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Potential antiinflammatory effects of interleukin 4: Suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2

(glucocorticoids/lymphokines/monocyte activation)

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ABSTRACT **Stimulated human monocytes/macrophages are a source of mediators such as tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and prostaglandin E_2 (PGE_2), which can modulate inflammatory and immune reactions. Therefore, the ability to control the production of such mediators by monocytes/macrophages may have therapeutic benefits, and it has been proposed that glucocorticoids may act in this way. Purified human monocytes, when stimulated *in vitro* with lipopolysaccharide (LPS) or with LPS and γ interferon (IFN-γ), produce TNF-α, IL-1, and PGE_2. Cotreatment of stimulated cells with the purified human lymphokine, interleukin 4 (IL-4 $\geq$ 0.1–0.5 unit/ml; 12–60 pM) dramatically blocked the increased levels of these three mediators; for TNF-α and IL-1, the inhibition was manifest at the level of mRNA. Thus, IL-4 can suppress some parameters of monocyte activation and, as for B cells, have opposite effects to IFN-γ. The effects of IL-4 on human monocytes are similar to those obtained with the glucocorticoid dexamethasone (0.1 μM).**

Tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and arachidonic acid metabolites, such as prostaglandins, are produced by stimulated monocytes/macrophages and have been implicated in many of the inflammatory, immunological, hematological, and metabolic changes occurring during infection and tissue injury (for reviews, see refs. 1 and 2). For example, TNF-α and IL-1 (IL-1α and IL-1β) are endogenous pyrogens (3) that induce proteases and alter arachidonic acid metabolism in a number of cell types (4), cause cartilage degradation *in vitro* (5), and induce the synthesis of hepatic acute-phase proteins (2). Prostaglandins, thromboxanes, and prostacyclins are likely to be involved in pain, edema, and other vascular changes (6).

Glucocorticoids, which are potent and widely used antiinflammatory drugs, inhibit monocyte/macrophage TNF-α and IL-1β production at the transcriptional and posttranscriptional levels (7, 8). The suppression by glucocorticoids of the production of prostanoids, most likely by blocking phospholipase A_2 activity (9), may also explain in part how these steroids are acting as antiinflammatory drugs (7, 10). However, the side effects of corticosteroid therapy have limited their use for long-term treatment (10), and alternative therapeutic agents are required.

Interleukin 4 (IL-4), a 20-kDa product from activated T lymphocytes, was originally described (and called B-cell stimulatory factor 1) by its ability to stimulate the entry of murine anti-IgG-activated B cells into the S phase of the cell cycle (11). However, this lymphokine also has a variety of stimulatory and inhibitory actions on B and T cells (for reviews, see refs. 12 and 13; also see refs. 14 and 15). Cells of the monocyte/macrophage lineage have receptors for IL-4; binding of IL-4 induces many products similar to those induced in B cells—e.g., major histocompatibility complex class I and class II antigens (16, 17) and CD23 (18). Recently, IL-4 was identified as a factor stimulating human monocyte differentiation *in vitro* in that it caused changes in monocyte morphology, up-regulated many differentiation-linked antigens, and reduced the capacity of the cells to secrete an uncharacterized IL-1-like activity (19). Other functions of monocytes/macrophages—e.g., antibody-dependent cell cytotoxicity—are unaffected by IL-4 (20).

We report here that purified recombinant human IL-4 inhibited the ability of human monocytes to produce TNF-α, IL-1, and prostaglandin E_2 (PGE_2). For TNF-α and IL-1β [the major form of IL-1 produced by human monocytes (21)], this inhibition occurred, at least in part, at the level of mRNA. The IL-4 effects were similar to those found with the corticosteroid dexamethasone (Dex).

MATERIALS AND METHODS

Monocyte Isolation. As previously described (22, 23), mononuclear cells were selected by centrifugation (170 $\times$ *g* for 30 min) of leukocyte-rich fractions (Melbourne Red Cross Blood Bank) on pyrogen-tested Lymphoprep (Nycomed, Oslo) and suspended in Hanks' balanced salt solution (Commonwealth Serum Laboratories, Melbourne, Australia) containing 0.21% sodium citrate, polymyxin B sulfate (Sigma) at 1 μg/ml, and neomycin sulfate at 70 μg/ml. Monocytes were isolated by countercurrent centrifugal elutriation (Beckman JE-6B Elutriation System) with a constant rotor speed (2000 rpm) but increasing pump rates from 8 to 22 ml/min. For each elutriation process, monocyte fractions were collected at a rate between 14.5 and 22.0 ml/min; monocyte enrichment $\geq$ 90% was confirmed by cell morphology on Giemsa-stained cytocentrifuged smears and by nonspecific esterase staining. Lymphocytes were the main contaminating cell type; polymorphonuclear cells always were 3% or less (22, 23).

