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Immunology

Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1β are differentially regulated in human blood mononuclear cells and mouse spleen cells

(cytokines/caspase 1/monocytes/macrophages/interferon γ)

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ABSTRACT **Interleukin (IL)-18, formerly called interferon γ (IFN-γ)-inducing factor, is biologically and structurally related to IL-1β. A comparison of gene expression, synthesis, and processing of IL-18 with that of IL-1β was made in human peripheral blood mononuclear cells (PBMCs) and in human whole blood. Similar to IL-1β, the precursor for IL-18 requires processing by caspase 1. In PBMCs, mature but not precursor IL-18 induces IFN-γ; in whole human blood stimulated with endotoxin, inhibition of caspase 1 reduces IFN-γ production by an IL-1β-independent mechanism. Unlike the precursor for IL-1β, precursor for IL-18 was expressed constitutively in PBMCs and in fresh whole blood from healthy human donors. Western blotting of endotoxin-stimulated PBMCs revealed processed IL-1β in the supernatants via an caspase 1-dependent pathway. However, in the same supernatants, only unprocessed precursor IL-18 was found. Unexpectedly, precursor IL-18 was found in freshly obtained PBMCs and constitutive IL-18 gene expression was present in whole blood of healthy donors, whereas constitutive IL-1β gene expression is absent. Similar to human PBMCs, mouse spleen cells also constitutively contained the preformed precursor for IL-18 and expressed steady-state IL-18 mRNA, but there was no IL-1β protein and no spontaneous gene expression for IL-1β in these same preparations. We conclude that although IL-18 and IL-1β are likely members of the same family, constitutive gene expression, synthesis, and processing are different for the two cytokines.**

After synthesis in human blood monocytes, the precursor of interleukin (IL) 1β (proIL-1β) remains primarily cytosolic until it is cleaved and transported out of the cell (for review, see ref. 1). A small amount of proIL-1β is secreted from intact cells in the absence of cell death (2). It has been proposed that export of mature IL-1β takes place through a membrane channel as part of the cleavage process (3). Release of mature IL-1β is linked to processing at the aspartic acid–alanine (residues 116 and 117) peptide cleavage by the IL-1β-converting enzyme (ICE) (4), also termed caspase 1. Agents such as nigericin and ATP result in a dramatic increase in processing and secretion of IL-1β in human monocytes (5–7) but do not directly increase ICE activity in cell-free systems.

These observations have not been established for IL-18 [formerly interferon-γ (IFN-γ) inducing factor], an ICE-dependent cytokine with structural similarities to IL-1β (8). Similar to proIL-1β, precursor IL-18 (proIL-18) does not contain a signal peptide required for the removal of the precursor amino acids with subsequent secretion (9). The N-terminal amino acid sequence of the secreted form of murine IL-18 was consistent with the sequence obtained after cleavage at an aspartic acid residue, a typical cleavage site for ICE. Therefore, it was not surprising that ICE cleaved proIL-18 (after aspartic acid) and resulted in the mature and active protein (10, 11). In addition to caspase 1, caspase 3 cleaves mature or proIL-18 after aspartic acids 71 and 76, resulting in inactive peptides (12). As reported for proIL-1β (13), proIL-18 may serve as a negative regulator by acting as a sink for the enzymatic activity of caspase 1 and caspase 3.

Unlike mice deficient in IL-1β, ICE-deficient mice have low levels of circulating IFN-γ after lipopolysaccharide (LPS) injection (10, 11), and like IFN-γ receptor-deficient mice, are resistant to LPS-induced death (14, 15). Compared with wild-type mice, splenocytes from ICE-deficient mice produced low levels of IFN-γ after LPS or zymosan (16). Most importantly, the reduced levels of IFN-γ in mice deficient in ICE are not due to a lack of IL-12. By comparison, there is no reduction in IFN-γ production in mice deficient in IL-1β (17). Therefore, ICE-deficient mice demonstrate that IFN-γ production can be independent of IL-12. The role of IL-12 in IFN-γ production appears to be due, in part, to the ability of IL-12 to increase IL-18 binding to T cells (18).

Because the ligands IL-18 and IL-1β and the receptor family for these two cytokines are similar (for review, see ref. 19), we have examined gene expression, synthesis, and processing of IL-18 in human peripheral blood mononuclear cells (PBMCs) and whole blood and compared these parameters to those of IL-1β in the same cells and under the same conditions. In addition, we examined these differences in primary mouse spleen cells.

