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Coordinated antiinflammatory effects of interleukin 4: Interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist

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ABSTRACT Interleukin 1 receptor antagonist (IL-1ra), a naturally occurring polypeptide with amino acid sequence homology to interleukin 1α (IL-1α) and interleukin 1β (IL-1β), prevents *Escherichia coli*-induced shock and death. Both IL-1 and IL-1ra are produced by monocytes stimulated with lipopolysaccharide (LPS). Because interleukin 4 (IL-4) suppresses IL-1 production, we investigated whether IL-4 modulated IL-1ra synthesis in LPS-stimulated human peripheral blood mononuclear cells. IL-1β and IL-1ra were measured by specific RIAs. IL-4 alone (0.01–100 ng/ml) did not stimulate IL-1β synthesis but rather induced IL-1ra (4.82 ± 0.94 ng/ml). LPS induced synthesis of both IL-1β (6.67 ± 1.06 ng/ml) and IL-1ra (10.77 ± 2.79 ng/ml). IL-4 suppressed LPS-induced IL-1β mRNA accumulation and synthesis. However, IL-4 acted synergistically with LPS in inducing IL-1ra. IL-4 enhanced LPS-induced IL-1ra mRNA accumulation 4-fold and IL-1ra protein synthesis nearly 2-fold. Moreover, IL-1ra mRNA levels were maximal after 6 hr of exposure to LPS but peaked within the first 3 hr in the presence of IL-4. IL-4 added as late as 12 hr after LPS stimulation still enhanced IL-1ra synthesis. In human peripheral blood mononuclear cells stimulated with IL-1α, IL-4 markedly suppressed IL-1β production but enhanced IL-1ra synthesis >2-fold. Because IL-4 favors synthesis of the natural antagonist IL-1ra over synthesis of the agonist IL-1, IL-4 may exert potent antiinflammatory effects on host responses to Gram-negative infections.

Interleukin 4 (IL-4), a 20-kDa polypeptide secreted by T cells and mast cells, has pleiotropic effects on hematopoietic cells (1). Human IL-4 promotes growth of activated B and T cells (2, 3), induces IgE production (4), and enhances expression of B cell surface antigens, including the low-affinity receptor for IgE (FcεRII/CD23) (5, 6) and class II major histocompatibility complex molecules (7). Nonlymphoid hematopoietic cells also express IL-4 receptors (8). IL-4 induces FcεRII on human monocytes (6, 9) but down-regulates the expression of all three Fcγ receptors (10). Furthermore, IL-4 induces *in vitro* differentiation of human monocytes—i.e., changes in morphology and enhanced expression of class II major histocompatibility complex antigens (11). Moreover, IL-4 suppresses interleukin 1 (IL-1) synthesis (12, 13).

IL-1, a 17-kDa cytokine primarily produced by monocytes and macrophages, plays a key role in several inflammatory processes. IL-1 induces fever, hypotension, expression of leukocyte adhesion molecules, and synthesis of cyclooxygenase products (14). Therefore, IL-1 is a likely mediator of the systemic effects of Gram-negative infections (15). Although IL-1α and IL-1β are distinct gene products, these two forms of IL-1 bind to the same receptors and share biological activities (14). A naturally occurring "IL-1 inhibitor" had been shown to block IL-1 activity by competing with IL-1 for occupancy of its receptor (16, 17). This IL-1 inhibitor has been recently cloned, expressed, and characterized (18–20). Because it binds to both IL-1 receptors (18, 21) without agonist activity (22), and inhibits IL-1 binding and biological activities (18, 23, 24), the IL-1 inhibitor has been renamed the IL-1 receptor antagonist (IL-1ra). Human IL-1ra has 19% amino acid homology to human IL-1α and 26% homology to human IL-1β (19) and is thought to be a member of the IL-1 gene family (25). IL-4 suppresses IL-1 and other monocyte-derived cytokines, including tumor necrosis factor α (12), IL-6 (26), and IL-8 (27). We, therefore, investigated whether IL-4 could also modulate IL-1ra gene expression and synthesis in human peripheral blood mononuclear cells (PBMC).