Monocyte Culture. Monocyte-rich fractions were pooled and resuspended in α-modified Eagle's medium (α-MEM; Flow Laboratories) supplemented with 20 mM 3-(*N*-morpholino)propanesulfonic acid (Sigma), 13.3 mM $NaHCO_3$, 2 mM glutamine, 50 μM 2-mercaptoethanol, 70 μg of neomycin sulfate per ml, and 1% fetal calf serum (Flow) (complete α-MEM) with an osmolarity of 290 mmol/kg (22, 23). Cells (0.8×10^6 to 1.0×10^6) were cultured in 1 ml of medium in 2-cm^2 tissue culture plastic wells (Linbro). Where indicated,

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Abbreviations: IL-1 and -4, interleukins 1 and 4; TNF-α, tumor necrosis factor α; PGE_2, prostaglandin E_2; IFN-γ, γ interferon; LPS, lipopolysaccharide; Dex, dexamethasone; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mAb, monoclonal antibody.

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the lymphokines (0.01 ml) were added at the following final concentrations: IL-4, 0.01–5.0 unit(s)/ml; IFN-γ, 100 units/ml (23). Lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4, purified by the Westphal method (Difco), was added to a final concentration of 100 ng/ml. Polymyxin B sulfate, which inhibits LPS binding to cell membranes, was added at 1 μg/ml to LPS-free cultures. Triplicate cultures for each test variable were incubated at 37°C in 5% CO_2/95% air for 4 or 18 hr and were terminated by the removal, centrifugation (170 $\times$ *g* for 7 min), and storage of the supernatant at −20°C until assay. In many experiments, the adherent cells, together with those pelleted by centrifugation of the culture media, were lysed by Zaponin (Coulter), and the nuclei were quantitated in a Coulter Counter (22, 23). In all experiments after 18 hr in culture, regardless of the lymphokines/reagents added, there was no change in the number of monocyte nuclei recovered; therefore, mediator activities released into the culture supernatants were expressed according to the number of cells at the beginning of the 18-hr culture.

Assays of TNF-α. *Bioassay.* TNF-α activity was measured as described (22, 23) with actinomycin D-treated L929 target cells. One unit of TNF-α activity was defined as the amount that caused 50% destruction (i.e., 50% absorbance change) of the L929 cells; the units of TNF-α activity in the monocyte culture supernatants were expressed as the reciprocal of the dilution necessary to achieve 50% cell cytotoxicity. For each assay, a dose–response curve using a recombinant TNF-α standard was constructed; 1 unit/ml $\approx$ 5 pM. The assay was sensitive to TNF-α levels of 0.1 pM. The blocking of cytotoxic activity in monocyte supernatants by an anti-TNF-α monoclonal antibody (mAb; 0.7 μg/ml) was confirmed in all assays (22, 23).

RIA. Immunoreactive TNF-α was measured as described (23). Standards or culture supernatants (0.1 ml) were mixed with 0.1 ml of diluted (1:400) rabbit antiserum to recombinant human TNF-α and were incubated for 24 hr. ^{125}I-labeled TNF-α (0.05 ml, 21.9 μCi/μg, 4500 pg/ml; 1 μCi = 37 kBq) was added and incubated for 5–6 hr at room temperature. Bound and free TNF-α were separated by adding 0.05 ml of a 50% slurry of protein A-Sepharose beads (Pharmacia). TNF-α levels $\geq$ 100 pg/ml could be detected.

Assays of IL-1. *Bioassay.* IL-1 was assayed by the murine thymocyte comitogenesis assay (22, 23). One unit of IL-1 activity was defined as the amount that stimulated 50% maximal thymocyte proliferation. IL-1 activity in the monocyte supernatants was measured at multiple dilutions (each dilution in duplicate) with the expressed activity being the reciprocal of the dilution giving half-maximal [^{3}H]thymidine incorporation. In each assay, a standard curve was constructed by using a recombinant IL-1β standard; a standard of 1 unit/ml was approximately equal to 5 pM. Specific antibodies to IL-1α and to IL-1β confirmed previous reports (21) that human monocytes secrete predominantly IL-1β. None of the lymphokines or reagents used to stimulate monocytes *in vitro* acted as a comitogen for thymocytes under the culture conditions described.