MATERIALS AND METHODS

Reagents. Recombinant human proIL-18 was expressed in *Escherichia coli* as the 24-kDa form and provided by Michael Su and Yong Gu (Vertex Pharmaceuticals, Cambridge, MA) as described (20). Recombinant human mature IL-18, murine mature IL-18 and murine proIL-18 were gifts from Y. Stabinsky (Peprotech, Rocky Hill, NJ). IFN-γ was a gift from Michael Palladino (Genentech). IL-1 receptor antagonist (IL-1Ra) was a gift from Vann Parker (Amgen Biologicals). IL-1β was a gift from Aldo Tagliabue (Sclavo, Siena, Italy). The reversible ICE inhibitor (21) was purchased from Alexis Biochemicals (San Diego, CA). Protease inhibitors and phytohemagglutinin were obtained from Sigma. For Western blotting, enhanced chemiluminescence reagents including Hybond, Western blotting detection reagents, AuroDye forte, and Hyperfilm were obtained from Amersham Life Sciences. The horseradish peroxidase- or alkaline phosphatase-conjugated

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Abbreviations: IL, interleukin; IFN-γ, interferon γ; PBMC, peripheral blood mononuclear cell; ICE, IL-1β-converting enzyme; LPS, lipopolysaccharide; IL-1Ra, IL-1 receptor antagonist.

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donkey anti-rabbit IgG were obtained from Jackson ImmunoResearch. Rabbit anti-human and anti-murine IL-18 were produced by immunizing rabbits with mature human or murine IL-18 in complete Freund's adjuvant (primary immunization) followed by several monthly booster injections in incomplete adjuvant.

PBMCs. These studies were approved by the Combined Colorado Investigational Review Board. All subjects gave consent. Platelet-depleted residual blood obtained from healthy volunteers after plateletpheresis or freshly obtained whole blood were used a source of PBMCs as described (20). Whole blood assays were performed with heparinized blood (20 units/ml, sodium heparin, Abbott) freshly obtained by venipuncture from fasting donors (22). For the measurement of IFN-γ, an electrochemiluminescence method was used as reported (22).

Western Blot Analysis. PBMCs that had been incubated were removed from plates by means of a cell scraper and centrifuged at 400 $\times$ *g* for 15 min. The cell pellets were resuspended in 200 μl of a lysis buffer [50 mM Tris·HCl, pH 7.6/100 mM NaCl/2 mM EDTA/2 mM EGTA/1 mM phenylmethylsulfonyl fluoride/aprotinin (10 μg/ml)/1% Nonidet P-40] and lysed for 30 min on ice. The cell debris was removed by centrifugation at 13000 $\times$ *g*. The cell supernatants (4–8 ml) were concentrated up to 40-fold by Centricon-10 concentrators (Amicon). Briefly, cell supernatants were added to the concentrators and centrifuged at 5000 $\times$ *g* in a Beckman J-21C centrifuge. SDS/PAGE was performed with 15% gels to separate proteins in the cell lysates and supernatants. The proteins were transferred onto nitrocellulose membranes by using a semi-dry technique (Hoefer Semi-Dry Transfer Unit, Hoefer). The buffer used in the system contained 25 mM Tris·HCl, 192 mM glycine, 15% methanol, and 0.1% SDS. After the transfer, nonspecific sites on the membranes were blocked with 5% nonfat milk in PBS. The blots were probed with primary antibody (1:200 or 1:400 dilution) overnight at 4°C followed by several washes in PBS/0.1% Tween 20 (PBST). A secondary horseradish peroxidase-labeled antibody (1:4000 dilution) was added for 1 h at room temperature, the blot was washed several times in PBST, and the antibody was detected by using enhanced chemiluminescence.

Steady-State mRNA Levels. Total RNA was isolated from PBMCs or freshly obtained heparinized human whole blood by using Tri-Reagent (Molecular Research Center, Cincinnati). To prepare cDNA, 0.5 μg of total RNA was reverse-transcribed by using random hexamer primers as templates. The reaction took place in a total volume of 20 μl containing the following components (purchased from Perkin–Elmer) at the final concentrations indicated: 5 mM $MgCl_2$, PCR buffer [1$\times$, 50 mM KCl and 10 mM Tris·HCl (pH 8.3)], all four dNTPs (each at 1 mM), 2.5 μM random hexamer primers, 20 units of RNase inhibitor, and 50 units of murine leukemia virus reverse transcriptase. The reaction was incubated at 42°C for 30 min and terminated by heating at 95°C for 5 min. For PCR, 2–4 μl of reverse transcription product was used in a total volume of 50 μl containing the following components (Perkin–Elmer, final concentrations): 1.7 mM $MgCl_2$, PCR buffer [1$\times$, 50 mM KCl and 10 mM Tris·HCl (pH 8.3)], all four dNTPs (each at 0.2 mM), 1 units of Ampli*Taq* polymerase, and forward (F) and reverse (R) primers (each at 20 μM). The following reaction sequence was performed on a thermocycler (Perkin–Elmer) for each PCR: 90°C for 5 min and 60°C for 5 min (1 cycle); followed immediately by 72°C for 1 min, 90°C for 1 min, and either 55°C (for IL-18) or 60°C (for IL-1β) 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 used 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. To ensure that differences between tubes were not the result of unequal concentrations of RNA, PCRs using GAPDH as the internal control were performed on each sample.

