Cytokine regulation of human monocyte interleukin-1 (IL-1) production *in vitro*. Enhancement of IL-1 production by interferon (IFN) gamma, tumour necrosis factor-alpha, IL-2 and IL-1, and inhibition by IFN-alpha

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SUMMARY

IL-1 production (secreted and cell-associated) was measured in monocyte cultures stimulated by a variety of agents *in vitro*. Monocytes either adherent to conventional plastic culture plates in serum-free conditions, or in suspension in culture medium containing serum were stimulated to produce IL-1 during culture. In non-adherent, serum-free conditions, monocytes produced very low or undetectable amounts of IL-1 during 20 h of culture. Lipopolysaccharide (LPS) induced equivalent amounts of secreted and cell-associated IL-1, although at very low concentrations more cell-associated IL-1 was produced. IL-1 production in response to LPS could be augmented by crude lymphokine, IFN-γ, or tumour necrosis factor (TNF) alpha. TNF-α preferentially augmented the production of cell-associated IL-1 in LPS-stimulated cultures. TNF-α induced a significant amount of IL-1 (mainly cell-associated) directly but could also induce IL-1 secretion when combined with IL-2 or IFN-γ, or when in the presence of serum. IL-2 acted synergistically with low concentrations of IFN-γ or IL-1 to induce significant levels of IL-1 production. IFN-α did not induce any IL-1 production, but was a potent inhibitor of IL-1 production induced by a variety of stimuli. These results suggest that IL-1 production may be enhanced or reduced by different cytokines at concentrations likely to be found in chronic inflammatory lesions.

Keywords interleukin-1 monocytes tumour necrosis factor-alpha inteferons interleukin-2

INTRODUCTION

The production of cytokines is thought to contribute to tissue destruction in chronic inflammatory joint disease (Henderson, Pettipher & Higgs, 1987). IL-1, IL-2, TNF-α, IFN-γ and IFN-α have all been identified in inflamed rheumatoid joints (Hopkins & Meager, 1988; Ruschen, Lemm & Warnatz, 1988; Di Giovine, Nuki & Duff, 1988; Buchan *et al.*, 1988). It is possible that some of these cytokines, even if present in relatively low amounts, may serve to enhance the production of IL-1 which is presumed responsible for much of the tissue destruction.

TNF-α (Dinarello *et al.*, 1986; Chantry *et al.*, 1989), IL-2 (Numerof, Aronson & Mier, 1988; Tilden & Dunlap, 1989; Herrmann *et al.*, 1989), IFN-α (Gerrard *et al.*, 1986) and even IL-1 itself (Dinarello *et al.*, 1987; Manson *et al.*, 1989; Dalton, Connor & Johnson, 1989) have been reported to induce IL-1 production by peripheral blood mononuclear cells. IFN-γ has been shown to enhance IL-1 production induced by lipopolysaccharide (LPS) (Newton, 1985; Gerrard *et al.*, 1986; Hart *et al.*,

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1989). High concentrations of cytokines were required to demonstrate direct IL-1 induction, usually by unfractionated mononuclear cells. In addition, cells were cultured on plastic in the presence of serum, conditions which are now thought to alter monocyte responses (Bayne *et al.*, 1986; Van der Meer *et al.*, 1988).

We used purified monocytes cultured in non-adherent, serum-free conditions to study the effect of cytokines on IL-1 production. We have found that adherence to plastic resulted in stimulation of IL-1 secretion and that exposure to serum resulted in the stimulation of cell-associated IL-1 production in non-adherent cultures. In the absence of serum or adherence to plastic and with minimal endotoxin contamination, TNF-α stimulated IL-1 production directly. IFN-α inhibited IL-1 production induced by a variety of stimuli. Other cytokines were effective in inducing significant IL-1 production only when used in certain combinations.

