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Interleukin-4 suppression of monocyte tumour necrosis factor-α production. Dependence on protein synthesis but not on cyclic AMP production

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SUMMARY

The molecular mechanisms by which human interleukin-4 (IL-4) down-regulates tumour necrosis factor-α (TNF-α) production by monocytes remain unknown. Other studies of IL-4 action in B lymphocytes and large granular lymphocytes (LGL) suggested that IL-4 may suppress mediator production by augmenting intracellular cyclic AMP (cAMP) levels. However, this study did not find evidence for involvement of a cAMP-dependent signalling pathway for expression of IL-4 activity in monocytes. IL-4 reduced TNF-α production by monocytes when IL-4 and lipopolysaccharide (LPS) were added concomitantly, or upon subsequent activation by LPS 16 hr after first exposure to IL-4. The continued presence of IL-4 at the time of LPS stimulation was not necessary; however, the suppressive effects of IL-4 were dependent on protein synthesis. This sustained activity of IL-4 for down-regulation of the production of inflammatory signals may be important for control *in vivo* of excessively activated monocytes/macrophages, and in therapy.

INTRODUCTION

Many laboratories have now shown a suppressive effect of recombinant human interleukin-4 (IL-4) on several monocyte functions, particularly those associated with a pro-inflammatory response. IL-4 down-regulates monocyte production of IL-1β and tumour necrosis factor-α (TNF-α)[1–4], PGE_2[1], IL-6[5], IL-8[6], H_2O_2[7], superoxide anions[8] and monocyte procoagulant activity.[9] Recently, IL-4 was reported to decrease monocyte adherence to both plastic and endothelial monolayers.[10] However, not all monocyte functions are suppressed. IL-4 stimulates human monocyte major histocompatibility complex (MHC) class II expression,[4] tissue-type plasminogen activator production[11] and expression of the Fcε type II receptor, CD23.[12] The suppressive effects of IL-4 on monocyte pro-inflammatory mediator production suggested that IL-4, or an agonist, may be used therapeutically as an anti-inflammatory agent. It was important to understand more about the mechanism of action of IL-4 on human monocytes.

The effects of IL-4 on monocyte pro-inflammatory mediator production are controlled at least in part at the messenger RNA (mRNA) level.[1–3] Recently, IL-4 was reported to decrease both the stability of IL-1β mRNA in monocytes, as well as monocyte transcription of the IL-1β gene.[13] However, the IL-4-induced signal transduction pathway in monocytes leading to these effects is not known. In contrast, IL-4 signalling in B lymphocytes[14] and large granular lymphocytes (LGL)[15] has been studied. In the former, IL-4 induces a novel signal transduction cascade involving an immediate and transient elevation of inositol 1,4,5-triphosphate and Ca^{2+} levels, followed by a sustained rise in cellular cyclic adenosine monophosphate (cAMP) levels.[14] In LGL, the IL-4 inhibitory effect on IL-2-driven cell differentiation, and the subsequent acquisition of their lytic potential, was dependent on elevated cAMP levels.[15] The inhibitory properties of cAMP for many monocyte activities has also been documented.[16,17] From these reports, it seemed possible that IL-4 signalling in monocytes may involve increased cAMP levels.

In this study, the involvement of cAMP and/or protein synthesis for expression of the inhibitory action of IL-4 on monocyte TNF-α levels was examined. The prophylactic effects of IL-4 when added 16 hr prior to cell activation were also investigated.

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MATERIALS AND METHODS

Reagents

Reagents were obtained as gifts as indicated: recombinant human IL-4 (lot no. 72787; Dr F. Lee, DNAX, Palo Alto, CA); recombinant human TNF-α, $2{\cdot}5 \times 10^7$ U/mg, and a monoclonal antibody (mAb) to TNF-α with a neutralization titre of 6000 U TNF-α/μg mAb (Dr G. R. Adolf, Boehringer Ingelheim, Vienna, Austria); polyclonal rabbit anti-TNF-α for ELISA (Professor I. F. C. McKenzie, University of Melbourne, Australia); recombinant human IFN-γ, 10^6 U/ml (lot no. 302, Ro 23–4400; Hoffmann-La Roche, Nutley, NJ); and mAb

(FMC32) to CD14 (Professor H. Zola, Flinders Medical Centre, South Australia).