MATERIALS AND METHODS

Human PBMC Culture. PBMC were separated from heparinized blood by centrifugation on Ficoll/Hypaque (Ficoll type 400, Sigma; Hypaque-M 90%, Winthrop Breon Laboratories) gradients. Cells were washed twice in 0.15 M NaCl and resuspended in ultrafiltered RPMI 1640 medium (Whittaker Bioproducts)/2 mM L-glutamine/penicillin at 100 units per ml/streptomycin at 100 μg/ml (GIBCO). PBMC (2.5×10^6 cells per ml) were stimulated with lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 (10 ng/ml; Sigma) or human recombinant IL-1α (10 ng/ml; provided by A. Tagliabue, Sclavo Research Center, Siena, Italy) with or without human recombinant IL-4 (0.01–100 ng/ml; provided by T. Nagabhushan, Schering–Plough, Bloomfield, NJ). PBMC cultures were incubated in 12 × 75 mm polypropylene round-bottom tubes (Becton Dickinson) for 24 hr at 37°C in a humidified atmosphere containing 5% CO_2.

Cytokine RIAs. After incubation, cultures were subjected to three freeze–thaw cycles. This procedure is optimal for recovery and measurement of total (cell-associated plus secreted) cytokines by specific RIAs as described for IL-1β (28) and IL-1ra (29). The sensitivities (defined as 95% binding) of RIAs for IL-1β and IL-1ra were 64 ± 5 (n = 3) and 138 ± 28 (n = 7) pg/ml, respectively.

RNA Isolation and Northern Analysis. PBMC were stimulated for 3, 6, 12, or 24 hr at 37°C in 50-ml polypropylene tubes with LPS (10 ng/ml) with or without IL-4 (0.1, 1, or 10 ng/ml). Total cellular RNA was then extracted from PBMC by lysis with 4 M guanidium isothiocyanate followed by ultracentrifugation on a 5.7 M cesium chloride gradient (30). Total RNA (20 μg) was subjected to electrophoresis in 6.6% formaldehyde (Sigma)/1.2% agarose (International Biotechnologies) gel, and transferred to nylon membranes (Hybond-N, Amersham) by capillary blotting. Serial dilutions of total RNA (2.50, 1.25, and 0.62 μg) were also applied directly on nylon membranes by using a microsample filtration man-

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Abbreviations: IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells.

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ifold (Minifold; Schleicher & Schuell). The membranes were exposed to short-wave UV light for 5 min to fix the RNA to the nylon matrix and treated for 2 hr at 42°C with prehybridization solution containing salmon sperm DNA at 10 mg/ml. Membranes were then treated at 42°C overnight with prehybridization solution containing salmon sperm DNA at 10 mg/ml and ^{32}P-labeled nucleic acid probe. The probes used were a 1075-base-pair (bp) fragment of human IL-1β precursor cDNA subcloned in pGEM2, an 800-bp fragment of human IL-1ra cDNA subcloned in pUC8 (from A. Shaw, Glaxo Institute for Molecular Biology, Geneva) and the full length of chicken β-actin cDNA subcloned in pGEM3 (from B. Huber, Tufts University, Boston). The DNA was labeled using [^{32}P]dCTP (3000 Ci/mmol; New England Nuclear; 1 Ci = 37 GBq) and a random-primed DNA-labeling kit (Boehringer Mannheim). After incubation, membranes were washed in 0.1% SDS/1× standard saline citrate at 42°C. Washed membranes were exposed to Kodak XAR-5 x-ray film (Kodak) at −70°C with an intensifying screen.

Statistical Analysis. Data were expressed as mean ± SEM of the indicated number of experiments. Differences between IL-4-treated groups and RPMI 1640 medium-treated groups were analyzed for significance by Student's *t* test for paired samples or two-way analysis of variance.

RESULTS

IL-4 Induces IL-1ra Synthesis in Human PBMC. IL-4 alone did not induce IL-1β synthesis (data not shown) but rather triggered IL-1ra synthesis in a dose-dependent manner (Fig. 1). As little as 1 ng of IL-4 per ml induced significant amounts of IL-1ra (2.47 ± 0.41 ng/ml, $P < 0.01$) when compared with basal levels. Maximal IL-1ra synthesis was observed for IL-4 at 10 ng/ml (4.82 ± 0.94 ng/ml, $P < 0.001$). No further increase in IL-1ra production was seen with IL-4 at 100 ng/ml (4.54 ± 0.75 ng/ml, $P < 0.001$). Unstimulated PBMC cultures contained IL-1β at 0.09 ± 0.01 ng/ml and IL-1ra at 0.34 ± 0.09 ng/ml (n = 4).