MATERIALS AND METHODS

Reagents
LPS from *Escherichia coli* 055.B5 (Difco-Bacto) was used as the

standard IL-1-inducing stimulus. Pure recombinant human IFN-γ (specific activity 2×10^7 U/mg) and TNF-α (specific activity 6×10^7 U/mg) were provided by Boehringer Ingelheim (Austria). Pure recombinant human IFN-α (2b, specific activity 2×10^8 U/mg) was provided by the Schering Corporation (Sydney, Australia). Crude lymphokine was generated from normal human lymphocytes enriched by elutriation. Lymphocytes 100×10^6 in 10 ml of RPMI culture medium were treated with 10^{-8} M PMA (Sigma) at 37°C for 3 h, washed twice and stimulated with 0·3 ml of concanavalin A (Con A)/Sepharose (Pharmacia) in a total volume of 20 ml in a 75-cm² flask for 24 h. Supernatants from three such cultures were pooled, aliquoted and stored at -70°C The lymphokine preparation did not contain any IL-2 activity (as assessed by the IL-2-dependent CTLL cell line) because the lymphocytes were monocyte depleted.

Polyclonal antisera raised against recombinant IL-1 (alpha-021150, beta-021100) were purchased from Cistron Technology (Pine Brook, NJ) and that against native IL-1 (EP200) from Genzyme (Boston, MA). Purified human IL-1 and recombinant human IL-1β (specific activity 10^7 U/mg) were purchased from Cistron Technology. Recombinant human IL-1α (specific activity 10^7 U/mg) and recombinant IL-2 (specific activity 2×10^6 U/mg) were purchased from Boehringer Mannheim (Sydney, Australia). Recombinant human IL-6 (specific activity 10^8 U/mg on the 7TD1 cell line) and antiserum to IL-6 were provided by Dr Gordon Wong (Genetics Institute, Cambridge, MA). Indomethacin (Sigma) was dissolved at 1 mg/ml in 5% sodium bicarbonate, sterilized by membrane filtration and diluted with culture medium.

With the exception of the crude lymphokine preparation, which stimulated thymocyte proliferation at concentrations greater than 1%, and IL-2, none of these cytokines had any direct thymocyte stimulatory activity, and they did not influence the activity of a standard IL-1 preparation over the concentration ranges used. Ten samples of serum with high titre of rheumatoid factor were pooled in order to investigate its ability to stimulate monocyte IL-1 production. Samples of normal human serum, normal recalcified platelet-poor plasma and fetal calf serum (FCS) were also tested.

Reagents were tested for endotoxin contamination using the *Limulus* lysate assay (E-toxate, Sigma, St Louis, MO) with a chromogenic substrate (LAL, Spectrozyme, American Diagnostica, Sydney, Australia). This assay detected LPS in the range of 10–1000 pg/ml. Sera (10%), TNF-α (10^6 U/ml), IFN-γ (10^5 U/ml), IFN-α (10^6 U/ml), and IL-1α (100 U/ml) were all negative. The IL-2 preparation at 200 U/ml contained 100 pg/ml of endotoxin, and the IL-1β preparation at 100 U/ml contained 60 pg/ml of endotoxin.

Monocyte isolation and culture

Venous blood (60–120 ml) was collected from healthy volunteers, using 0·38% sodium citrate as anti-coagulant. Monocyte isolation and enrichment were achieved by density gradient and counterflow centrifugation steps as previously described (Danis *et al.*, 1987). FCS (GIBCO) at 0·2% (containing less than 1 pg/ml of endotoxin contamination) was used in the elutriation buffer instead of 0·1% bovine serum albumin (containing 0·7 ng/ml endotoxin). The elutriator was also washed with 0·5% Etoxa-Clean (Sigma) prior to use. Recovery of monocytes was >85% and purity was >80% by the criterion of latex ingestion.

