Interleukin-13 (IL-13) Induces IL-1 Receptor Antagonist Gene Expression and Protein Synthesis in Peripheral Blood Mononuclear Cells: Inhibition by an IL-4 Mutant Protein

By Edouard Vannier, René de Waal Malefyt, Adriana Salazar-Montes, Jan E. de Vries, and Charles A. Dinarello

Interleukin-13 (IL-13), a 10-kD polypeptide secreted by activated T cells and mast cells, has multiple biologic activities on human B cells and monocytes. IL-13 promotes growth of precultivated B cells, induces germine transcripts and directs naive B cells to switch to IgE and IgG3 synthesis. IL-13 induces expression of the low-affinity receptor for IgE (FcεRI/CD23), and upregulates class II major histocompatibility complex (MHC) expression on both B cells and monocytes. In monocytes, IL-13 downregulates Fcy receptor surface expression and inhibits synthesis of inflammatory cytokines, including tumor necrosis factor α, IL-1β, IL-6, and IL-8. Moreover, IL-13 suppresses synthesis of IL-12, a critical cytokine for differentiation of uncommitted T cells towards the Th phenotype. Interestingly, all these biologic activities are shared with IL-4. Like IL-4, IL-13 may thus favor humoral over cell-mediated immunity.

In addition to its inhibitory effect on IL-1β synthesis, IL-13 reduces IL-1 activity by both inducing synthesis of IL-1 receptor antagonist (IL-1Ra) in resting monocytes and enhancing IL-1Ra synthesis in activated monocytes. IL-1Ra is a naturally occurring polypeptide with significant amino acid homology to IL-1α and IL-1β and is a member of the IL-1 gene family. IL-1Ra binds to both type I and type II IL-1 receptors without agonist activity, prevents IL-1 binding to its receptors, and inhibits IL-1 biologic activities.

We have recently shown that IL-13, like IL-4, enhances IL-1Ra synthesis in lipopolysaccharide (LPS)-stimulated monocytes, but suppresses IL-1β synthesis in the same cells. More recently, Muzzio et al have shown that IL-13 induces IL-1Ra synthesis in resting monocytes. However, these investigators suggested that IL-13, unlike IL-4, increases IL-1Ra mRNA stability rather than IL-1Ra gene transcription. We now report that a single amino acid mutant form of IL-4 (hIL-4.Y124D) is a weak inducer of IL-1Ra synthesis in human peripheral blood mononuclear cells (PBMC). However, hIL-4.Y124D inhibits IL-1Ra synthesis induced by either IL-13 or IL-4, indicating that both cytokines induce IL-1Ra synthesis via binding to a common subunit of their respective receptors. Accordingly, IL-13, like IL-4, enhanced IL-1Ra mRNA steady-state levels in PBMC stimulated with either LPS or IL-1α, but failed to affect IL-1Ra mRNA stability. Furthermore, IL-13, like IL-4, induced transcriptional activation of the IL-1Ra gene, but reduced spontaneous IL-1β gene transcription.

MATERIALS AND METHODS

Reagents. Human and mouse recombinant IL-13 were generated and purified as described. Human recombinant IL-4 was a gift from Dr T. Nakagahshian (Schering-Plough, Bloomfield, NJ). The human IL-4 mutant protein hIL-4.Y124D, in which Tyr at position 124 was replaced by Asp, was produced and purified as described. Endotoxin levels, as determined by the Limulus amebocyte lysate assay (Whittaker M.A. Bioproducts, Walkersville, MD), were typically less than 25 endotoxin units/mL. Purity of these recombinant proteins was higher than 95%. Human recombinant IL-1α was provided by Genzyme, Inc (Cambridge, MA). LPS from Escherichia coli O55:B5 was purchased from Sigma Chemical Co (St Louis, MO). Actinomycin D was purchased from Calbiochem (La Jolla, CA).