Monocyte isolation

The method for isolating peripheral blood monocytes by countercurrent centrifugal elutriation has been described previously.[1,11,18] Mononuclear cells were first selected by centrifugation (170 ***g***, 30 min) of 'buffy coats' (Adelaide Red Cross Blood Transfusion Service) on pyrogen-tested Lymphoprep (Nycomed, Oslo, Norway). Cells were washed twice in Hanks' balanced salt solution containing 0·21% sodium citate, 1 μg/ml polymyxin B sulphate (Sigma Chemical Co., St Louis, MO), 100 U/ml penicillin and 100 μg/ml streptomycin, and elutriated (Beckman JE-6B Elutriation System, Beckman Instruments, Fullerton, CA) in the same solution. Monocyte enrichment was assessed by morphology on Giemsa-stained cytocentrifuge smears and staining for CD14 with mAb FMC32.[19] Lymphocytes, and not polymorphonuclear leucocytes, were the main cell contaminant, and contaminating cells were always $<20\%$ of total.

Monocyte culture

Monocyte-rich fractions were pooled and resuspended in RPMI-1640 medium (Flow Laboratories, McLean, VA) supplemented with 2 mM MOPS (Sigma), 13·3 mM $NaHCO_3$, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin (subsequently referred to as 'complete RPMI'), with an osmolality of 290 mmol/kg H_2O. Unless otherwise indicated, 10^6 cells were cultured in 1 ml complete RPMI supplemented with 1% (v/v) foetal calf serum in 2-cm^2 tissue culture plastic wells (Linbro, Flow; adherent cultures) or 70×11 mm polyethylene tubes (Nunc Minisorp, cat. no. 466982, Roskilde, Denmark; non-adherent cultures). With the exception of the preparations for cAMP measurement, the following reagents, in a volume of 10 μl, were added at the initiation of culture, or after 16 hr to give final concentrations of: IL-4, 10 U/ml; interferon-γ (IFN-γ), 500 U/ml; lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4, purified by the Westphal method (Difco, Detroit, MI), 500 ng/ml; PGE_2 (Sigma), 0–250 ng/ml; cycloheximide (CHX; Sigma), 0·25 μg/ml; dibutyryl cAMP (dbcAMP; Sigma), 0–100 μM. Triplicate cultures for each test variable were incubated at 37° in 5% CO_2. To terminate, culture supernatants were removed and centrifuged to remove non-adherent cells; remaining monolayers were incubated with 1 ml cold NaCl containing 20 mM HEPES. Supernatants were stored frozen until assay of inflammatory mediators; monolayers were lysed by freeze thawing.

By trypan blue staining, all cells were viable after culture for 40 hr. In the cultures which were not washed to remove reagents, cell numbers recovered did not vary. Thus, levels of monocyte mediators have been expressed according to the number of cells at the beginning of the culture period. However, as we have previously shown that neither LPS nor IL-4 can affect [^{3}H]-leucine incorporation into total protein,[1] measurement of [^{3}H]leucine incorporation was used as a measure of cell numbers remaining in the culture dishes after extensive washing of the cells ([4,5-^{3}H]leucine, cat. no. TRK 510; Amersham International, Amersham, Bucks, U.K.; 24 hr pulse; 2 μCi/well). In these experiments, any non-adherent cells were returned after washing to the original cultures. For replicate monocyte cultures incubated with CHX, IL-4, CHX with IL-4 or control medium, washed extensively after 16 hr and subsequently incubated for 24 hr with LPS (see Table 2), [^{3}H]leucine incorporation was not significantly decreased in the IL-4-pretreated cultures. Because this procedure did not actually count the cells after washing, TNF-α levels in the washed, 40-hr cultures were quantified as ng/ml.