Because LPS stimulates IL-1ra synthesis in PBMC (29) and because IL-4 suppresses LPS-induced IL-1 synthesis (12), we investigated whether endotoxin contamination could account for the IL-4-induced IL-1ra synthesis. Before exposure to PBMC, IL-4 preparations (0.1, 1, or 10 ng/ml) were preincubated with or without polymyxin B at 5 μg/ml. LPS (10 ng/ml) was used as a positive control. Polymyxin B suppressed LPS-induced synthesis of IL-1ra (91% inhibition) and IL-1β (92% inhibition) (Table 1). However, polymyxin B did not affect IL-4-induced IL-1ra synthesis.

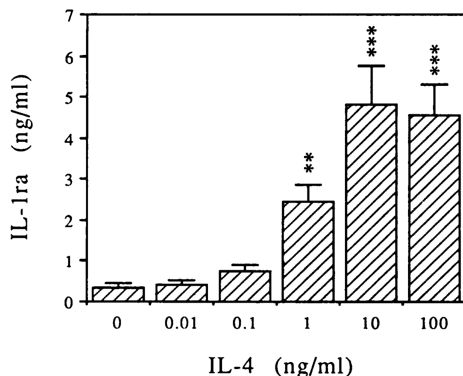

FIG. 1. IL-4 induces IL-1ra synthesis by PBMC. PBMC were incubated for 24 hr with IL-4 (0.01–100 ng/ml). Differences in IL-1ra synthesis were analyzed for significance by analysis of variance (**, $P < 0.01$; ***, $P < 0.001$). Data are expressed as mean ± SEM for four donors.

Table 1. Effect of polymyxin B on IL-1ra and IL-1β levels from IL-4-treated PBMC cultures

Stimulus (ng/ml)	IL-1ra, ng/ml		IL-1β, ng/ml	
	− PMB	+ PMB	− PMB	+ PMB
None	0.39	0.49	<0.07	0.07
LPS (10)	8.80	0.79	3.02	0.23
IL-4 (0.1)	0.50	0.64	<0.07	0.09
IL-4 (1)	1.77	1.55	0.09	<0.07
IL-4 (10)	3.57	3.07	<0.07	0.09

Increasing concentrations of IL-4 were preincubated for 1 hr at room temperature with or without polymyxin B sulfate (PMB; Pfizer) at 5 μg/ml. LPS was used as a positive control. PBMC (2.5 × 10^6 cells) were then added to these samples and incubated for 24 hr at 37°C.

Effect of IL-4 on LPS-Induced Synthesis of IL-1ra and IL-1β. LPS induced synthesis of IL-1ra (10.77 ± 2.79 ng/ml) and IL-1β (6.67 ± 1.06 ng/ml). IL-4 suppressed LPS-induced IL-1β synthesis but enhanced IL-1ra production in a dose-dependent manner (Fig. 2). IL-4 at 1 ng/ml significantly reduced IL-1β synthesis (62% inhibition, $P < 0.001$), whereas maximal suppression (93% inhibition, $P < 0.001$) was observed with IL-4 at 10 ng/ml. IL-1β synthesis was reduced to the same extent by IL-4 at 100 ng/ml (94% inhibition, $P < 0.001$). In contrast, LPS-induced IL-1ra synthesis was increased 1.6-fold by IL-4 at 10 ng/ml. No further increase in LPS-induced IL-1ra synthesis was seen with IL-4 at 100 ng/ml. Similar effects of IL-4 were seen when PBMC were stimulated with LPS at 100 pg/ml or 1 ng/ml (data not shown).

We next investigated whether IL-4 and LPS had additive or synergistic effects on IL-1ra synthesis. PBMC were collected from nine different donors. PBMC stimulated with either IL-4 (10 ng/ml) or LPS (10 ng/ml) produced IL-1ra at 3.36 ± 0.49 ng/ml and 9.79 ± 1.08 ng/ml, respectively. However, IL-1ra levels measured in PBMC stimulated with both IL-4 and LPS were significantly higher (18.69 ± 1.68 ng/ml) than the calculated sum of IL-1ra levels from PBMC stimulated with either IL-4 or LPS (13.14 ± 1.46 ng/ml; $P < 0.01$).