Purified monocytes were cultured in liquid suspension in 70×11 mm Minisorp tubes (Nunc, Roskilde, Denmark) which had been sterilized by ethylene oxide. The culture medium was RPMI 1640 buffered with 20 mM HEPES (Ultrol, Calbiochem-Behring) and 10 mM sodium bicarbonate, and supplemented with 0·5 mg/ml lactalbumin hydrolysate (contaminating endotoxin level 4 pg/0·5 mg, batch number 742576, Difco), 10 μg/ml bovine insulin, 10 μg/ml human transferrin (Sigma), 60 μg/ml penicillin and 100 μg/ml streptomycin. Indomethacin at 10^{-6} M was also included in the culture medium to obviate the inhibitory effect of prostaglandin generation. Endotoxin-free water was prepared using the Milli-Q/UF system coupled to a Milli-RPO15 unit (Millipore). This was verified using the *Limulus* test described above.

Monocytes were cultured at 10^6/ml in a total volume of 0·4 ml in tubes fitted with a loose cap in 5% CO_2 in air in a Hotpac humidified incubator. After 20 h the tubes were centrifuged at 200 *g* for 10 min and the supernatants were harvested. After one wash, the cells were resuspended in 0·4 ml of fresh culture medium and disrupted by sonication for 10 sec at 50 W using a Labsonic 2000 ultrasonic homogeniser (B. Braun Instruments, Sydney, Australia) with a 4-mm titanium needle probe. This was used to estimate the cell-associated IL-1 activity. All samples were stored at -20°C prior to assay. In some cases, monocytes were cultured in 96-well or 24-well flat-bottomed polystyrene plates (Linbro, Flow). The variation in IL-1 production in replicate cultures was 25%. The viability of cells after culture was >90% as determined by trypan blue dye exclusion.

IL-1 assay (standard)

A micro-modification of the thymocyte co-stimulation assay was used to measure IL-1. Thymocytes were obtained from 3–5-week-old CBA/H/T6-T6 mice and cultured at $7·5 \times 10^6$/ml in 20 μl hanging-drop cultures (Micro-multi Test Plate, Disposable Products, Sydney, Australia). The culture medium consisted of RPMI 1640 with 10% FCS, 2×10^{-5} M 2-mercapto-ethanol (Sigma), 4 μg/ml PHA-P (Difco) and antibiotics. Test samples were serially diluted in RPMI containing 1% FCS prior to addition to the culture plates using an Eppendorf multipipette with a 0·5 ml Combitip. The cells were cultured for 72 h with a pulse of 18·5 KBq of tritiated thymidine (247·9 GBq/mmol, New England Nuclear, Boston, MA) during the last 16 h, using a 50-μl Hamilton syringe and a PB600 dispenser (Hamilton, Reno, NV). They were harvested on a Micro Harvester (Flow) and counted on a Packard liquid scintillation counter.

Samples were assayed in triplicate and in three to five two-fold dilutions, and the 50% maximum stimulation titre was determined by interpolation of the line of best fit using a computer program for geometric regression. Results were quantified relative to an arbitrary internal reference standard designated to have 300 U/ml of activity, although its 50% maximum titre ranged from 0·25 to 1·0% or 400 to 100 U as defined by reciprocal titre. Because four reference standards were run with each assay, and the rank order of activity remained the same despite variations in the reciprocal titres, the artibrary designation of units was considered to be more reliable than the reciprocal titre for inter-assay quantitative comparisons. The coefficient of variation in results could thereby be reduced from 45% to less than 30%. Intra-assay variability was

estimated at 16%. Variation within a single culture plate was 11% and between different plates in the same assay 14%.

Samples were diluted in a range between 0·08 and 50% in order to find a dose-dependent range for quantification. Purified human IL-1 (quoted as 1000 U/ml) obtained from Cistron Technology yielded 100 U/ml and 250 U/ml activity, respectively in this assay.