The sequences and cycle number are indicated as follows: IL-18 (F), 5′-GCTTGAATCTAAATTATCAGTC-3′ (35 cycles); IL-18(R), 5′-GAAGATTCAAATTGCATCTTAT-3′; IL-12p40 (F), 5′-CAGCAGTTGGTCATCTCTTG-3′ (35 cycles); IL-12p40 (R), 5′-CCAGCAGGTGAAACGTCCA-3′; IL-1β (F), 5′-ATGGCAGAAGTACCTAAGCTCGC-3′ (28 cycles); IL-1β (R), 5′-ACACAAATTGCATGGTGAAGTCAGTT-3′; IFN-γ (F), 5′-ATGAAATATACAAGTTATATCTTGGCTTT-3′ (35 cycles); IFN-γ (R), 5′-GATGCTCTTCGACCTCGAAACAGCAT-3′; GAPDH (F), 5′-ACCACAGTCCATGCCATCAC-3′ (23 cycles); GAPDH (R), 5′-TCCACCACCCTGTTGCTGTA-3′. The PCR products were separated on a 2% agarose gel containing 0.5$\times$ Tris/borate/EDTA (TBE) containing 50 mM Tris, 45 mM boric acid, 0.5 mM EDTA (pH 8.3), and ethidium bromide (0.5 μg/ml), visualized by UV illumination, and photographed. The predicted sizes of the PCR products were 341 bp, 420 bp, 802 bp, and 452 bp for IL-18, IL-12, IL-1β IFN-γ, and GAPDH, respectively.

Mouse Studies. C57BL6 males (8 weeks old) were left untreated or injected with LPS (100 μg) i.p. Spleens were removed immediately after cervical dislocation, snap frozen in liquid nitrogen, and homogenized in Tri-Reagent. Total RNA and protein were extracted by the manufacturer's instructions. Western blots and reverse transcription-coupled PCR were performed as those described for human PBMCs, except that rabbit anti-mouse IL-18 was used at 1:200 dilution. The primer sequences for murine IL-18 and murine IL-1β are as follows: IL-8 (F), 5′-ACTGTACAACCGCAGTAATACGG-3′; IL-18 (R), 5′-AGTGAACATTACAGATTTATCCC-3′; IL-1β (F), 5′-CTCCATGAGCTTTGTACAAGG-3′; IL-1β (R), 5′-TGCTGATGTACCAGTTGGGG-3′. The annealing temperature was 55°C for IL-18 and IL-1β and 60°C for GAPDH. Thirty cycles were used for IL-18 and IL-1β and 25 for GAPDH.

RESULTS

Influence of IL-18 Versus IL-1β on IFN-γ Production from Whole Human Blood. As shown in Fig. 1*A*, phytohemagglutinin-induced IFN-γ was markedly enhanced by the presence of mature IL-18, whereas proIL-18 was inactive. The addition of mature human IL-1β (1, 10, or 100 ng/ml) did not augment IFN-γ production under these same conditions (n = 3). We next examined the role of endogenous IL-18 and IL-1β in IFN-γ production induced by LPS. For these studies, a whole blood assay was used to measure LPS-induced IFN-γ production. To distinguish a role for IL-1β from IL-18, saturating concentrations of IL-1Ra or a specific inhibitor of ICE was added to the whole blood cultures. As shown in Fig. 1*B*, blocking IL-1 receptors did not influence the production of IFN-γ induced by LPS compared with the effect of the ICE inhibitor. The results suggest that biologically active IL-18 was the likely cytokine participating in the augmentation of LPS-induced IFN-γ in whole blood cultures.