Human PBMC culture. Blood was drawn from healthy human volunteers who had not ingested histamine receptor antagonists or cyclooxygenase inhibitors for at least 2 weeks. The study was approved by the Human Investigative Review Committee of the New England Medical Center Hospitals. PBMC were separated from heparinized blood by centrifugation on Ficoll-Hypaque (Ficoll Type 400 [Sigma]; Hypaque-M 90% [Winthrop Breon Laboratories, New York, NY]) gradients. Cells were washed twice in 0.15 mol/L NaCl and resuspended at 5 × 105 cells/mL in ultraltrified RPMI culture medium 1640 (Whittaker M.A. Bioproducts) supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (GIBCO Laboratories, Grand Island, NY). PBMC (2.5 × 106 cells/mL in RPMI) were stimulated with LPS (10 ng/mL) or human IL-1α (10 ng/mL) in the absence or presence of murine IL-13, human IL-13, or human IL-4. PBMC cultures (0.5 mL final volume) were incubated in 12 × 75 mm polystyrene round bottom tubes (Becton

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Dickinson and Co, Lincoln Park, NJ) for 24 hours at 37°C in a humidified atmosphere containing 5% CO₂.

In other experiments, PBMC were stimulated for 24 hours with either IL-4 (3 ng/mL) or IL-13 (50 ng/mL) in the absence or presence of the human IL-4 mutant protein hIL-4.Y124D. Cells were exposed to hIL-4.Y124D at the time of their stimulation by IL-4 or IL-13.

Cytokine radioimmunoassays (RIA). After incubation, cultures were subjected to three freeze-thaw cycles. This procedure is optimal for recovery and measurement of total (cell-associated + secreted) cytokines by specific RIA as described for IL-1β and IL-1Ra. The sensitivities (defined as 95% binding) of the RIAs for IL-1β and IL-1Ra were typically 76 and 150 pg/mL, respectively. In one experiment, IL-1β levels were assessed using an enzyme-linked immunosorbent assay (ELISA) kit (Cistron Biotechnology, Pine Brook, NJ).

Northern analysis. PBMC were stimulated for 6 hours at 37°C in 50 μL polypropylene tubes with either LPS (10 ng/mL) or IL-1α (10 ng/mL) in the absence or presence of human IL-13 (100 ng/mL). Total cellular RNA was then extracted from PBMC by lysis with 4 mol/L guanidium isothiocyanate, followed by ultraacentrifugation on a 5.7 mol/L cesium chloride gradient. Total RNA (10 μg) was subjected to electrophoresis in 6.6% formaldehyde (Sigma) and 1.2% agarose (International Biotechnology Inc, New Haven, CT) gel and transferred to nylon membranes (Hybond-N; Amersham, Arlington Heights, IL) by capillary blotting. The membranes were exposed to short-wave UV light for 5 minutes to fix the RNA to the nylon matrix and treated for 24°C with prehybridization solution. Membranes were then treated at 42°C overnight with prehybridization solution containing 32P-labeled nucleic acid probe. The probes used were a 600-bp fragment of human IL-1Ra cDNA subcloned into pUC8, a 1,075-bp fragment of human IL-1P precursor cDNA subcloned into pGEM2, and the full-length chicken β-actin cDNA subcloned in pGEM3 (gift of Dr Brigitte Huber, Tufts University, Boston, MA). The DNA was labeled with 32P-dCTP (3,000 Ci/mmol; New England Nuclear, Boston, MA) using a random-primed DNA labeling kit (Boehringer Mannheim, Mannheim, Germany). After incubation, membranes were washed in 0.1% sodium dodecyl sulfate (SDS), 0.1% SSC at 42°C. Washed membranes were exposed to x-ray film (XAR5; Kodak, Rochester, NY) at -70°C with an intensifying screen.

Reverse transcriptase-polymerase chain reaction (RT-PCR). PBMC were stimulated for 1.5, 3, 6, 12, or 24 hours at 37°C with LPS (10 ng/mL) in the absence or presence of human IL-13 (100 ng/mL). Total cellular RNA was extracted by lysis with TRI-Reagent (Molecular Research Center, Cincinnati, OH) followed by a back-extraction with chloroform and precipitation in isopropanol. The DNA was labeled with 32P-dCTP (3,000 Ci/mmol; New England Nuclear, Boston, MA) using a random-primed DNA labeling kit (Boehringer Mannheim, Mannheim, Germany). After incubation, membranes were washed in 0.1% sodium dodecyl sulfate (SDS), 0.1% SSC at 42°C. Washed membranes were exposed to x-ray film (XAR5; Kodak, Rochester, NY) at -70°C with an intensifying screen.