Monocyte culture for cAMP measurements

Monocytes were cultured, and cAMP extracted from the same cells, as previously described for B cells.[14] Briefly, $2{\cdot}5 \times 10^6$ cells in 0·25 ml complete RPMI containing 10% foetal calf serum were incubated at 37° in 5% CO_2 with 125 ng LPS, together with 2·5 U IL-4, 0·06–63 ng PGE_2 or medium alone. This concentration of IL4 has been shown previously to maximally inhibit monocyte TNF-α production.[1] These reactions in cell-non-adherent polyethylene tubes were terminated after 0, 1, 5, 10, 20 or 30 min incubation by addition of 0·75 ml ice-cold ethanol and the samples were sonicated for 30 seconds. Cell debris were removed by centrifugation at 3000 ***g*** for 10 min, and the supernatants dried in a rotary vacuum overnight. For each experiment, duplicate tubes were terminated at each time-point. In one experiment, LPS was omitted from tubes terminated after 10 min. In all experiments, similarly treated non-adherent cells (10^6 cells in 1-ml cultures) were incubated for 18 hr in order to associate cAMP induction with TNF-α levels.

Assessment of cAMP levels

Monocyte cAMP was measured using a [^{3}H]-cAMP kit assay (TRK432, Amersham) according to the kit procedures. Briefly, the cell residues were resuspended in the assay buffer, 50 mM Tris-HCl, pH 7·5, 4 mM EDTA. cAMP competed with [^{3}H]-cAMP for binding to the cAMP binding protein before removal of any unbound material by charcoal. Replicates of the supernatants were measured in a β-scintillation counter.

Maintenance of LPS-free conditions

During monocyte isolation and subsequent culture, extreme care was taken to limit LPS contamination of isolation fluids.[1,11,18] These precautions included use of disposable plasticware whenever possible, soaking of glassware in E-Toxaclean (Sigma), and filtering of buffers and culture medium through Zetapore filters (Cuno, Meriden, CT). Levels of LPS <50 pg/ml in all reagents were confirmed in the Limulus lysate assay (Whittaker Bioproducts, Walkersville, MA).

Assay of TNF-α

Immunoreactive TNF-α was measured by a sandwich ELISA as previously described[1,20] using a monoclonal anti-TNF-α plate-binding antibody, a second rabbit anti-TNF-α polyclonal antibody, followed by a biotinylated anti-rabbit IgG (cat. no. B9642; Sigma Immunochemicals) and an ExtrAvidin peroxidase conjugate (cat. no. E2886, Sigma Immunochemicals). The assay was sensitive to levels of TNF-α of $>0{\cdot}04$ ng/ml.

Statistical analysis

The significance of differences between treatment groups was assessed by using a two-tailed Student's *t*-test. The results were considered significantly different if $P<0{\cdot}05$.

RESULTS

Cyclic AMP levels and IL-4 activity

In previous studies of resting B lymphocytes and LGL,[14,15] IL-4 enhanced cAMP levels two- to fivefold with maximal levels detected after 10–20 min exposure. For monocytes incubated with LPS (500 ng/ml), with or without IL-4 (10 U/ml), there was no detectable difference in monocyte cAMP levels over a 20-min incubation period; Fig. 1 shows the mean monocyte cAMP-±SEM) for cells cultured in duplicate from each of three cell donors. In contrast, for all three monocyte preparations, PGE_2 (100 ng/ml) significantly enhanced monocyte cAMP levels after 5, 10 and 20 min exposure ($P < 0.05$; Fig. 1). For a further donor, induction of cAMP in LPS-treated monocytes after exposure to IL-4 and PGE_2 for 1, 10 and 30 min, was investigated. IL-4 was without effect at any time-point, but PGE_2-induced cAMP levels after 1 min were more than double those measured after 10 min, suggesting maximal cAMP levels induced by PGE_2 before the first regular measurement at 5 min. After 30 min exposure, PGE_2-induced cAMP levels were reduced to insignificant levels. There was also no evidence for LPS-induced cAMP levels (data not shown).