ELISA. An adaption of the assay described by Kenney *et al.* (24) was used. Polyvinyl chloride microtiter wells (Dynatech) were coated overnight at 4°C with 0.05 ml of an anti-IL-1β mAb (IgG1; 30 μg/ml in 50 mM $NaHCO_3$, pH 9.0), and nonspecific binding was blocked with 2.5% bovine serum albumin. Samples or standards (0.05 ml) were added for 2 hr, followed by biotinylated anti-IL-1β mAb (0.05 ml, 1 μg/ml) for 2 hr and streptavidin–biotinylated horseradish peroxidase complex (diluted 1:1000, 0.05 ml; Amersham) for 1.5 hr. Between each procedure, the wells were washed with phosphate-buffered saline containing 0.1% Tween-20. The substrate mixture, 0.05% 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt (Sigma)/0.09% H_2O_2/0.1 M trisodium citrate, pH 4.6, was added for 30 min. IL-1 levels were quantitated by absorbance readings at 414 nm. An IL-1β dilution curve was prepared for each assay by using an IL-1β standard from the National Institute for Biological Standards and Control, Hampstead, London; the assay was sensitive to 0.4 ng of IL-1β per ml.

Assay of PGE_2. Levels of PGE_2 in monocyte culture supernatants ($\geq$0.03 ng/ml) were determined by immunoassay using competitive adsorption to dextran-coated charcoal (PGE_2 ^{3}H/RIA Kit, Seragen) (22, 23). All determinations were performed in duplicate, and the mean was used to calculate PGE_2 levels by interpolation from a standard curve.

Assay of Protein Synthesis. Monocytes were cultured as outlined above except that leucine-free α-MEM (Flow) was used. [4,5-^{3}H]Leucine (Amersham TRK 510; 5 μCi/well) was added at the start of the 16-hr incubation time (16-hr pulse) or for the last 5 hr of this culture period (5-hr pulse). Adherent cells and cells pelleted by centrifugation of the medium were lysed with 0.5 ml of 0.2 M NaOH. CCl_3COOH-insoluble protein was harvested onto glass fiber filters, and the radioactivity was measured (25).

Detection of mRNA. Total cellular RNA from 4-hr monocyte cultures was prepared as described (26) and fractionated (5 μg per lane) on a formaldehyde-containing 1% agarose gel (27) prior to transfer to GeneScreen*Plus* nylon membrane (DuPont). Transfer of RNA, hybridizations, and labeling of cDNAs were as outlined (28), except the hybridizations were performed at 45°C. The relative radioactivity for bands on autoradiograms was estimated by laser scanning densitometry (LKB Ultrascan); the relative intensity of bands for the monocyte mediators, TNF-α and IL-1, was compared with the intensity scan of the autoradiogram for the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Maintenance of LPS-Free Conditions. All equipment was of a plastic disposable nature whenever possible (22, 23). Glassware was soaked in 1% E-Toxiclean (Sigma) and, after washing, was heated to 240°C. All buffers and media were filtered through Zetapor membranes (AMF Cuno). LPS levels $\leq$ 10 pg/ml in all reagents were confirmed in the Limulus lysate assay (Commonwealth Serum Laboratories).

Reagents. Recombinant human IL-4 (>400 units/μg) (29) was obtained from A. Van Kimmenade, DNAX (Palo Alto, CA). Activity of 1 unit/ml was defined to give half-maximal growth of phytohemagglutinin-activated T cells. Reagents were obtained as gifts from the following people: recombinant human IFN-γ at 1.5 $\times$ 10^7 units/mg (E. Hochuli, Hoffmann–La Roche, Basel); recombinant human TNF-α at 2.5 $\times$ 10^7 units/mg and a mAb to TNF-α with a neutralization titer of 6000 units of TNF-α per μg of mAb (G. R. Adolf, Ernst-Boehringer Institut, Vienna); polyclonal rabbit anti-TNF-α for the RIA (M. Vadas and J. Gamble, Institute for Medical and Veterinary Science, Adelaide, Australia); recombinant human IL-1β standard at 2.5 $\times$ 10^7 units/mg (P. L. Simon, Smith Kline & French, Swedeland, PA); recombinant human IL-1α at 10^7 units/mg (P. Lomedico, Hoffman–La Roche, Nutley, NJ); polyclonal antibodies to IL-1α (goat) and to IL-1β (rabbit) (R. Chizzonite, Hoffmann–La Roche, Nutley, NJ, and A. R. Shaw, Glaxo, Geneva, respectively); for the ELISA, a mAb to IL-1β (H6) and the biotinylated form of another anti-IL-1β mAb (H67) (A. C. Allison, Syntex, Palo Alto, CA); and cDNA probes for TNF-α and IL-1β (W. Kohr, Genentech, South San Francisco, and U. Gubler, Hoffmann–La Roche, Nutley, NJ).