Differential Processing and Secretion of IL-1β and IL-18. We assessed the nature of IL-1β and IL-18 processing and secretion in the same PBMCs 24 h after LPS stimulation. The molecular sizes of the forms of these cytokines in the cell supernatants and lysates were determined by Western blotting using several donors; the results of two representative donors are shown in Fig. 2. In cell supernatants after LPS stimulation, the major IL-1β species observed was the mature 17-kDa form. The presence of the ICE inhibitor led to a marked decrease in the amount of mature IL-1β with a slight accumulation of the precursor form (Fig. 2*A*). However, in the same supernatants of control or LPS-stimulated cells, the mature form of IL-18 was not readily detectable (Fig. 2*B*). Instead, the precursor

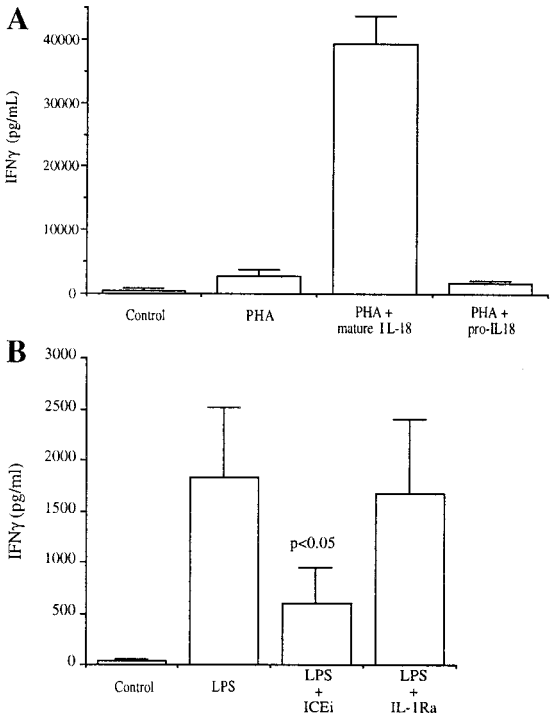

FIG. 1. Effect of IL-18 on IFN-γ induction. (*A*) Enhancing effect of mature IL-18 (10 nM) on phytohemagglutinin- (10 μg/ml) induced production of IFN-γ in human PBMCs. ProIL-18 was also added at 10nM. PBMCs were stimulated for 72 h, and IFN-γ was assayed on supernatants by ECL assay. Data are the mean ± SEM ($n = 3$). (*B*) Effect of reversible ICE inhibitor (ICEi) and IL-1Ra on LPS-induced IFN-γ production in whole human blood. Whole blood was stimulated with LPS (10 ng/ml) in the presence of ICEi (10 μM) or IL-1Ra (10 μg/ml) and after 24 h, the concentration of IFN-γ was measured in the whole blood after lysis in 0.5% Triton X-100. Data are the mean ± SEM ($n = 3$). A paired Student's *t* test was used to assess significance between LPS and LPS + ICEi.

form of IL-18 was observed in supernatants of both unstimulated and stimulated cells. There was no obvious difference in the amount of secreted proIL-18 under control or stimulated conditions. There was also no variation in the accumulation of the precursor form of IL-18 in LPS-stimulated cells in the presence of the ICE inhibitor.

We next studied the molecular forms of IL-1β and IL-18 in cell lysates. After 24 h of incubation, no proIL-1β was observed in unstimulated cells. On LPS stimulation there was the expected increase in the IL-1β precursor form at 31–33 kDa and no detectable mature IL-1β was noted (Fig. 2*C*). Also in the lysates, an early 28-kDa cleavage form of proIL-1β was observed. This cleavage likely takes place at the upstream ICE site in proIL-1β (21). In the same lysates, the precursor form of IL-18 (24 kDa) was readily observed in both unstimulated and stimulated cells and there was no apparent difference between the two conditions (Fig. 2*D*). These Western blots were stained for total protein with AuroDye to ensure that there was equal loading of protein.

Constitutive Presence of IL-18 in PBMCs. The PBMCs used in the above experiments were obtained as a by-product after plateletpheresis, and hence PBMCs could have been activated during the procedure to express IL-18 but not IL-1β. Therefore, PBMCs were obtained from freshly drawn heparinized blood from three donors and the cells were lysed immediately and prepared for electrophoresis (Fig. 3). Under these circumstances, the major form of IL-18 was again the precursor form (as shown in Fig. 2*D*). In each donor, however, the mature form was also present in these cell lysates. By Western blotting, there was no IL-1β in these freshly obtained lysates (data not