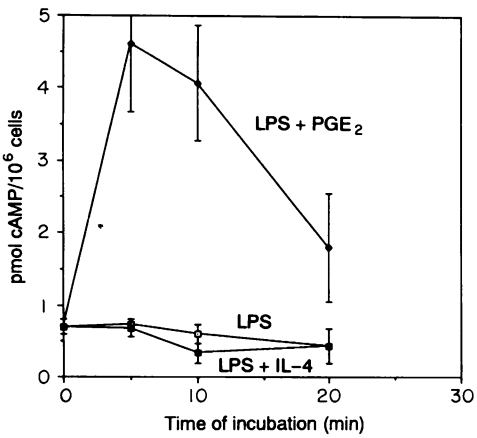

Figure 1. Monocyte cAMP levels. Monocytes were incubated for 0, 5, 10 or 20 min with LPS alone (500 ng/ml), or together with IL-4 (10 U/ml) or PGE_2 (100 ng/ml). Each point represents the mean level (±SEM) of cAMP for monocytes incubated in duplicate for each of three separate experiments; the absence of error bars indicates that the SEM was too small for visual presentation.

After first confirming a dose-dependent suppression of monocyte TNF-α by dbcAMP (data not shown), the sensitivity of the assay linking increased cAMP with decreased TNF-α levels was investigated. When supernatants from triplicate identically treated cultures from each of three monocyte donors were harvested after 18 hr, IL-4 (10 U/ml) suppressed LPS-induced TNF-α by 25, 53 and 73%, respectively. Levels of PGE_2 approximately equal to 25 ng/ml were necessary to reduce LPS-induced TNF-α to levels detected in response to IL-4 (10 U/ml) ($n = 3$). In titrations of the concentration of PGE_2 required for suppression of LPS-induced TNF-α, for three donors, 2·5 ng/ml PGE_2 suppressed LPS-induced TNF-α by 15±8% (mean-±SEM), with little suppression detected in response to 0·25 ng/ml PGE_2. Figure 2a demonstrates that for a 10-min exposure time, concentrations of PGE_2 >0·25 ng/ml were required for induction of cAMP by LPS-treated monocytes. Figure 2b confirms that for the same cell population, suppression of monocyte TNF-α levels by increasing concentrations of PGE_2 were directly related to the PGE_2-induced cAMP levels. This suggested that the assay for changes in cAMP provided a sensitive system for detection of modulators of TNF-α production that may operate through changes in cAMP.

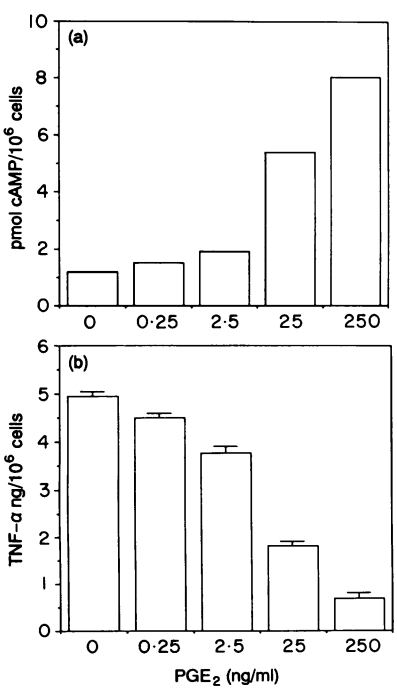

Figure 2. Effect of increasing PGE_2 concentrations on monocyte cAMP and TNF-α levels. Replicate cultures of monocytes from a single donor were incubated with LPS (500 ng/ml), together with increasing concentrations of PGE_2 (0·25–250 ng/ml) for 10 min for cAMP measurement (a) and for 18 hr for assessment of TNF-α levels (b). Duplicate cultures were assessed for cAMP measurement; a mean value is shown. Triplicate cultures were assessed for TNF-α measurement; mean values ± SEM are shown.