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RESULTS

IL-13 induces IL-1Ra synthesis. In human PBMC, murine IL-13 alone did not induce IL-1β synthesis (data not of each specific primer. First strand cDNAs were amplified for 24 cycles using IL-1Ra primers, 20 cycles using IL-1β primers, and 30 cycles using G3PDH primers. Amplification was performed as follows: 1 cycle at 94°C for 1 minute; followed by 20-30 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes; and 1 cycle at 72°C for 7 minutes. Amplified products were subjected to electrophoresis in 1.5% agarose gel containing ethidium bromide (0.3 μg/mL). Product size was compared to molecular weight standards (1 kb DNA ladder from Gibco Life Technologies). The molecular weight markers shown in lanes “L” of Fig 7 contain the following number of base pairs: 2036, 1636, 1018, the doublet 517/3308, 517/3308, and 298 (from top to bottom).

Nuclear run-on assays. PBMC (4 × 10⁷ cells per condition) were stimulated for 3 hours at 37°C with either human IL-13 (100 ng/mL) or human IL-4 (10 ng/mL). Cells were lysed in ice-cold hypotonic buffer (10 mMol/L HEPES pH 7.6, 10 mMol/L NaCl, 3 mMol/L MgCl₂) containing 0.5% Nonidet P-40. Nuclei were pelleted at 760g for 5 minutes at 4°C and resuspended in 50 μL of ice-cold freezing buffer (40% glycerol, 50 mMol/L HEPES, pH 7.6, 5 mMol/L MgCl₂, 0.1 mMol/L EDTA). Nuclear suspensions were then added to 35 μL of transcription reaction mix [12.14 mMol/L HEPES, pH 7.6, 6.07 mMol/L MgCl₂, 0.36 mMol/L KCl, 0.8 mMol/L (NH₄)₂SO₄, 2.43 mg/mL heparin, 1.14% sarkosyl, 0.71 μL/μL RNAsin, 5.71 mMol/L DTT] containing 1 mMol/L ATP, 1 mMol/L CTP, 1 mMol/L GTP, and 50 μCi α³²P-UTP (3,000 Ci/mmol; New England Nuclear) and incubated for 30 minutes at 30°C. The reaction mix was then incubated for 30 minutes at 30°C in the presence of RNase-free DNase I (54 μg/mL) to ensure complete DNA degradation. Proteins were subsequently digested by proteinase K (0.52 mg/mL) during an incubation at 50°C for 1 hour. Nuclear transcripts were added with yeast tRNA and ammonium acetate and extracted with a mixture of phenol/chloroform/isoamyl alcohol. The upper aqueous phase was then extracted with chloroform and precipitated in ice-cold 100% ethanol. RNA was resuspended in diethylpyrocarbonate (DEPC)-treated water and purified through two additional cycles of neutralization with ammonium acetate, precipitation in 100% ethanol, and washes in 70% ethanol. After the first of these two cycles, RNA was resuspended in DNase buffer (200 U/mL RNAsin, 5 mMol/L MgCl₂, 1 mMol/L CaCl₂, 20 mMol/L HEPES, pH 7.6) containing RNase-free DNase I (35 mMol/L). After the last of these two cycles, RNA was resuspended in DEPC-treated water. Nuclear transcripts were hybridized at 42°C for 48 hours to nitrocellulose filters on which 5 μg of denatured plasmid DNA had been immobilized. The probes used were a 900-bp fragment of human IL-1Ra cDNA subcloned in Bluescript (gift of Dr Michael F. Smith Jr, University of Colorado Health Sciences Center, Denver, CO), the full length of human IL-1β precursor cDNA subcloned in the Okayama-Berg expression vector, and the full-length chicken β-actin cDNA subcloned in pGEM3. pGGL2 basic was used as an internal control vector (Promega, Madison, WI). Filters were washed 4 times in 0.1% SDS, 2× SSC for 30 minutes at 65°C and twice in 2× SSC for 10 minutes at 25°C. Filters were subsequently washed in 2× SSC containing 10 μg/mL RNase A for 30 minutes at 25°C and 4 times in 2× SSC for 2 to 3 minutes at 25°C. Washed filters were exposed to x-ray film for 2 days at -70°C with an intensifying screen.

Statistical analysis. Data are expressed as mean ± SEM of the estimated number of experiments. Differences between cytokine-treated groups and RPMI-treated groups were analyzed for significance by Student’s t-test for paired samples or two-way analysis of variance (ANOVA).

