpectedly revealed that IL-18 may actually suppress PGE_2 synthesis. The mechanism of any suppressive effect of IL-18 on PGE_2 synthesis is likely the result of IL-18-induced IFN-γ production from T-cells and natural killer cells in the PBMC preparations. Because PBMC contain a mixture of cells similar to those found in submuscosal tissues and lymph nodes, these observations are relevant to local immune responses. IFN-γ suppresses spontaneous and IL-1-induced PGE_2 production in PBMC in a dose-dependent manner (28, 37, 38). IFN-γ also inhibits the COX-2 gene expression (28), reducing cellular responses dependent on endogenous PGE_2. For example, in synovial cells cultured from patients with rheumatoid arthritis, IFN-γ down-regulates IL-1-induced PGE_2, collagenase release, and cell growth (39). In addition, IFN-γ reduces IL-1α-induced production of IL-1β (31, 32, 40), IL-1-induced proliferation of human vascular smooth muscle cells (41), IL-1-induced metalloproteinase gene expression (42), and IL-1-stimulated bone resorption in neonatal mouse calvaria (43).

The role of IFN-γ in the suppression of PGE_2 synthesis by IL-18 was shown both directly and indirectly in this study. Directly, we found that anti-IFN-γ-antibody reverses the suppression of IL-1β-induced PGE_2 production by IL-18. Indirectly, in the presence of IL-18BP, IL-1β-induced PGE_2 production was significantly enhanced. It was previously shown by Dayer and coworkers that monocytes, not lymphocytes, are the source of PGE_2 in human PBMC cultures (17), However, similar to the present observations, adding purified lymphocytes to monocyte cultures resulted in a 40–60% suppression of PGE_2 production but an increase in IL-1 synthesis (17). Moreover, the absence of a suppressive effect of IL-18 on LPS-induced PGE_2 production, observed in this study, supports the hypothesis that the mechanism for IL-18 suppression of IL-1β-induced PGE_2 production in PBMC is via IFN-γ. Unlike IL-1β-induced PGE_2 synthesis, LPS-induced PGE_2 production is not reduced by IFN-γ (29, 30).

Although IL-18 alone does not induce IFN-γ production from T-lymphocytes (44), the presence of secondary stimulants, particularly IL-12, mitogens, or microbial agents, is required for IL-18-induced IFN-γ production (10). Two mechanisms may account for stimulatory effects of IL-12 on IFN-γ production in PBMC. First, IL-12 up-regulates production of IL-18 (45), and, second, IL-12 increases the responsiveness of T- and B-cells to IL-18 by up-regulation of IL-18Rα chain mRNA expression (10).

In rats, administration of COX inhibitors increase immune-mediated colitis (46). As a potent immunomodulator, PGE_2 promotes Th2 responses in dendritic and Th0 cells (47, 48) and inhibits the production of Th1 lymphokines by T-cells. PGE_2 also down-regulates expression of the IL-12 receptor, decreases responsiveness of human PBMC to IL-12 (49), and reduces the production of IL-12 and IFN-γ in PBMC (50).

In the present study, we observed suppression of IL-12-induced IFN-γ production in PBMC by IL-18BP. Because IL-18BP is specific for neutralizing the biological activities of IL-18 (20, 21), these results are consistent with IL-18 being present in freshly obtained PBMC (27). The fact that IL-18BP increases both spontaneous and IL-1β-induced PGE_2 production supports the concept that IL-18 and IL-12 from freshly isolated PBMC affects PGE_2 production in these cells via IFN-γ. In addition, we used whole human blood production of PGE_2 stimulates by *S. epidermidis*. Similar to the augmentation of PGE_2 production in PBMC stimulated with IL-12, there was increased production of PGE_2 in these cultures when IL-18BP was present (Fig. 7). Thus, these observations in whole blood also support the concept that endogenous IL-18 suppresses PGE_2 induced by an exogenous, pro-inflammatory microbial agent. Because *S. epidermidis* production of IFN-γ in whole blood is IL-18-dependent (24), we conclude that the IL-18 suppression of PGE_2 is via IFN-γ in these cultures. Because IL-18BP is constitutively expressed in humans, its role in PGE_2 synthesis may affect the progression and severity of autoimmune diseases, particularly in inflammatory bowel disease.

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