Addition of IL-4 to monocytes 16 hr before LPS stimulation

Many laboratories have detected decreased inflammatory mediator production when monocytes were incubated concomitantly with LPS and IL-4.[1-4] In this study, we have examined the prophylactic action of IL-4. Adherent monocytes from four donors (86±5% enriched) were incubated overnight with medium alone, IL-4 or LPS. Sixteen hours later, cells were treated with IL-4 (10 U/ml), LPS (500 ng/ml) or a combination of both IL-4 and LPS (concentrations as above). TNF-α immunoreactive protein levels were measured in the culture supernatants after a further 24 hr incubation. Table 1 shows that the inhibitory action of IL-4 for monocyte TNF-α production persisted during incubation for 16 hr prior to LPS stimulation ($P < 0.05$ for Donors 1, 2 and 4; $P = 0.09$ for Donor 3). In fact, for the three donors for whom monocyte preincubation with IL-4 was compared with concomitant addition of IL-4 with LPS to monocytes at 16 hr, there was no significant difference in TNF-α levels between the different treatment groups ($P > 0.05$).

Table 1. The effect of IL-4 on TNF-α immunoreactive levels in monocyte culture supernatants

	TNF-α ng/10^6 cells*			
Addition at time 0	—	—	IL-4	—
Addition at time 16 hr	—	LPS	LPS	LPS+IL-4
Donor				
1	0·04±0·02†	21·2±0·1	14·8±1·4+	11·9±0·7§¶
2	0·09±0·01	3·5±0·1	1·9±0·2+	1·9±0·2§¶
3	0·01±0·01	4·6±0·7	2·5±0·1	2·6±0·1¶
4	0·02±0·01	14·7±0·5	8·6±0·2+	not done

*Monocyte cultures were terminated after a total incubation of 40 hr and TNF-α immunoreactive protein levels were measured in the supernatants.

†Mean±SEM, $n=3$.

‡$P<0·05$ for difference between TNF-α levels stimulated by LPS at 16 hr, with or without prior incubation with IL-4 at time 0.

§$P<0·05$ for difference between TNF-α levels stimulated by LPS at 16 hr, with or without addition of IL-4 at 16 hr.

¶$P>0·05$ for TNF-α levels of monocytes stimulated with both LPS and IL-4 at 16 hr, compared with IL-4 added at 0 hr and LPS at 16 hr.

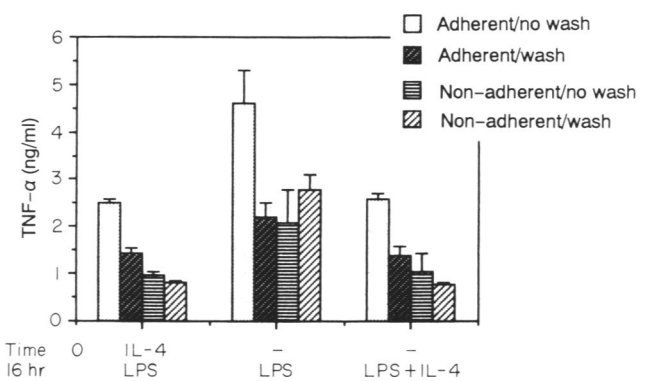

Figure 3. Effect of removal of IL-4 prior to LPS stimulation. Both adherent and non-adherent monocytes were incubated for 16 hr with IL-4 (10 U/ml), or in medium alone, before extensive washing with warmed medium and incubation for a further 24 hr with LPS (500 ng/ml), with or without IL-4 (10 U/ml). Mean TNF-α levels (ng/ml, ±SEM) in supernatants from triplicate cultures are shown; the absence of error bars indicate that the SEM was too small for visual representation.