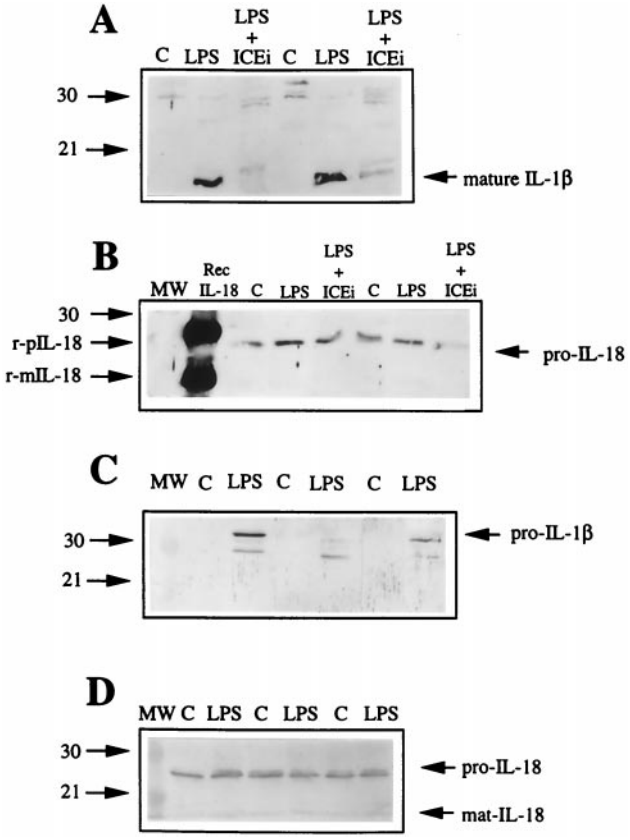

FIG. 2. Effect of ICE inhibitor on IL-1β and IL-18 processing and release. PBMCs from two separate donors were stimulated with LPS (100 ng/ml) in the presence or absence of 10 μM ICE inhibitor (ICEi). The letter C above the lanes indicates the unstimulated control PBMCs. After 24 h at 37°C, the supernatants were removed and concentrated in a Centricon device, electrophoresed in two separate gels, and blotted (in duplicate). (*A*) Western blotting with anti-human IL-1β. (*B*) Duplicate blot to that in *A* but developed with anti-human IL-18. (*C*) Cell lysates from the same cultures used in *A* but developed with anti-human IL-1β. (*D*) Same cultures as used in *A* but developed with anti-human IL-18. Molecular mass makers (in kDa) are indicated by arrows on left side of figure. r-pIL-18 and r-mIL-18 indicate recombinant pro- and mature IL-18, respectively.

shown). In earlier studies from this laboratory (20), proIL-1β or mature IL-1β are also not present in freshly obtained lysates of PBMCs when measured by specific ELISA for each IL-1β form. The lack of a mature IL-18 form in the 24-h supernatants from LPS-stimulated PBMCs (see Fig. 2*B*) may be due to

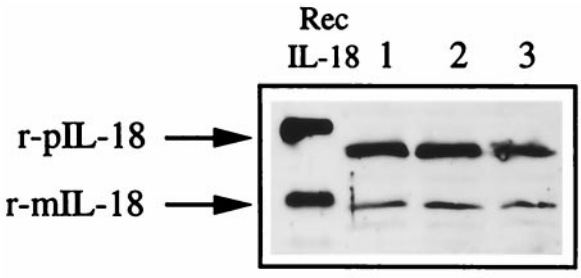

FIG. 3. Detection of IL-18 in freshly isolated human PBMCs. Whole blood from three donors was obtained and PBMCs were isolated. Without further processing, cells were counted and centrifuged at 250 × *g*, and lysis buffer was immediately added. The lysates were subjected to SDS/PAGE with 15% gels and Western blotting with horseradish peroxidase-conjugated anti-rabbit IgG. Recombinant human pro- and mature IL-18 are indicated as in Fig. 2. Each lane represents a different human donor.

secretion of mature IL-18 and binding to T cells present in PBMCs. Because these findings were unanticipated, we validated the antibodies used for the Western blot analysis. The specificity of the antibody was established by absorbing the antibody with excess of precursor IL-18 in a duplicate Western blot. There was a diminution in the intensity of recombinant pro-IL-18, mature IL-18, and the natural forms produced by LPS-stimulated PBMCs.

Constitutive Expression of Steady-State IL-18 mRNA. Because there appeared to be constitutive expression of the IL-18 protein (see Fig. 3), we compared IL-18 mRNA in isolated PBMCs and in freshly obtained whole blood from the same donors. As shown in Fig. 4, under both conditions and in both donors, IL-18 mRNA was present. However, for IL-1β in these same preparations, there was detectable mRNA in PBMCs but not whole blood. This lack of gene expression for IL-1β has been reported from our laboratory (23) and has also been observed for IL-8 when RNA is prepared directly from whole blood (24). In contrast, gene expression for several cytokines is stimulated during the isolation procedures for PBMCs (24). The number of PCR cycles required to demonstrate IL-18 was not different from that used for PBMCs. We confirmed that the amplicon observed in the whole blood was IL-18 by direct DNA sequencing. Comparison with the published sequence for human IL-18 showed that there was a near perfect match.