Expression of Results. Unless otherwise indicated, mean values $\pm$ SEM for measurements in supernatants from triplicate cultures have been presented. The significance of differences was assessed by using a two-tailed Student *t* test; results were considered significantly different when $P < 0.05$.

RESULTS

Effect of IL-4 on Levels of Monocyte TNF-α Activity. There was no TNF-α activity detected in the supernatants of human monocytes cultured for 18 hr with IL-4 [0.01–5.0 unit(s)/ml]. In contrast, IL-4 at concentrations as low as 0.1 unit/ml suppressed the TNF-α activity induced by LPS (Fig. 1, $P < 0.05$ for 0.1 unit of IL-4 per ml). Decreasing concentrations of IL-4 were investigated for monocytes from two additional donors; for both, 0.1 unit of IL-4 per ml was sufficient to inhibit significantly LPS-induced TNF-α activity. Maximal inhibitory activity of IL-4 was consistently seen with ≈2.5 units/ml (300 pM); when results for monocytes from a number of donors were examined, IL-4 at 2.5 units/ml reduced the mean TNF-α activity induced by LPS from 27.2 (± 10.7) units per 10^6 cells to 1.5 (± 0.7) units per 10^6 cells (± SEM; $n = 10$, $P < 0.01$). IL-4 did not affect the L929 cytotoxicity assay. This inhibitory effect of IL-4 on monocyte TNF-α activity was seen as early as 4 hr after simultaneous incubation of the cells with IL-4 and LPS; for the two donors investigated at this time point, mean TNF-α activities decreased from 54 and 5.3 units per 10^6 cells to 14 and 0.9 unit(s) per 10^6 cells, respectively, in the presence of 2.5 units of IL-4 per ml.

IFN-γ, although not able to induce TNF-α activity, can synergize strongly with LPS to increase the TNF-α activity of human monocytes (22, 30). Addition of IL-4 suppressed the TNF-α activity resulting from the synergistic action of LPS/IFN-γ; for monocytes from five donors, 2.5 units of IL-4 per ml reduced TNF-α activities from 840 (± 314) units per 10^6 cells to 462 (± 273) units per 10^6 cells (± SEM; $P < 0.05$). For the three donors investigated, addition of 0.5 unit of IL-4 per ml was sufficient for significant suppression of the TNF-α activity induced by LPS/IFN-γ ($P < 0.05$).

Effect of IL-4 on Immunoreactive TNF-α Levels. To confirm that differences in the production of TNF-α protein were responsible for the decreases in TNF-α activity rather than inhibitors or an effect of IL-4 on the detection of TNF-α bioactivity, we determined whether IL-4 lowered the TNF-α levels as measured by RIA. An inhibitory effect of IL-4 was again observed (Fig. 2) for the same samples for which results are presented in Fig. 1. In response to IL-4 at 2.5 units/ml, immunoreactive TNF-α levels induced by LPS decreased from 1.14 (± 0.55) ng per 10^6 cells to 0.09 (± 0.04) ng per 10^6 cells (± SEM; $n = 7$, $P < 0.01$). IL-4 also significantly suppressed TNF-α protein levels induced by LPS/IFN-γ from 6.46 (± 1.41) ng per 10^6 cells to 1.47 (± 0.55) ng per 10^6 cells (± SEM, $n = 5$, $P < 0.01$). No immunoreactive TNF-α was detected for unstimulated control monocytes or those treated with IL-4 alone [0.01–5.0 unit(s)/ml].