IL-1 Assay (IL-2 independent)

In order to assay IL-1 in the presence of IL-2, the following modifications were made. An excess of human recombinant IL-2 (10 U/ml) was added to the thymocyte culture medium, zinc chloride (0·1 mM) was added to enhance sensitivity and reduce the requirement for PHA (used at 0·5 µg/ml; Winchurch, 1988). Addition of up to 20 U/ml of additional IL-2 did not affect this assay for IL-1. Commercial preparations of recombinant IL-1 (both alpha and beta) had reciprocal titres as quoted by the companies (1000 U/ml) so one of these was used as a reference standard and was designated to have 1000 U/ml of IL-1 activity. The reference standard used previously was measured at 1110 U/ml by the modified assay so the previous assay procedure underestimated the activity by a factor of 3·7. The inter-assay variation was 13%. Samples were diluted in a range between 0·005 and 50% using ten-fold dilution steps, and the IL-1 activity was quantitated as before.

Imunoassays for IL-1

Antibody capture assays for IL-1α and IL-1β were developed using polyclonal sheep antisera provided by Dr S. Poole (National Institute for Biological Standards and Control, UK). The IgG fractions were isolated using protein-G Sepharose 4 Fast Flow (Pharmacia). A portion of this was used as the capture antibody and a portion was biotinylated and used as the tracer antibody. IgG (1 mg/ml in 0·1 M NaCHO₃, pH 8) was allowed to react for 1 h at room temperature with α-biotinyl-amino-hexanoyl-*n*-hydroxy-sucinimide ester (Bac-1, Bresatec, Adelaide). Bac-1 was dissolved at 1 mg/ml in DMSO and 0·12 ml was added per ml of IgG.

Nunc-certified Maxisorp plates were coated with IgG specific for either IL-1α or Il-1β (5 µg/ml and 10 µg/ml, respectively, in carbonate buffer; pH 9·6) by overnight incubation at 4°C. The plates were blocked by the addition of 1% BSA in PBS, which was also the diluent for the biotinylated IgG (1 µg/ml and 20 µg/ml of antibody to IL-1α and IL-1β, respectively) and the streptavidin-peroxidase (1/2000 dilution, Amersham) used in subsequent incubations at 37°C for 30 min each. The washing buffer was PBS with 0·05% Tween 20 (Sigma). Samples were incubated for 30 min at 37°C in a CO₂ incubator but other incubations were in plain air. The peroxidase substrate was ABTS (Boehringer Manheim), and the absorbance at 405 nm was read on a Titertek Multiskan MCC (Flow). Results were calibrated with serial dilutions of known quantitities of recombinant IL-1. The range of detection was 0·1 to 40 ng/ml for both assays. The two assays were specific for their respective isotypes and also showed no reactivity to TNF-α, IL-2 or IL-6. The variation in quantification was 6·7% and 6·5% for IL-1α and IL-1β, respectively.

Statistical analysis

The paired Student's *t*-test and the Wilcoxon signed rank test were used as indicated in the figures. Data are represented as mean ± s.e.m.

Table 1. Neutralization of thymocyte-stimulatory activity (IL-2 independent assay) of recombinant IL-1 (β and α) and monocyte IL-1 (supernatants and cell lysates) by pre-incubation (3 h at 37°C) with antisera directed against recombinant cytokines (IL-1α, IL-1β and IL-6) or against purified native IL-1 (nIL-1)

Stimulus	Antiserum specificity				
	None	rIL-1α	rIL-1β	nIL-1	rIL-6
None	40 000	—	—	—	—
rIL-1β	73 900	80 500	37 900	67 600	81 600
Supernatant 1	59 400	59 750	44 600	40 350	53 700
Supernatant 2	56 500	58 300	45 300	42 200	56 300
Supernatant 3	87 800	83 400	58 200	49 200	76 700
Supernatant 4	81 400	73 200	50 500	43 900	67 500
rIL-1α	75 350	48 700	85 300	39 650	52 340
Lysate 1	63 100	49 400	65 200	38 800	68 900
Lysate 2	71 000	59 900	82 100	43 900	81 500
Lysate 3	62 400	51 800	71 600	42 300	63 740
Lysate 4	73 900	63 200	75 000	49 400	72 200

Normal rabbit IgG was the control. Results are expressed as the mean ct/min of ³H-thymidine uptake, and the s.d. were less than 10%. The dilution of antiserm to IL-6 used here was sufficient to neutralize 5 ng/ml of IL-6 as tested in the hybridoma growth assay.