Removal of IL-4 prior to LPS stimulation

We next questioned whether the continued presence of IL-4 was necessary for expression of down-regulatory effects of IL-4 on monocyte TNF-α production. Both adherent and non-adherent cells from the same monocyte donor were incubated with IL-4 for 16 hr. Replicate wells from each group were washed three times with warmed serum-free culture medium prior to LPS addition; any cells recovered in the washings were returned to the original adherent cell cultures. For the cultures in non-adherent tubes, all cells were pelleted and resuspended upon each wash. In the control 'no wash' wells, IL-4 or medium remained present. TNF-α immunoreactive protein levels for one of thee representative experiments are shown in Fig. 3. Irrespective of whether the monocytes were adherent or non-adherent, or whether the IL-4 was removed prior to LPS stimulation, addition of IL-4 16 hr prior to cell activation reduced LPS-induced TNF-α levels to a similar extent as that measured when IL-4 was added concomitantly with LPS at 16 hr.

Table 2. Removal of IL-4 inhibitory effects on monocyte TNF-α production by cycloheximide (CHX)

	TNF-α ng/ml*			
Addition at time 0	—	CHX	IL-4	CHX+IL-4
Addition at time 16 hr	LPS	LPS	LPS	LPS
Donor				
1	3·9±0·1†	8·7±0·1	2·1±0·1	10·1±0·9
2	8·7±0·3	11·0±1·4	4·9±0·1	8·3±0·8

*Adherent monocyte cultures were terminated after a total incubation of 40 hr. Cells were washed three times with warm medium prior to addition at 16 hr. TNF-α immunoreactive protein levels were measured in the supernatants as described in the Materials and Methods

†Mean±SEM, $n=3$.

Monocytes from two further donors were incubated with IL-4 at time 0, together with CHX (0·25 μg/ml). After culture for 16 hr at 37° in 5% CO_2, the cell monolayers were washed three times with warmed medium before addition of LPS, and incubation for a further 24 hr. Table 2 shows that the sustained suppressive effect of IL-4 was no longer detected in the CHX-treated cultures, suggesting IL-4-induced protein synthesis was necessary for the expression of the suppressive effects of IL-4.

DISCUSSION

Several studies have suggested an anti-inflammatory role for IL-4 in suppression of monocyte inflammatory mediator production.[1-4] In comparison with IL-4, glucocorticoids have similar suppressive effects on monocyte pro-inflammatory mediator production. However, IL-4 and glucocorticoids operate by different signalling pathways, principally because their suppressive activities on monocyte IL-1 and TNF production are cumulative, and IL-4, but not glucocorticoids. induce monocyte tissue-type plasminogen activator production.[11,21] In the current study, we attempted to gain a more detailed understanding of the mechanism by which IL-4 can suppress monocyte pro-inflammatory functions, in particular the production of TNF-α.

Reports of increased cAMP levels being linked with IL-4-dependent functional changes in both B lymphocytes and LGL led to the initial hypothesis that IL-4 similarly increased cAMP levels in human monocytes.[14,15] This did not seem an unreasonable hypothesis because other inhibitory agents for monocyte mediator production (e.g. PGE_2, forskolin, IBMX) are associated with increased cAMP levels.[22] However, in our studies of multiple donors, we were unable to link IL-4 inhibitory activity on monocyte TNF-α production with elevated cAMP (Fig. 1).

We used PGE_2 as a positive inductant of cAMP, and we showed that levels of PGE_2 that could significantly increase cAMP were approximately equal to those required for a significant suppression of monocyte TNF-α immunoreactive protein (Fig. 2). There is therefore a striking difference between IL-4 signalling in monocytes and that in B lymphocytes and LGL. Similarly, it has been reported that IL-4 stimulates different signalling pathways in murine and human B cells.[14]

Previous studies have concentrated on the control of IL-1 production by IL-4.[3,13] It was reported that IL-4 was most inhibitory when added concomitantly, or up to 4 hr prior to cell activation.[3] IL-4 did not diminish IL-4 receptors.[3] In this study, we have shown that the inhibitory effects of IL-4 for monocyte TNF-α production are equally as potent when added 16 hr prior to, as when added together with, the activating agent LPS. Furthermore, IL-4 need not be present during subsequent monocyte activation (Fig. 3). The inability to negate the suppressive effects of IL-4 by extensive washing of the cells after IL-4 exposure suggested that the biochemical mechanisms involved in IL-4 suppression of monocyte TNF-α production were relatively stable for 16 hr. This was in sharp contrast to the quick- but short-acting stimulatory processes associated with AU-rich regions and induction of the pro-inflammatory mediators for an acute reaction.[23] Similar results to those reported for TNF-α, namely a sustained suppressive effect of IL-4 for 16 hr, were found for monocyte IL-1β production (data not shown).