RESULTS

IL-13 induces IL-1Ra synthesis. In human PBMC, murine IL-13 alone did not induce IL-1β synthesis (data not
shown) but rather stimulated IL-1Ra synthesis in a dose-dependent manner (Fig 1). IL-13 at 10 ng/mL induced significant amounts of IL-1Ra (1.52 ± 0.47 ng/mL, P < .01) when compared with basal levels (0.48 ± 0.10 ng/mL, n = 3). A further increase in IL-1Ra synthesis was observed when IL-13 was used at either 100 ng/mL (3.28 ± 0.45 ng/mL, P < .001) or 500 ng/mL (4.68 ± 0.47 ng/mL, P < .001). Moreover, IL-1Ra levels induced by IL-13 at 500 ng/mL were significantly higher (P < .01) than those observed for IL-13 at 100 ng/mL. Because in vivo levels of IL-13 are unlikely to be as high as 500 ng/mL, regulation of cytokine synthesis was subsequently studied using IL-13 at concentrations up to 100 ng/mL.

We next compared the potencies of human and murine IL-13 in our in vitro model. IL-1Ra synthesis was induced to the same extent by murine IL-13 (3.55 ± 0.22 ng/mL) and human IL-13 (4.57 ± 0.80 ng/mL, n = 4, P > .05) when used at the same concentration of 100 ng/mL.

IL-13 and IL-4 share a common pathway in inducing IL-1Ra synthesis. Because IL-4 induces IL-1Ra synthesis without inducing IL-1β synthesis, we investigated whether IL-13 and IL-4 could display additive or synergistic effects in inducing IL-1Ra synthesis. IL-4 was used at 1 ng/mL because this concentration elicited a 50% maximal induction of IL-1Ra synthesis. As shown in Fig 2, the addition of IL-13 at concentrations of 1 or 10 ng/mL did not affect IL-4–induced IL-1Ra synthesis (P > .05). However, IL-4–induced IL-1Ra synthesis was significantly increased (P < .001) in the presence of a higher concentration of IL-13 (100 ng/mL). Nevertheless, IL-1Ra levels induced by IL-13 alone at 100 ng/mL did not significantly differ from those induced by the same concentration of IL-13 in the presence of IL-4 (P > .05).

Because IL-13 and IL-4 had no additive or synergistic effects in inducing IL-1Ra synthesis, we next investigated the effect of the human IL-4 mutant protein hIL-4.Y124D on IL-1Ra synthesis induced by either IL-13 or IL-4. As shown in Fig 3, hIL-4.Y124D alone induced IL-1Ra synthesis in a dose-dependent manner. Maximal induction of IL-1Ra synthesis (1.60 ± 0.29 ng/mL, P < .01) was observed at 1 μg/mL. Thus, hIL-4.Y124D exhibited a partial agonist activity because, in the same PBMC preparations, native IL-4 used alone at a suboptimal concentration (5 ng/mL) induced 3.49 ± 0.70 ng/mL IL-1Ra (n = 3). Furthermore, hIL-4.Y124D inhibited the IL-13–induced IL-1Ra synthesis in a dose-dependent manner (Fig 3). hIL-4.Y124D at 0.3 μg/mL marginally suppressed IL-1Ra synthesis (21% inhibition, P < .01). A further reduction in IL-13–induced IL-1Ra synthesis (31% inhibition, P < .001) was observed when hIL-4.Y124D was used at 1 μg/mL. At a higher concentration of 3 μg/mL, hIL-4.Y124D reduced IL-1Ra levels (45% inhibition, P < .001) in IL-13–stimulated cells to those observed in the presence of the mutant protein alone. Similarly, hIL-4.Y124D at 3 μg/mL inhibited IL-1Ra synthesis in IL-4 (3 ng/mL)–stimulated cells to the extent of that seen in cells exposed to the mutant protein alone (data not shown).