Effect of IL-4 on LPS-Induced mRNA Accumulation for IL-1ra and IL-1β. Unstimulated PBMC did not express detectable levels of mRNA for IL-1β or IL-1ra (Fig. 3). IL-1β and IL-1ra protein levels from these cultures were, respectively, 0.07 and 0.64 ng/ml. IL-4 alone (1 and 10 ng/ml) increased IL-1ra mRNA levels and induced IL-1ra protein synthesis (2.16 and 2.91 ng/ml, respectively). However, IL-4 induced neither IL-1β protein synthesis nor accumulation of mRNA.

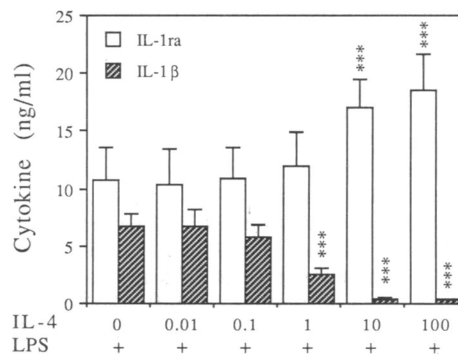

FIG. 2. Effect of IL-4 on the LPS-induced synthesis of IL-1ra and IL-1β. PBMC were stimulated with LPS (10 ng/ml) in the presence or absence of IL-4 (0.01–100 ng/ml). Differences in cytokine synthesis were analyzed for significance by analysis of variance (***, $P < 0.001$). Data are expressed as mean ± SEM for four donors.

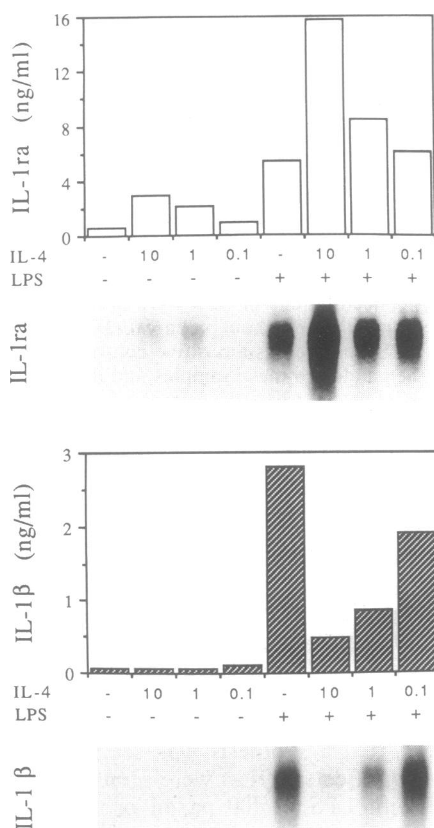

FIG. 3. Effect of IL-4 on LPS-induced mRNA accumulation for IL-1ra (*Upper*) and IL-1β (*Lower*). PBMC were stimulated with either IL-4 alone (0.1–10 ng/ml) or LPS (10 ng/ml) in the absence or presence of IL-4. Comparable amounts of total RNA were detected in all lanes by ethidium bromide staining (data not shown). mRNA levels for IL-1ra and IL-1β were analyzed 6 hr after LPS stimulation by Northern blotting (below). Protein levels from PBMC cultures at the same 6-hr time point were assayed by RIA (above). Northern blots and protein levels of one experiment representative of two donors are shown.