IL-18 and IL-1β Gene Expression and LPS-Induced Synthesis in the Mouse Spleen. Because the data unexpectedly showed regulation of IL-18 distinct from that of IL-1β in human PBMCs and whole blood, we examined the same parameters in the murine spleen. First, we wanted to confirm that similar to the human, the mouse expresses constitutive IL-18 at the level of steady-state mRNA. To accomplish this, three mice were instantly sacrificed by cervical dislocation and the spleens were homogenized in Tri-Reagent. As shown in Fig. 5*B*, IL-18 mRNA was expressed in the spleens of three mice, whereas in the same tissue there was no expression of IL-1β. However, upon stimulation with LPS, there was an increase in IL-1β but only a modest increase in IL-18. We also assessed IL-18 synthesis. As shown in Fig. 5*A*, freshly obtained mouse spleen contained pre-formed proIL-18. This is similar to the data observed in freshly obtained human PBMCs shown in Fig. 3. After LPS treatment, the amount of proIL-18 in the spleen cells decreased, but this probably is the result of processing and external transport.

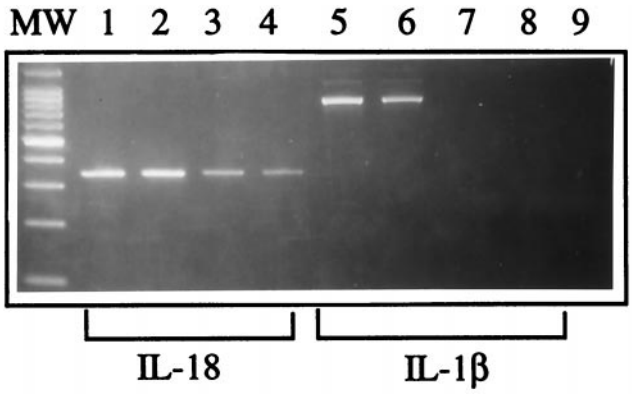

FIG. 4. Differential presence of steady-state IL-18 and IL-1β mRNA by reverse transcription-coupled PCR. Whole blood and PBMCs were obtained from two donors. Lanes 1 and 2 depict the IL-18 PCR product in unstimulated PBMCs immediately after separation from the whole blood of two donors. Lanes 3 and 4 show the IL-18 PCR product in whole blood from the same donors. Lanes 5 and 6 show the IL-1β PCR product in unstimulated PBMCs immediately after separation from the whole blood of two donors. Lanes 7 and 8 show the IL-1β PCR product in whole blood from the same donors. Lane 9 is the water control.

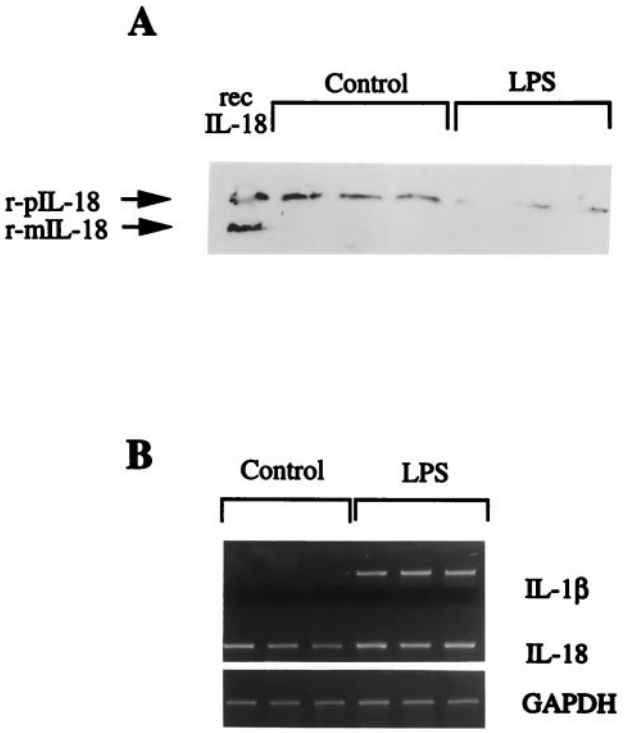

FIG. 5. Differential expression of IL-18 and IL-1β in mouse spleen. Spleens from three C57BL6 mice were removed. (*A*) IL-18 as assessed by Western blotting. The molecular mass markers (in kDa) of murine proIL-18 (pIL-18) or mature (mIL-18) are shown. Under the bracket labeled control, each lane represents spleen cells from a different mouse. Under the bracket labeled LPS, the mice were injected 2 h previously with LPS (100 μg). (*B*) Gene expression for IL-1β and IL-18 under conditions identical to those in *A*.