IL-13 differentially regulates IL-1Ra and IL-1β synthesis in LPS- or IL-1α–stimulated cells. Unstimulated PBMC cultures contained IL-1Ra at 0.54 ± 0.07 ng/mL and IL-1β at less than 0.08 ng/mL (n = 6). LPS (1 ng/mL) induced synthesis of IL-1Ra (11.19 ± 1.43 ng/mL) and IL-1β (6.62 ± 1.11 ng/mL). As shown in Fig 4, IL-13 reduced LPS-induced IL-1β synthesis but upregulated IL-1Ra synthesis in a dose-dependent manner. IL-13 at 1 ng/mL did not modify LPS-induced synthesis of either IL-1Ra or IL-1β. At 10 ng/mL, IL-13 marginally enhanced IL-1Ra synthesis (13% increase, P < .05) without affecting IL-1β synthesis. How-
ever, at 100 ng/mL, IL-13 both enhanced IL-1Ra synthesis (33% increase, \( P < .001 \)) and reduced IL-1β synthesis (69% inhibition, \( P < .01 \)). Similar effects of IL-13 at 100 ng/mL were observed when PBMC were stimulated with LPS either at 100 pg/mL (22% increase IL-1Ra, \( P < .01; 73\% \) inhibition IL-1β, \( P < .05; n = 6 \)) or 10 ng/mL (22% increase IL-1Ra, \( P < .001; 64\% \) inhibition IL-1β, \( P < .001; n = 10 \)).

We also investigated the effect of IL-13 on IL-1Ra and IL-1β synthesis in IL-1-stimulated cells. Unstimulated PBMC cultures contained IL-1Ra at 0.56 ± 0.06 ng/mL and IL-1β at less than 0.08 ng/mL (\( n = 7 \)). IL-1α (10 ng/mL) stimulated synthesis of both IL-1Ra (3.34 ± 0.69 ng/mL) and IL-1β (1.06 ± 0.20 ng/mL). IL-13 differentially regulated IL-1α-induced synthesis of IL-1Ra and IL-1β (Fig 5). IL-13 at 10 ng/mL marginally enhanced IL-1Ra synthesis (22% increase, \( P < .05 \)) but did not modify IL-1β synthesis. However, at 100 ng/mL, IL-13 significantly enhanced IL-1Ra synthesis (79% increase, \( P < .001 \)) but also markedly inhibited IL-1β synthesis (83% inhibition, \( P < .001 \)).

Effect of IL-13 on steady-state levels for IL-1Ra and IL-1β mRNA in LPS- or IL-1α-stimulated PBMC. After 6 hours of culture, unstimulated PBMC did not express detectable levels of mRNA for IL-1β or IL-1Ra (Fig 6). IL-1β and IL-1α protein levels from these cultures were 0.08 and 0.48 ng/mL, respectively. IL-13 alone (100 ng/mL) increased IL-1Ra mRNA levels and induced IL-1Ra protein synthesis (2.17 ng/mL). However, IL-13 induced neither IL-1β protein synthesis nor accumulation of IL-1β mRNA.

IL-13 markedly suppressed IL-1β mRNA accumulation in cells stimulated with LPS or IL-1α (Fig 6). Accordingly, IL-13 reduced IL-1β synthesis induced by either stimulus. In contrast, IL-13 dramatically enhanced LPS-induced IL-
IL-13 INDUCES IL-1Ra GENE TRANSCRIPTION

**Fig 6.** Effect of IL-13 on LPS- or IL-1α–induced mRNA accumulation for IL-1Ra and IL-1β. PBMC were stimulated with either LPS (10 ng/mL) or IL-1α (10 ng/mL) in the absence (−) or presence (+) of human IL-13 (100 ng/mL). mRNA levels for IL-1Ra (upper panel), IL-1β (middle panel), and β-actin (lower panel) were analyzed 6 hours after cell stimulation using Northern blotting (below). Protein levels in the same 6-hour PBMC cultures were assayed by RIA (above). Northern blots and protein levels of one experiment representative of two donors are shown.

1Ra mRNA accumulation. In these cells, IL-13 also enhanced IL-1Ra synthesis. However, we did not observe a significant increase in steady-state levels for IL-1Ra mRNA 6 hours (Fig 6) or 16 hours (data not shown) after cell stimulation with IL-1α. Nevertheless, IL-1Ra mRNA levels in IL-1α–stimulated cells were marginally enhanced in the presence of IL-13 when compared with those observed in cells stimulated with IL-13 alone (Fig 6). In the same cell cultures, IL-13 and IL-1α had additive effects on IL-1Ra synthesis.