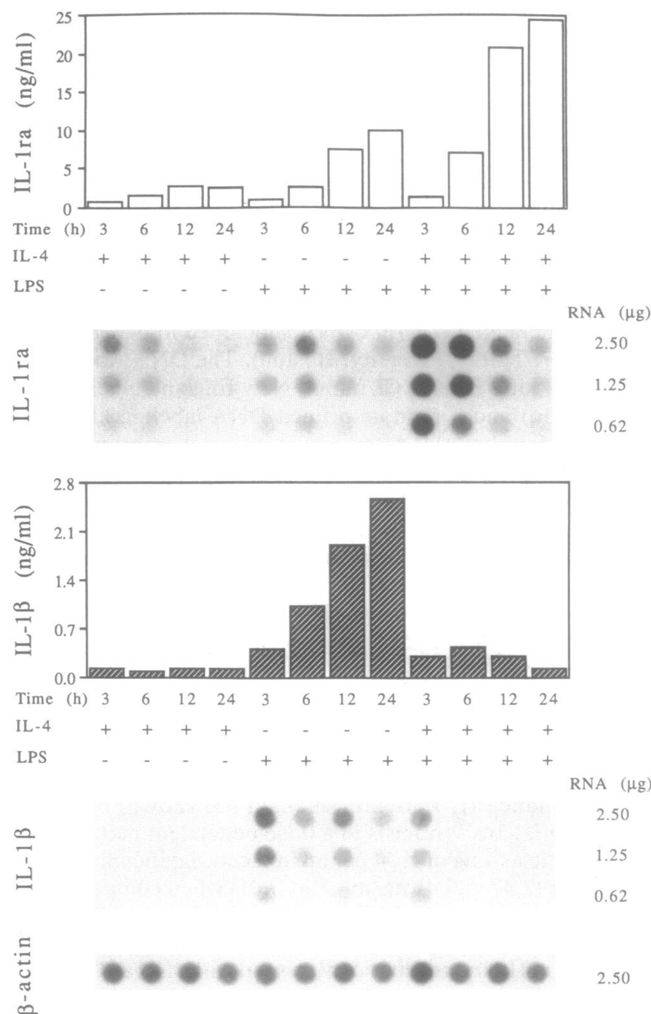

FIG. 4. Kinetics of the IL-4-mediated regulation of IL-1ra (*Upper*) and IL-1β (*Lower*) gene expression in LPS-stimulated PBMC. PBMC were stimulated with either IL-4 alone (10 ng/ml) or LPS (10 ng/ml) in the absence (−) or presence (+) of IL-4. mRNA levels for IL-1ra, IL-1β, and β-actin were analyzed 3, 6, 12, and 24 hr after LPS stimulation by dilutional analysis. Serial dilutions of total RNA (2.50, 1.25, and 0.62 μg) for each experimental point are shown. Protein levels from PBMC cultures at the corresponding time points were assayed by RIA. Dilutional analyses and protein levels of one experiment representative of three donors are shown.

IL-4 suppressed LPS-induced mRNA accumulation for IL-1β but enhanced IL-1ra mRNA levels (Fig. 3). However, IL-4 was more potent at reducing IL-1β mRNA levels than enhancing those for IL-1ra. IL-4 (10 ng/ml) dramatically suppressed mRNA accumulation for IL-1β in LPS-stimulated PBMC. At 1 ng/ml, IL-4 still reduced IL-1β mRNA levels but to a lesser extent (2-fold by dilutional analysis, data not shown). In contrast, IL-4 (10 ng/ml) markedly increased the steady-state mRNA levels for IL-1ra in LPS-stimulated PBMC (4-fold by dilutional analysis).

IL-4 Accelerates IL-1ra mRNA Accumulation and Protein Synthesis in LPS-Stimulated PBMC. mRNA accumulation for IL-1β was not observed in IL-4-treated PBMC (Fig. 4). However, IL-1ra mRNA reached peak levels within the first 3 hr and gradually returned to basal levels. IL-1ra protein levels increased during the first 12 hr of incubation with IL-4 and then reached a plateau.

The kinetics of LPS-induced mRNA accumulation for IL-1ra and IL-1β differed (Fig. 4). IL-1ra mRNA levels were maximal after 6 hr of exposure to LPS and gradually returned to basal levels. In contrast, IL-1β mRNA levels were maximal within the first 3 hr after LPS stimulation. IL-1β and IL-1ra protein levels in LPS-stimulated PBMC increased throughout the 24 hr culture (Fig. 4). With IL-4 treatment, IL-1ra mRNA levels in LPS-stimulated PBMC were increased >4-fold and reached peak levels earlier—i.e., within the first 3 hr. IL-1ra protein levels from IL-4-treated PBMC were higher at 6, 12, and 24 hr after exposure to LPS, 2-fold greater when compared at each time point to untreated cells (Fig. 4). IL-4 slightly reduced IL-1β mRNA levels 3 hr after LPS but markedly suppressed those seen at 6, 12, and 24 hr. Finally, IL-4 completely suppressed IL-1β protein synthesis in LPS-stimulated PBMC cultures (Fig. 4).