Influence of IFN-γ on Kinetics of IL-12 and IL-18 mRNA Expression. The influence of IFN-γ on cytokine synthesis in human PBMCs is well studied and is dependent on the stimulant used (25, 26). With specific regard to IL-12, IFN-γ enhances LPS-induced expression of p70 and p40 IL-12 proteins in cell supernatants (27). In Fig. 6*A*, as has been previously documented (27), we confirm that there is no constitutive expression of IL-12 p40 mRNA. After stimulation by LPS, there is detectable mRNA at 3–6 h after stimulation. IFN-γ alone did not induce IL-12 mRNA, and when coincubated with LPS, there was no obvious change in expression by comparison to LPS alone. IL-18, by contrast, was constitutively expressed, and there was a moderate enhancement within 1 h of stimulation with LPS that was sustained for up to 6 h and declined to baseline by 24 h (Fig. 6*B*). The addition of IFN-γ did not influence IL-18 expression in response to LPS at any of the time points tested.

DISCUSSION

The regulation of IL-18 gene expression, synthesis, processing, and secretion appears to be distinct from that of IL-1β. For example, there was constitutive expression of both mRNA and protein for IL-18 in freshly obtained unstimulated PBMCs, whereas this was not observed for IL-1β in the same cells. In addition, these unexpected findings were also observed in murine spleen cells snap frozen immediately after sacrifice. Therefore, the precursor form of IL-18 compared with precursor IL-1β is pre-formed distinctly in PBMCs from healthy humans and in mouse spleen cells. Two other members of the IL-1 family, namely IL-1α (28) and the intracellular form of the IL-1Ra (29, 30), are constitutively expressed in human and murine skin.

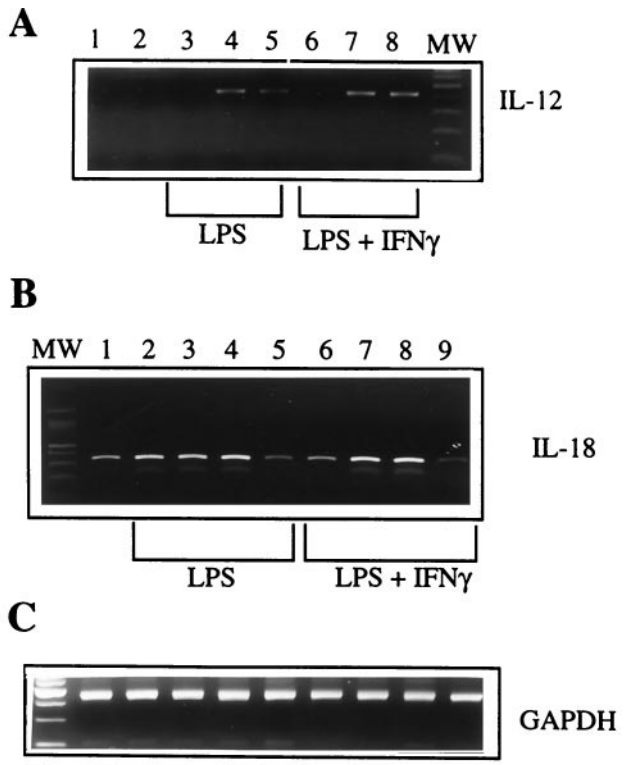

FIG. 6. Effect of IFN-γ on time course of LPS-induced expression of IL-12 and IL-18 (*A*) IL-12. PBMCs were stimulated with LPS (100 ng/ml) with and without IFN-γ (250 units/ml). Lanes: 1, unstimulated PBMCs; 2, IFN-γ; 3 and 6, after 1 h; 4 and 7, after 3 h; 5 and 8, after 6 h. (*B*) IL-18. Lanes: 1, unstimulated PBMCs; 2 and 6, after 1 h; 3 and 7, after 3 h; 4 and 8, after 6 h; 5 and 9, after 24 h.