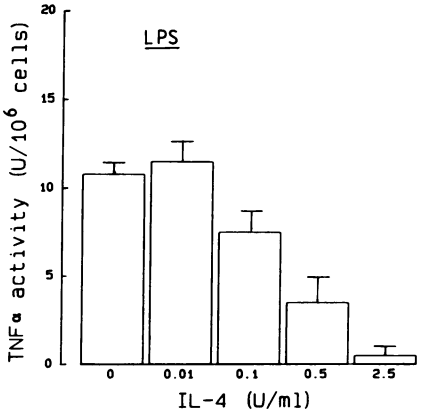

FIG. 1. Effect of IL-4 on TNF-α activity of stimulated human monocytes. Monocytes from a single donor were incubated as described for 18 hr with LPS (100 ng/ml) and IL-4 [0–2.5 unit(s)/ml]. The values shown represent the mean activities ± SEM in the supernatants of triplicate cultures.

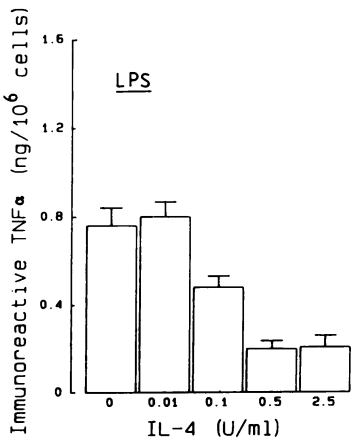

FIG. 2. Effect of IL-4 on TNF-α immunoreactive protein produced by human monocytes stimulated with LPS. The same supernatants for which TNF-α activities are shown in Fig. 1 were assayed for TNF-α levels by RIA. The results are means ± SEM for triplicate cultures.

Effect of IL-4 on TNF-α mRNA Levels. IL-4 lowered the increased TNF-α mRNA levels resulting from the action of LPS and LPS/IFN-γ (Fig. 3*A*); Fig. 3*B* shows that the intensities of the different bands were not significantly different when probed for GAPDH. The intensities of the radioactive bands after hybridization with the TNF-α probe were estimated by laser scanning densitometry and expressed as a function of the intensity of the corresponding GAPDH bands. IL-4 lowered the relative TNF-α mRNA levels in the LPS-treated and LPS/IFN-γ-treated cultures by 65% and 60%, respectively; it was also observed in Fig. 3*A* that the TNF-α mRNA levels in control cultures were lowered by IL-4. RNA blots from other donors showed similar reductions in TNF-α mRNA in response to IL-4.

Effect of IL-4 on IL-1 Levels. To determine the specificity of the inhibitory action of IL-4, we examined the effect of IL-4 on monocyte-derived IL-1 activity. IL-4 alone [0.01–5.0 unit(s)/ml] did not stimulate detectable IL-1 activity. Fig. 4 shows that IL-4 suppressed the expression of IL-1 activity by

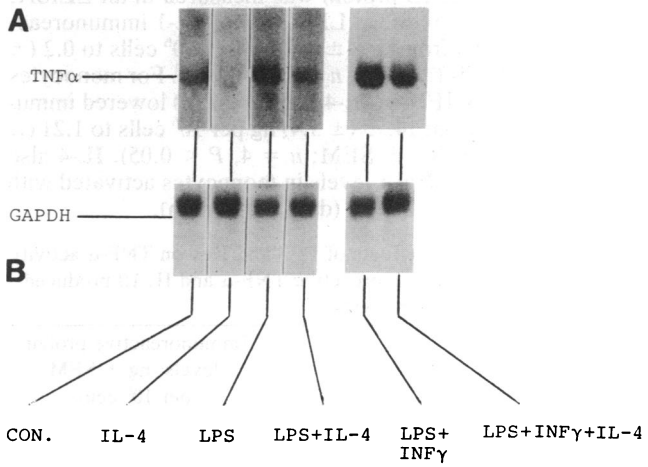

FIG. 3. Effect of IL-4 on mRNA levels of human monocytes. Monocytes from a representative donor were cultured for 4 hr as described with no added stimuli (Con.), with IL-4 (2.5 units/ml), with LPS (100 ng/ml) without or with IL-4 (2.5 units/ml), or with LPS (100 ng/ml)/IFN-γ (100 units/ml) without or with IL-4 (2.5 units/ml). (*A*) TNF-α. Exposure time for the autoradiography was 4 hr for the control, IL-4-treated, and LPS (± IL-4)-treated cells and 1.5 hr for other groups. (*B*) Internal standard GAPDH.