**Time-course of IL-1Ra and IL-1β gene expression in LPS-stimulated PBMC: effect of IL-13.** Because monocytes primarily express the secreted form of IL-1Ra over its intracellular counterpart,33 we studied the effect of IL-13 on steady-state levels of mRNA coding for the secreted form of IL-1Ra using the RT-PCR technique (Fig 7). IL-13 alone (100 ng/mL) induced a rapid accumulation of IL-1Ra mRNA in PBMC (upper row, left panel). IL-1Ra mRNA levels increased as early as 1.5 hours after exposure to IL-13 and reached peak levels within the first 3 hours of stimulation. The accumulation of IL-1Ra mRNA was transient because IL-1Ra mRNA levels gradually returned to basal levels within 24 hours of culture. IL-1Ra protein levels rapidly increased during the first 6 hours of exposure to IL-13 and gradually increased thereafter (upper histogram, left segment). In contrast, IL-13 failed to induce a detectable accumulation of IL-1β protein (lower histogram, left segment) or IL-1β mRNA (middle row, left panel) within the 24 hours of culture.

In LPS-stimulated PBMC, IL-13 enhanced IL-1Ra mRNA and protein levels (Fig 7, upper row, upper, middle, and right panels). Accumulation of IL-1Ra mRNA in LPS-stimulated cells was markedly increased in the presence of IL-13 at 1.5 and 3 hours of culture. This increase was also observed after 6 hours of culture, although to a lesser extent. Accordingly, IL-13 enhanced IL-1Ra protein levels in LPS-stimulated cells at each time point throughout the 24 hours of culture (upper histogram, upper and right segments). In contrast, IL-13 reduced IL-1β mRNA in LPS-stimulated cells (upper row, upper middle, and right panels). IL-13 remained without noticeable effect on IL-1β mRNA levels at the early time points of 1.5 and 3 hours. However, IL-13 markedly suppressed IL-1β mRNA levels in LPS-stimulated cells at 6, 12, and 24 hours of culture. A similar effect on IL-13 on LPS-induced IL-1β synthesis was observed (lower histogram, middle and right segments). IL-13 failed to modify accumulation of IL-1β protein within the first 3 hours after exposure to LPS, but dramatically suppressed any further IL-1β synthesis.

**Effect of IL-13 on the stability of IL-1Ra and IL-1β mRNA.** Because unstimulated PBMC did not express detectable levels of IL-1Ra mRNA, we studied the effect of IL-13 on IL-1Ra mRNA stability in LPS-stimulated PBMC. Cells were stimulated for 6 hours with LPS (10 ng/mL). Actinomycin D was then added to the cultures to block any further transcription. As shown in Fig 8, exponential regression analyses indicated that IL-13 (100 ng/mL) failed to affect the half-life of IL-1Ra mRNA in LPS-stimulated cells (2.7 hours in the absence of IL-13 v 2.5 hours in the presence of IL-13). In contrast, IL-13 reduced the half-life of IL-1β mRNA in these same cells (2.9 hours in the absence of IL-13 v 1.7 hours in the presence of IL-13).

**Effect of IL-13 on transcriptional activation of genes coding for IL-1Ra and IL-1β: comparison with IL-4.** Because
IL-13 failed to modify IL-1Ra mRNA stability, we next investigated whether IL-13, like IL-4, could modulate IL-1Ra gene expression at the transcriptional level. PBMC were stimulated for 3 hours with either IL-13 (100 ng/mL) or IL-4 (10 ng/mL). Nuclei were purified and nuclear run-on assays performed. As shown in Fig 9, transcription of the IL-1Ra gene in control, unstimulated cells was below the sensitivity of our assay. IL-13 induced IL-1Ra gene transcription. A comparable transcriptional activity of the IL-1Ra gene was observed in cells stimulated with IL-4. We also studied the effects of IL-13 and IL-4 on transcriptional activation of the IL-1β gene. Although we could not detect any significant accumulation of IL-1β mRNA in unstimulated cells using the RT-PCR technique, IL-1β gene transcription took place in control cells as indicated by the nuclear run-on assay. As expected, IL-13 and IL-4 reduced the spontaneous transcriptional activation of the IL-1β gene.

DISCUSSION

In these studies, we show that IL-13 has selective and reciprocal effects on the synthesis of two members of the IL-1 gene family, i.e., IL-1Ra and IL-1β. IL-13 alone induced mRNA and protein synthesis for IL-1Ra, but not for IL-1β. IL-13 suppressed IL-1β mRNA and protein levels in PBMC stimulated with LPS or IL-1α, but enhanced IL-1Ra mRNA and protein levels in these same cells. Thus, IL-13, like IL-4, inversely regulates IL-1Ra and IL-1β synthesis.

IL-13 and IL-4 displayed neither synergistic nor additive effect in inducing IL-1Ra synthesis, supporting the concept that IL-13 and IL-4 share a common signal transduction pathway.