In addition to these differences in constitutive expression in whole blood and PBMCs, stimulation of PBMCs with LPS did not lead to a marked increase of pro- or mature IL-18 compared with IL-1β. In the mouse, LPS stimulation resulted in only a modest increase in steady-state IL-18 mRNA compared with IL-1β in the same cells. These differences exist despite the similarities in three-dimensional structure (8) and the requirement for ICE (10, 11) for processing of the IL-1β and IL-18 precursors. Nevertheless, inhibition of ICE activity with the use of a specific inhibitor of this enzyme diminished LPS-induced IFN-γ production in whole blood. Because LPS-induced IFN-γ production was independent of IL-1, we conclude that a reduction in the processing and secretion of IL-18 by the ICE inhibitor accounted for the reduction in IFN-γ. We excluded a role for mature IL-1β (and also IL-1α) in this particular experiment by blocking IL-1 receptors with IL-1Ra. Although it has been reported that IL-1α could coinduce IFN-γ production in murine splenocytes (natural killer cells) in the presence of either IL-12 or IL-2 (31–33), we used saturating concentrations of IL-1Ra, which prevented any IL-1 receptor signaling.

IL-18 acts synergistically with mitogens and other cytokines to augment IFN-γ production (9, 18, 34); however, in the present study, we demonstrate that proIL-18 is biologically not active on PBMCs in this response. Thus recombinant proIL-18 is similar to recombinant proIL-1β in lacking significant biological activity until processed by ICE. Nevertheless, in the milieu of natural inflammation, proIL-1β released from dying cells is vulnerable to cleavage by a variety of extracellular enzymes, such as cathepsin and elastase, which are able to generate biologically active forms of IL-1β with nearly the same potency as that of the mature form generated by ICE (35). In addition, mice deficient in ICE produce biologically active forms of IL-1β in a model of local inflammation (36). There is also evidence that the precursor IL-1β found in human epidermis can be processed extracellularly by an enzyme(s) other than ICE (37, 38). Thus, proIL-18, which was found in supernatants of PBMCs after 24 h of culture, may also be cleaved by non-ICE enzymes in the context of inflammation.

The regulation of IL-18 is clearly different from that described for IL-1β. We were able to show that there is preexistent precursor detectable in lysates of both unstimulated and LPS-stimulated cells. This observation was not the result of an artifact because freshly isolated PBMCs appeared to express proIL-18 in the absence of prolonged mechanical stimulation. In this instance, the presence of mature IL-18 was evident. We exclude the possibility that the latter result is an artifact because protease inhibitors were used in the lysis procedure. In addition, there has been the recent purification of functional mature IL-18 from THP-1 cell lysates (12).

These overall results suggest that there is, as for IL-1β, a dissociation between processing and secretion of IL-18. The efficiency of processing may produce a sufficient number of active molecules for a biological response but at the same time also limit secretion to control excessive activity of the cytokine. Interestingly, limited post-translational maturation applies to ICE *per se* (39). In PBMCs and related cells, there is a pre-formed pool of precursor ICE protein. On activation with LPS, there is slight increase in mRNA levels for ICE, as well as maturation of a small amount of ICE, sufficient for the processing the precursor of IL-1β. The calculated ratio of precursor to mature ICE was 100.

Our observations on IL-18 regulation are similar to descriptions of constitutive expression of IL-16 and the presence of preformed protein (precursor and mature forms) in $CD8^+$ T cells and eosinophils (40–42). The PBMC isolation procedures used by most laboratories are thought to account for the presence of mRNA for IL-1β and IL-18. However, we pursued this further and were able to demonstrate that expression for IL-18 mRNA also occurred in freshly isolated PBMCs and in whole blood. By comparison, IL-1β mRNA was not detected in whole blood from the same donors as established (23). Furthermore, the kinetics of mRNA expression were different for the two cytokines. By comparison to IL-1β, there was no dramatic enhancement of IL-18 mRNA expression after LPS stimulation and, in addition, there appeared to be a down-regulation of mRNA below basal levels by 24 h (Fig. 6). The regulation of IL-12 expression also differed from that of IL-18 in that p40 mRNA was inducible by 3 h after stimulation, but like IL-18 this expression was unaffected by IFN-γ.

In murine keratinocytes and in a murine keratinocyte cell line, constitutive gene expression for IL-18 has been reported (43). This is similar to the observation that in human and mouse keratinocytes, IL-1α is also constitutively expressed and keratinocytes contain large amounts of preformed IL-1α protein but not IL-1β protein (28). Like IL-1α (44), the promoters for IL-18 are "TATA-less" (45). The IL-18 promoter activity upstream of exon 2 acts constitutively (45). Therefore, it is not unexpected that in our studies we find IL-18 mRNA constitutively expressed even in whole blood freshly obtained from healthy donors, where no procedural artifact is introduced. The finding that the 3′ untranslated region of human IL-18 lacks the AUUUA destabilization sequence (45) is also consistent with the less than dramatic increase in IL-18 mRNA after LPS compared with that of IL-1β in which LPS reduces the degradation of IL-1β mRNA, allowing for more sustained levels of the polyadenylated species and translation into protein (46).

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