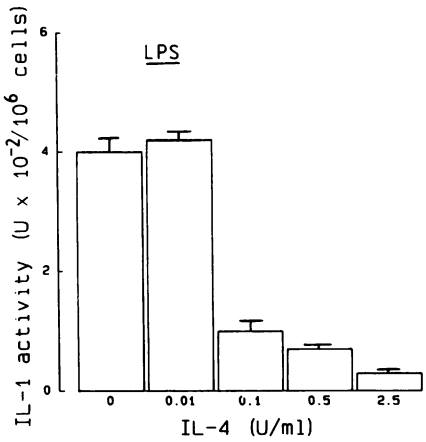

FIG. 4. Effect of IL-4 on IL-1 activity of stimulated human monocytes. Monocytes from a representative donor were incubated for 18 hr as described with LPS (100 ng/ml) and IL-4 [0–2.5 unit(s)/ml]. Results are means ± SEM for the supernatants of triplicate cultures. The IL-1 activities were measured in the same supernatants for which TNF-α activities are shown in Fig. 1.

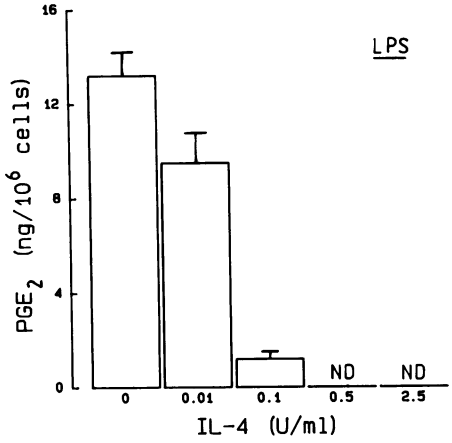

FIG. 5. Effect of IL-4 on the PGE_2 levels produced by stimulated human monocytes. Monocytes from a single donor were incubated as described for 18 hr with LPS (100 ng/ml) and IL-4 [0 to 2.5 unit(s)/ml]. The PGE_2 levels were measured in the same supernatants for which TNF-α and IL-1 activities are shown in Figs. 1 and 4. The results are means ± SEM for triplicate cultures.

monocytes activated with LPS in a dose-dependent manner ($P < 0.01$ at 0.1 unit of IL-4 per ml). IL-4 at 0.1 unit/ml significantly reduced LPS-induced IL-1 activities for all donors investigated ($n = 3$). In response to 2.5 units of IL-4 per ml, the IL-1 activity induced by LPS was suppressed from 214 (± 69) units per 10^6 cells to 3.4 (± 3.4) units per 10^6 cells (± SEM, $n = 10$, $P < 0.01$). Significant inhibition by IL-4 of LPS-induced IL-1 activity was seen after exposure for 4 hr; for two donors studied, IL-4 (2.5 units/ml) suppressed LPS-induced mean IL-1 activities from 57 and 93 units per 10^6 cells to 8 and 13 units per 10^6 cells, respectively.

The IL-1 activity induced by LPS/IFN-γ was reduced by 2.5 units of IL-4 per ml from 1672 (± 454) units per 10^6 cells to 156 (± 124) units per 10^6 cells (± SEM; $n = 5$, $P < 0.01$). For two of three donors examined with decreasing concentrations of IL-4, 0.5 unit of IL-4 per ml was necessary to significantly suppress the activity induced by LPS/IFN-γ; 0.1 unit of IL-4 per ml was sufficient in the third donor. The effect of IL-4 was not due to inhibition of the IL-1 bioassay. The suppressive effect of IL-4 was also observed when immunoreactive IL-1β protein was measured in an ELISA; IL-4 (2.5 units/ml) reduced LPS-induced IL-1 immunoreactive protein levels from 0.9 (± 0.2) ng per 10^6 cells to 0.2 (± 0.2) ng per 10^6 cells (± SEM, $n = 4$, $P < 0.01$). For monocytes treated with LPS/IFN-γ, IL-4 (2.5 units/ml) lowered immunoreactive IL-1 from 14.75 (± 5.4) ng per 10^6 cells to 1.21 (± 0.73) ng per 10^6 cells (± SEM; $n = 4$, $P < 0.05$). IL-4 also suppressed IL-1β mRNA levels in monocytes activated with LPS with or without IFN-γ (data not shown).