Fig 7. Kinetics of the IL-13-mediated regulation of IL-1Ra and IL-1β gene expression in LPS-stimulated PBMC. PBMC were stimulated either with human IL-13 alone (100 ng/mL) or with LPS (10 ng/mL) in the absence (−) or presence (+) of IL-13. mRNA levels for the secreted form of IL-1Ra (upper row), IL-1β (middle row), and G3PDH (lower row) were analyzed by RT-PCR at 1.5, 3, 6, 12, and 24 hours. Amplified products were subjected to electrophoresis side with the 1-kb DNA ladder from GIBCO Life Technologies (lane L in each panel). The expected PCR products for the secreted form of IL-1Ra, IL-1β, and G3PDH comprised 329, 802, and 983 bp, respectively. Protein levels from PBMC cultures at the corresponding time points were assayed by RIA for IL-1Ra and ELISA for IL-1β.
pathway. We further investigated the commonality between IL-13 and IL-4 activities using the mutant hIL-4 protein hIL-4.Y124D. Although substitution of Tyr124 by Asp at the carboxyl terminus of the fourth α helix of IL-4 results in nearly the same high-affinity receptor binding as the wild-type IL-4, this IL-4 variant has no detectable activity in a T-cell proliferation assay. However, this mutant retains a partial agonist activity in more sensitive B-cell assays, ie, induction of IgE synthesis or surface expression of CD23. In agreement with the latter reports, hIL-4.Y124D alone was a weak inducer of IL-1Ra synthesis. Nevertheless, hIL-4.Y124D inhibited both IL-13- and IL-4-induced IL-1Ra synthesis, indicating that IL-13 and IL-4 may induce IL-1Ra synthesis via binding to a common subunit of their respective heterodimeric receptors. Monoclonal antibodies directed against the 130-kD chain of the high-affinity IL-4 receptor (IL-4Ra) have been shown to block the antiproliferative effect of IL-13 on human B-cell precursors and the suppressive effect of IL-13 on IL-6 synthesis in LPS-stimulated human monocytes. Furthermore, recent studies have established that the IL-2 receptor γc chain, previously regarded as the common subunit to the IL-4 and IL-13 receptors, mediates some of the biologic activities of IL-4, but does not participate in the formation of IL-13 receptor complexes. Thus, IL-4Ra likely engages the IL-13/IL-1 receptor complex and may participate in signal transduction leading to IL-1Ra gene expression.

Fig. 8. Effect of IL-13 on the half-lives of IL-1Ra and IL-1β mRNA in LPS-stimulated PBMC. PBMC were stimulated with LPS (10 ng/mL) or RPMI 1640 culture medium (control) for 6 hours. Relative steady-state mRNA levels were determined by actinomycin D pulse-chase experiments. Relative mRNA levels were measured at 1, 2, and 4 hours after pulse labeling. IL-13 did not affect the half-life of β-actin mRNA (data not shown). Data are plotted as log of percent remaining cytokine mRNA versus time of decay in hours. IL-1Ra and IL-1β mRNA half-lives were determined by regression analyses.

Fig. 9. Effect of IL-13 on transcriptional activation of genes coding for IL-1Ra and IL-1β: comparison with IL-4. PBMC (4 x 10⁶ cells/condition) were stimulated for 3 hours with human IL-13 (100 ng/mL) or human IL-4 (10 ng/mL). Unstimulated cells are indicated by the letter "C" (for control). Nuclei were isolated and nuclear run-on assays performed as described in the Materials and Methods. Nuclear transcripts were analyzed for the presence of mRNA encoding IL-1Ra, IL-1β, or β-actin. The pGL2 basic vector served as an internal control.

4.Y124D inhibited both IL-13- and IL-4-induced IL-1Ra synthesis, indicating that IL-13 and IL-4 may induce IL-1Ra synthesis via binding to a common subunit of their respective heterodimeric receptors. Monoclonal antibodies directed against the 130-kD chain of the high-affinity IL-4 receptor (IL-4Ra) have been shown to block the antiproliferative effect of IL-13 on human B-cell precursors and the suppressive effect of IL-13 on IL-6 synthesis in LPS-stimulated human monocytes. Furthermore, recent studies have established that the IL-2 receptor γc chain, previously regarded as the common subunit to the IL-4 and IL-13 receptors, mediates some of the biologic activities of IL-4, but does not participate in the formation of IL-13 receptor complexes. Thus, IL-4Ra likely engages the IL-13/IL-1 receptor complex and may participate in signal transduction leading to IL-1Ra gene expression.