Addition of cycloheximide to the monocyte cultures prevented the expression of inhibitory activity of IL-4 on monocyte TNF-α expression (Table 2). As previously reported,[24] cycloheximide also superinduced TNF-α production by stabilizing TNF-α mRNA. This suggested that the expression of IL-4 activity 16 hr after first exposure to IL-4 was dependent on synthesis of an IL-4 regulatory protein(s). The present result is similar to the dependence on protein synthesis reported for IL-4 suppression of monocyte IL-1 production.[13] IL-4 suppression of LPS-induced IL-8 mRNA levels was also shown to be removed by CHX.[6]

We,[1] and others,[2,3] have found decreased steady-state TNF-α mRNA levels for IL-4-treated monocytes. As run-on experiments have found no IL-4 suppression of LPS-induced TNF-α gene transcription,[13] IL-4 must operate by reducing the stability of TNF-α mRNA. The results of the current study support the notion that a protein is responsible for expression of the destabilizing effects of IL-4 on TNF-α mRNA. Further characterization of this protein controlling TNF-α mRNA stability is the subject of ongoing studies. By manipulating the times of exposure to IL-4 and the persistence of the effects of IL-4, we are trying to understand better the kinetics of production of the protein(s) and its half-life. It is possible that IL-4 decreases TNF-α mRNA levels in IL-2-stimulated LGL[15] by a similar mechanism and that this inhibition is complementary to cAMP effects.

It is unknown to what extent IL-4 may be anti-inflammatory *in vivo*. It may provide a natural dampening signal for many inflammatory situations once they have run their course. If we are to harness this anti-inflammatory activity of IL-4 for therapeutic use, it is important that we gain a greater understanding of the mechanisms of action of this molecule. Our studies emphasize that IL-4 signals the production, by a protein-dependent pathway, of a regulatory factor which can manifest its activity for suppression of TNF-α at least 16 hr later. This property must be beneficial to the profile of IL-4 as an anti-inflammatory agent.