Table 1. Comparative effects of IL-4 and Dex on TNF-α activity and on the levels of immunoreactive TNF-α and IL-1β produced by stimulated human monocytes

Addition to monocyte culture	TNF-α activity, units ± SEM per 10^6 cells	Immunoreactive protein levels, ng ± SEM per 10^6 cells	
		TNF-α	IL-1β
LPS	47 ± 13	0.30 ± 0.03	1.1 ± 0.3
LPS/IL-4	7 ± 3	ND	ND
LPS/Dex	2 ± 2	ND	ND

Monocytes from a representative donor were incubated as described for 18 hr with LPS (100 ng/ml). Where indicated, IL-4 was added at 2.5 units/ml and Dex was added at 0.1 μM. TNF-α activity and immunoreactive TNF-α and IL-1β levels were measured as described. Results ± SEM are from supernatants of triplicate cultures. ND, not detected.

Effect of Glucocorticoids on TNF-α and IL-1 Levels. Dex (0.1 μM) and IL-4 (2.5 units/ml; 300 pM) were both potent in inhibiting the stimulatory effect of LPS (Table 1) and LPS/IFN-γ (data not shown) on monocyte-derived TNF-α activity and levels of TNF-α and IL-1β immunoreactive protein (Table 1). IL-1 activities for Dex-treated monocytes are not shown because Dex was suppressive to thymocyte activation, resulting in inhibition of the IL-1 bioassay.

Effect of IL-4 on PGE_2 Levels. IL-4 alone [0.01–5.0 unit(s)/ml] did not stimulate PGE_2 production by human monocytes. However, IL-4 inhibited PGE_2 production by activated monocytes (Fig. 5) in a manner very like that seen for the production of TNF-α (Figs. 1 and 2) and of IL-1 (Fig. 4). In response to IL-4 (2.5 units/ml), PGE_2 levels induced by LPS decreased from 14.7 (± 5.7) ng per 10^6 cells to 0.02 (± 0.02) ng per 10^6 cells (± SEM; $n = 6$, $P < 0.01$). Similar results were obtained for monocytes cotreated with IL-4 and LPS/IFN-γ; unlike TNF-α and IL-1 activities, IFN-γ was not synergistic with LPS for increased PGE_2 levels (23). IL-4 (2.5 units/ml) suppressed LPS-induced PGE_2 levels after exposure for only 4 hr; for two donors, mean PGE_2 levels decreased from 2.4 and 1.1 ng per 10^6 cells to 0.1 ng per 10^6 cells and undetectable levels. Dex (0.1 μM) also dramatically reduced monocyte PGE_2 production; for one donor, IL-4 (2.5 units/ml) reduced LPS-induced levels from 4.9 ± 0.2 ng per 10^6 cells (mean ± SEM) to nondetectable levels, while Dex (0.1 μM) reduced levels to 1.2 ± 0.4 ng per 10^6 cells.

Table 2. Effect of IL-4 and Dex on protein synthesis by activated human monocytes

Addition to monocyte culture	[^{3}H]Leucine incorporation, cpm × 10^{-3} ± SEM	
	16-hr pulse	5-hr pulse
LPS	252.3 ± 10.1	Not done
LPS/IL-4	223.8 ± 4.3	Not done
LPS/Dex	281.8 ± 17.9	Not done
LPS/IFN-γ	279.0 ± 9.5	113.6 ± 1.7
LPS/IFN-γ/IL-4	300.2 ± 8.9	114.5 ± 5.1
LPS/IFN-γ/Dex	291.2 ± 11.3	110.4 ± 2.9

Monocytes were incubated with [^{3}H]leucine from the beginning of a 16-hr culture period (16-hr pulse) or for the last 5 hr of this same culture period (5-hr pulse). LPS was at 100 ng/ml; IFN-γ at 100 units/ml; IL-4, at 2.5 units/ml; and Dex, at 0.1 μM. Cells of triplicate cultures were lysed with 0.2 M NaOH, and cpm were determined in CCl_3COOH-insoluble material. Results are means ± SEM ($n = 6$).