Induction of IL-1Ra synthesis by IL-13 was observed at concentrations 10-fold higher that those required to observe a similar effect with IL-4. Interestingly, IL-13 induces a rapid tyrosine phosphorylation and activation of raf-1 kinase in the human monocytic progenitor cell line U937 at a concentration of 0.1 ng/mL. However, a similar profile of protein phosphorylation is observed in human monocytes when concentrations of IL-13 are higher than 5 ng/mL. Because differences in responsiveness of human monocytes to IL-4 and IL-13 cannot be related to surface expression of IL-4Ra, the common chain to their respective receptors, one might speculate that differentiation along the monocytic lineage is accompanied by a reduced expression of the IL-13 receptor chain or a decreased affinity of the IL-13 receptor complex.

IL-4 alone induces mRNA accumulation for IL-1Ra by increasing transcriptional activity of the IL-1Ra gene with no effect on the half-life of its mRNA. Similarly, IL-4 enhances IL-1Ra mRNA steady-state levels in LPS-stimulated monocytes without affecting mRNA half-life. In agreement with these findings, IL-1Ra mRNA does not contain AU-rich sequences typically found in the 3'-untranslated regions of unstable mRNAs. Recently, Muzio et al have
reported that IL-13 induces IL-1Ra synthesis by increasing the stability of its mRNA. Surprisingly, they based their conclusion on a comparison of the IL-1Ra mRNA half-life in unstimulated monocytes with that calculated from IL-13-stimulated cells. However, we were unable to observe any significant accumulation of IL-1Ra mRNA in unstimulated PBMC. We therefore investigated the effect of IL-13 on the stability of IL-1Ra mRNA in LPS-stimulated cells. These cells, IL-13 failed to affect the half-life of IL-1Ra mRNA. However, like IL-4,16 IL-13 reduced the half-life of IL-1β mRNA in LPS-stimulated cells. We then investigated the effect of IL-13 on transcriptional activation of the genes coding for IL-1Ra and IL-1β. Like IL-4, IL-13 alone induced transcriptional activation of the IL-1Ra gene, but reduced the spontaneous IL-1β gene expression. These results are in agreement with the inhibitory activity of hIL-4.Y124D on IL-1Ra synthesis induced by either IL-13 or IL-4 because the commonality of their regulatory effects on cytokine gene expression likely relies on their binding to a common receptor subunit critical for signal transduction.26,31

IL-4 reduces self-induction of IL-1 and upregulates IL-1–induced synthesis of IL-1Ra.13 We now report that IL-13, like IL-4, differentially regulated IL-1Ra and IL-1β synthesis in IL-1α–stimulated PBMC. Furthermore, IL-13 enhanced IL-1Ra mRNA accumulation in IL-1α–stimulated cells but dramatically reduced IL-1β mRNA accumulation in these same cells. Similarly, IL-13 enhanced IL-1Ra mRNA levels in LPS-stimulated PBMC, but suppressed IL-1β mRNA accumulation in these cells, thus extending our preliminary observation that IL-13 differentially regulates IL-1Ra and IL-1β synthesis in LPS-stimulated monocytes.7 Because IL-13 favors synthesis of IL-1Ra over the synthesis of IL-1β regardless of the inflammatory stimulus, our present results suggest that IL-13 could exert its anti-inflammatory properties in various inflammatory settings.

The anti-inflammatory properties of IL-13 may be of therapeutic interest in diseases in which IL-1 is thought to be a key mediator, such as adult myeloid leukemia,38 inflammatory bowel disease,39 and rheumatoid arthritis.40 Regarding the latter condition, IL-4 has been shown to reduce chronic synovial inflammation in a rat model of arthritis.41 Because administration of recombinant human IL-4 to cancer patients results in increased levels of circulating IL-1Ra,14,42 future clinical trials should confirm the anti-inflammatory properties of IL-13 we observed in vitro. However, because IL-13 does not act on T cells,26 IL-13 should display a more selective repertoire of biologic activities than IL-4.

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Interleukin-13 (IL-13) induces IL-1 receptor antagonist gene expression and protein synthesis in peripheral blood mononuclear cells: inhibition by an IL-4 mutant protein

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