Time Dependence of IL-4 on LPS-Induced IL-1ra and IL-1β Synthesis. Because of the difference in the kinetics of LPS-induced mRNA accumulation for IL-1ra and IL-1β, we compared the effects of delayed addition of IL-4 on LPS-induced synthesis of IL-1ra and IL-1β. All PBMC cultures were assayed 24 hr after exposure to LPS. Fig. 5 shows that IL-4 added together with LPS dramatically suppressed IL-1β synthesis (95% inhibition, $P < 0.01$) but enhanced IL-1ra synthesis (59% increase, $P < 0.05$). IL-4 up-regulated IL-1ra synthesis to the same extent whether added together with LPS or within the first 9 hr of culture (45–73% increase, $P < 0.05$) because IL-1ra levels from those cultures were not significantly different ($P > 0.05$). When added as late as 12 hr, IL-4 still enhanced IL-1ra production (35% increase, $P < 0.001$). In contrast, IL-1β synthesis was reduced by IL-4 to a lesser extent as the time of its addition after LPS stimulation

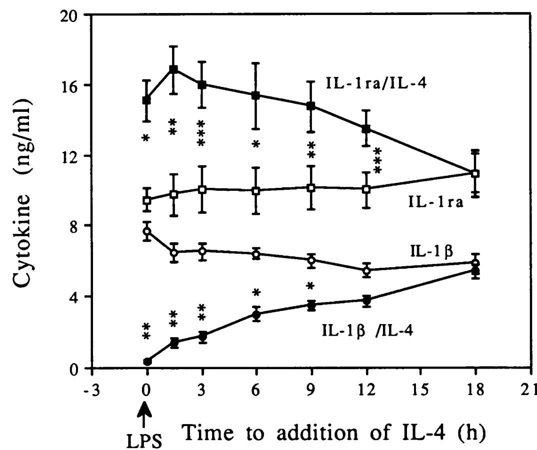

FIG. 5. Time dependence of the IL-4-mediated effects on LPS-induced synthesis of IL-1ra and IL-1β. PBMC were stimulated with LPS (10 ng/ml). IL-4 (10 ng/ml, closed symbols) or control medium (open symbols) was added together with LPS (t = 0, arrow) or at various time points (1.5, 3, 6, 9, 12, or 18 hr) after LPS. All PBMC cultures were assayed for IL-1ra (squares) and IL-1β (circles) 24 hr after LPS stimulation. Differences in cytokine synthesis between the IL-4-treated group and the control group were analyzed for significance by Student's t test for paired samples (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) at each time point. Data are expressed as mean ± SEM for three donors.

increased (Fig. 5). IL-1β synthesis was markedly suppressed by addition of IL-4 within the first 3 hr of culture (74–95% inhibition, $P < 0.01$). IL-4 still reduced IL-1β synthesis when added after 6 or 9 hr, although to a lesser extent (53% and 41% inhibition, respectively, $P < 0.05$).

Effect of IL-4 on IL-1ra and IL-1β Synthesis in IL-1α-Stimulated PBMC. IL-1α (10 ng/ml) stimulated synthesis of both IL-1ra (2.51 ± 0.73 ng/ml, n = 4) and IL-1β (0.76 ± 0.11 ng/ml) (Fig. 6). IL-4 markedly suppressed (86% inhibition, $P < 0.001$) IL-1α-induced synthesis of IL-1β (0.11 ± 0.02 ng/ml, n = 4) but also significantly enhanced (133% increase, $P < 0.05$) IL-1α-induced synthesis of IL-1ra (5.86 ± 1.07 ng/ml, n = 4). However, IL-1ra levels measured in PBMC stimulated with both IL-4 and IL-1α were not significantly different from the calculated sum of IL-1ra levels in PBMC cultures stimulated with either IL-4 or IL-1α (7.33 ± 1.55 ng/ml).

DISCUSSION

In these studies, we demonstrate that IL-4 has selective and reciprocal effects on the synthesis of the related gene products IL-1ra and IL-1β. IL-4 alone induced mRNA and protein synthesis for IL-1ra but not for IL-1β. IL-4 suppressed IL-1β production in PBMC stimulated with either LPS or IL-1α but up-regulated IL-1ra synthesis in both cases. Moreover, IL-4 enhanced IL-1ra mRNA levels in LPS-stimulated PBMC but reduced IL-1β mRNA levels. Thus, IL-1β and IL-1ra are differentially regulated by IL-4 at both mRNA and protein levels. The transcriptional regulatory sequences in the 5′-flanking region of the IL-1ra gene are likely to differ from those of IL-1β.