Effect of IL-4 on Monocyte Protein Synthesis. IL-4 (2.5 units/ml) did not suppress total protein synthesis over a 16-hr culture period (Table 2); there was also no change in cell viability (estimated by trypan blue exclusion) or cell number over this period (data not shown). Thus, the effects of IL-4 did not appear to reflect a toxic effect on cellular metabolism. In contrast to IFN-γ (22), IL-4 did not increase the spreading of monocytes on plastic dishes over a 16-hr culture period.

DISCUSSION

We have shown that IL-4 at levels $\geq$ 0.5 unit/ml ($\geq$ 60 pM), and for some donors $\geq$ 0.1 unit/ml, significantly inhibited the production of TNF-α, IL-1β, and PGE_2 by human monocytes. For many donors, 2.5 units of IL-4 per ml suppressed the induction of the three mediators by LPS (100 ng/ml) to nondetectable levels. Specificity of the action of IL-4 for suppression of only certain monocyte products is indicated because total protein synthesis was not lowered after incubation with IL-4 for 16 hr (Table 2). For TNF-α and IL-1β, the decrease was manifest at the level of secreted protein (Fig. 2) and of mRNA (Fig. 3). The inhibitory effect of IL-4 on the three proinflammatory mediators occurred relatively quickly, decreases being observed during a 4-hr experiment.

Our data show that IFN-γ and IL-4 have opposite effects on the production of TNF-α and IL-1 by LPS-stimulated human monocytes. These observations are consistent with the opposite actions of IL-4 and IFN-γ on stimulation of B-cell functions (for a review, see ref. 13). The exception was for monocyte PGE_2 production, for which IL-4 was suppressive (Fig. 5) and IFN-γ added with LPS was without a consistent effect (23). It should also be noted that IFN-γ and IL-4 compete when present in the same cultures for the control of TNF-α and IL-1 production. There is evidence that IFN-γ and IL-4 are produced by distinct subsets of murine helper T-cell lines (31), suggesting that different subsets of cells are activated for lymphokine secretion. Alternatively, lymphokines may be randomly produced by T cells (32). For human T cells, the situation is unknown.

T lymphocytes are susceptible to both stimulatory and inhibitory actions of IL-4 (12, 13, 15). In this study, monocytes were isolated to 90% purity or more by countercurrent centrifugal elutriation; lymphocytes were the main contaminating cell in these preparations. It remains possible that IL-4 was indirectly controlling monocyte activity by first activating lymphocytes to secrete alternative modulatory molecules. However, for studies in which increasing numbers of lymphocytes were added, the IL-4-induced suppression did not increase and suggested a direct inhibitory effect of IL-4 on monocyte activation (data not shown).

IL-4 (2.5 units/ml) and Dex (0.1 μM) inhibited the TNF-α and IL-1 levels of activated monocytes to a similar degree (Table 1). As for the steroids (7, 8), IL-4 was suppressive for TNF-α (Fig. 3) and IL-1β mRNA. The actions of corticosteroids on monocyte mediator production may form a significant part of their antiinflammatory action (7, 10). We suggest that IL-4 also might have antiinflammatory properties. IL-4, or perhaps even IL-4 receptor agonists, might have less side effects and might be used therapeutically in conjunction with lower doses of corticosteroids than are now used. Whether IL-4 acts in the same way as the glucocorticoids in their suppression of gene transcription and reduction of TNF-α mRNA and IL-1 mRNA stability (7, 8) or on the expression of phospholipase A_2 activity for prostaglandin synthesis (9, 10) remains to be determined.

The actions of another antiinflammatory drug, indomethacin, on mediator production by activated human monocytes can be contrasted to those of IL-4. This cyclooxygenase inhibitor at 0.1 μM or more enhances LPS-induced monocyte TNF-α and IL-1 synthesis; cyclooxygenase products, such as prostaglandins, provide a negative signal (23, 33–35). Indomethacin, although suppressing the production of cyclooxygenase products, may have some of its clinical usefulness as an antiinflammatory agent lessened because of the induction of proinflammatory mediators. It is possible that similar or even lower doses of cyclooxygenase inhibitors might be more effective as antiinflammatory agents if the production of TNF-α and IL-1 were suppressed by additional immunotherapy—e.g., by IL-4.

Up until now the actions of IL-4 on human monocytes have generally been considered to be stimulatory (16–18). The present data show that IL-4 can also inhibit some parameters of human monocyte activation. Thus, another function is added to the list of the pleiotropic effects of IL-4. The results of this study suggest that IL-4 may indeed be a powerful, previously unrecognized antiinflammatory agent.

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