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REFERENCES

1. Hart P.H., Vitti G.F., Burgess D.R., Whitty G.A. & Hamilton J.A. (1989) Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2. *Proc. natl. Acad. Sci. U.S.A.* **86,** 3803.
2. Essner R., Rhoades K., McBride W.H., Morton D.L. & Economou J.S. (1989) IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J. Immunol.* **142,** 3857.
3. Donnelly R.P., Fenton M.J., Finbloom D.S. & Gerrard T.L. (1990) Differential regulation of IL-1 production in human monocytes by IFN-γ and IL-4. *J. Immunol.* **145,** 569.
4. Te Velde A.A., Klomp J.P.G., Yard B.A., De Vries J.E. & Figdor C.G. (1988) Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. *J. Immunol.* **140,** 1548.
5. Cheung D.L., Hart P.H., Vitti G.F., Whitty G.A. & Hamilton, J.A. (1990) Contrasting effects of IFN-γ and IL-4 on the IL-6 activity of stimulated human monocytes. *Immunology*, **71,** 70.
6. Standiford T.J., Streiter R.M., Chensue S.W., Westwick J., Kasahara K. & Kunkel S.L. (1990) IL-4 inhibits the expression of IL-8 from stimulated human monocytes. *J. Immunol.* **145,** 1435.
7. Lehn M., Weiser W.Y., Engelhorn S., Gillis S. & Remold H.G. (1989) IL-4 inhibits H_2O_2 production and antileishmanial capacity of human cultured monocytes mediated by IFN-γ. *J. Immunol.* **143,** 3020.
8. Abramson S.L. & Gallin J.J. (1990) IL-4 inhibits superoxide production by human mononuclear phagocytes. *J. Immunol.* **144,** 625.
9. Szabo G. & Miller-Graziano C.L. (1990) Induction and regulation of monocyte procoagulant activity. *Transplantation*, **50,** 301.
10. Elliott M.J., Gamble J.R., Park L.S., Vadas M.A. & Lopez A.F. (1991) Inhibition of human monocyte adhesion by interleukin-4. *Blood*, **77,** 2739.
11. Hart P.H., Burgess D.R., Vitti G.F. & Hamilton J.A. (1989) Interleukin-4 stimulates human monocytes to produce tissue-type plasminogen activator. *Blood*, **74,** 1222.
12. Te Velde A.A., Rousset F., Peronne C., De Vries J. & Figdor C.G. (1990) IFN-α and IFN-γ have different regulatory effects on IL-4-induced membrane expression of FcεRIIb and release of soluble FcεRIIb by human monocytes. *J. Immunol.* **144,** 3052.
13. Donnelly R.P., Fenton M.J., Kaufman J.D. & Gerrard T.L. (1991) IL-1 expression in human monocytes is transcriptionally and posttranscriptionally regulated by IL-4. *J. Immunol.* **146,** 3431.
14. Finney M., Guy G.R., Michell R.H., Gordon J., Dugas B., Rigley K.P. & Callard R.E. (1990) Interleukin 4 activates human B lymphocytes via transient inositol lipid hydrolysis and delayed cyclic adenosine monophosphate generation. *Eur. J. Immunol.* **20,** 151.
15. Blay J.-Y., Branellec D., Robinet E., Dugas B., Gay F. & Chouaib S. (1990) Involvement of cyclic adenosine monophosphate in the interleukin 4 inhibitory effect on interleukin 2-induced lymphokine-activated killer generation. *J. clin. Invest.* **85,** 1909.
16. Hart P.H., Whitty G.A., Piccoli D.S. & Hamilton J.A. (1989) Control by IFN-γ and PGE_2 of TNF-α and IL-1 production by human monocytes. *Immunology*, **66,** 376.

17. KNUDSEN P.J., DINARELLO C.A. & STROM T.B. (1986) Prostaglandins post-transcriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. *J. Immunol.* **137,** 3189.
18. HART P.H., WHITTY G.A., PICCOLI D.S. & HAMILTON J.A. (1988) Synergistic activation of human monocytes by granulocyte–macrophage colony-stimulating factor and interferon-γ. Increased TNF-α but not IL-1 activity. *J. Immunol.* **141,** 1516.
19. HANCOCK W.W., ZOLA H. & ATKIN R.C. (1983) Antigenic heterogeneity of human mononuclear phagocytes: immunohistologic analysis using monoclonal antibodies. *Blood,* **62,** 1271.
20. HART P.H., COOPER R.L. & FINLAY-JONES J.J. (1991) IL-4 suppresses IL-1β, TNF-α and PGE_2 production by human peritoneal macrophages. *Immunology,* **72,** 344.
21. HART P.H., WHITTY G.A., BURGESS D.R., CROATTO M. & HAMILTON J.A. (1990) Augmentation of glococorticoid action on human monocytes by interleukin-4. *Lymphokine Res.* **9,** 147.
22. ENDRES S., FULLE H.-J., SINHA B., STOLL D., DINARELLO C.A., GERZER R. & WEBER P.C. (1991) Cyclic nucleotides differentially regulate the synthesis of tumour necrosis factor-α and interleukin-1β by human mononuclear cells. *Immunology,* **72,** 56.
23. COLLART M.A., BELIN D., VASSALLI J.-D., DEKOSSODO S. & VASSALLI P. (1986) γInterferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes, which are controlled by short-lived repressors. *J. exp. Med.* **164,** 2113.
24. ZUCKERMAN S.H., EVANS G.F. & GUTHRIE L. (1991) Transcriptional and post-transcriptional mechanisms involved in the differential expression of LPS-induced IL-1 and TNF mRNA. *Immunology,* **73,** 460.