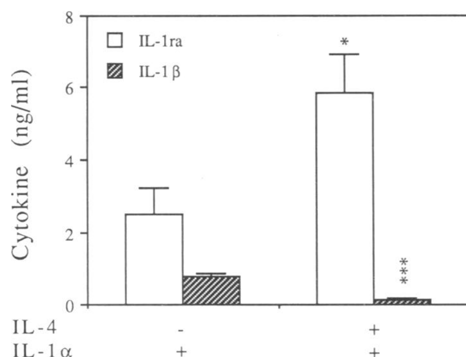

FIG. 6. Effect of IL-4 on IL-1α-stimulated synthesis of IL-1ra and IL-1β. PBMC were stimulated with IL-1α (10 ng/ml) in the absence (−) or presence (+) of IL-4 (10 ng/ml). Differences in cytokine synthesis were analyzed for significance by Student's t test for paired samples (*, $P < 0.05$; ***, $P < 0.001$). Data are expressed as mean ± SEM for four donors.

IL-4 and LPS had synergistic effects on IL-1ra synthesis at both mRNA and protein levels, suggesting that a common signal transduction pathway is activated by IL-4 and LPS. Furthermore, IL-1ra mRNA levels in LPS-stimulated PBMC reached a plateau earlier in the presence of IL-4. In addition, IL-4 increased both the magnitude and the rate of IL-1ra synthesis in LPS-stimulated PBMC. In contrast, IL-4 and IL-1α had additive effects on IL-1ra synthesis. Therefore, IL-1ra synthesis may be initiated via at least two distinct activation pathways.

Previous studies have shown that IL-4 reduces IL-1 synthesis in monocytes stimulated with either LPS or *Staphylococcus aureus* (31). In contrast, interferon γ up-regulates LPS-induced IL-1 synthesis but reduces self-induction of IL-1 (32). Furthermore, interferon γ has therapeutic value in some patients with rheumatoid arthritis (33, 34). Recently, IL-1ra has been shown to reduce joint swelling in a rat model of arthritis (35). Because IL-4 reduces self-induction of IL-1 and up-regulates IL-1-induced synthesis of IL-1ra, IL-4 may also exert beneficial effects in rheumatoid arthritis. Interestingly, low amounts of IL-4 have been found in synovial fluids from patients with rheumatoid arthritis, a disease characterized by intense T cell infiltration of synovium (36).

Induction of IL-1ra by IL-4 offers insight into the selective eosinophil accumulation in allergic disorders. Most allergen-specific T cell clones from atopic donors produce high levels of IL-4 and IL-5 but little interferon γ (37). Interestingly, overexpression of IL-4 in transgenic mice is associated with tissue infiltrates of eosinophils (38). IL-4 increases the surface expression of vascular cell adhesion molecule 1 on endothelial cells, a ligand for very late activation antigen 4 on eosinophils (39). Moreover, IL-4 suppresses the IL-1-induced surface expression of endothelial leukocyte adhesion molecule 1 and intercellular adhesion molecule 1, adhesion molecules involved in neutrophil recruitment. Induction of IL-1ra synthesis by IL-4 may, therefore, contribute to the preferential recruitment of eosinophils over that of neutrophils. Whether IL-4 induces IL-1ra synthesis in endothelial cells remains to be elucidated.

Because IL-4 favors synthesis of the natural antagonist IL-1ra over synthesis of the agonist IL-1, IL-4 may have beneficial effects in diseases where IL-1 is thought to be a key mediator, such as acute myeloblastic leukemia (40), inflammatory bowel disease (41), and Gram-negative sepsis (15). It is tempting to speculate that IL-4 may be useful as a therapeutic agent in septic shock because delayed addition of IL-4 still up-regulates the LPS-induced synthesis of IL-1ra. Finally, IL-4 may also improve survival in Gram-negative sepsis by suppressing synthesis of tumor necrosis factor α (12), a critical mediator of septic shock (42).

There are presently no data supporting a role for IL-4 in animal models of endotoxic shock. However, large doses (30–45 μg/kg per day) of recombinant IL-4 have been given i.v. to patients with refractory malignancies (43), and the coordinated antiinflammatory effects of IL-4 described herein could occur at doses which are tolerable by humans. More complete understanding of the potential beneficial effects of IL-4 in Gram-negative sepsis and other clinical conditions awaits further investigation.

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