RESULTS

Specificity of the IL-1 assays

The specificity of the thymocyte assays were checked using neutralising antisera. Results were identical in both assay procedures used and representative data are shown in the Table 1. The thymocyte-stimulating activites of monocyte supernatants were neutralized by antibodies to IL-1β, and monocyte cell lysates by antibodies to IL-1α. Neither were affected by antibodies to IL-6, although the activity of IL-1α was partially inhibited by this antiserum. Recombinant IL-6 (0·025–250 ng/ml) did not have any direct effect on thymocyte proliferation, and incorporation of IL-6 (up to 25 µg/ml) did not affect the quantification of rIL-1 or monocyte-derived IL-1 activities (data not shown).

IL-1 production in non-adherent cultures

Monocytes cultured for 20 h in Minisorp tubes did not adhere and could be recovered in single cell suspension with >90% viability. The background level of IL-1 production in this system was below the limits of detection in the standard IL-1 assay, in contrast to cultures adherent to plastic in which background stimulation was about 25% of maximum stimulation achieved by LPS (Fig. 1). The dose-response curves for LPS stimulation were also shifted to the left by about an order of magnitude in the non-adherent cultures. LPS stimulation resulted in parallel expression of secreted and cell-associated IL-1 activity, although cell-associated IL-1 was more readily induced by low concentrations of LPS (Fig. 2a).

Effect of cytokines and serum on IL-1 production

IFN-γ (1–100 U/ml), IFN-α (10–1000 U/ml) and crude lymphokine (0·001–0·1%) did not induce any IL-1 production as detected by the standard IL-1 assay. The ability of LPS, TNF-α and serum (pooled, rheumatoid factor positive) to induce IL-1 production are compared in Fig. 2. Rheumatoid factor positive
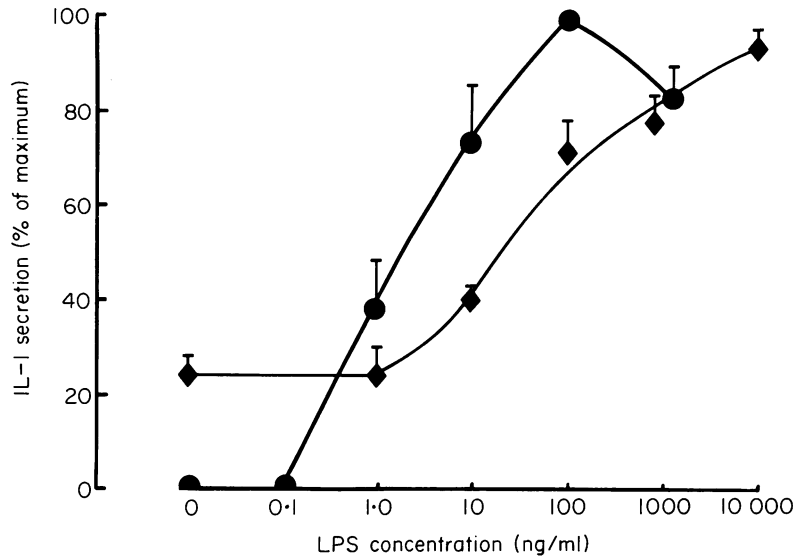
Fig. 1. The induction of monocyte IL-1 secretion *in vitro*. Comparison between adherent (♦) and non-adherent (●) cultures. Data from 11 and five healthy subjects, respectively.
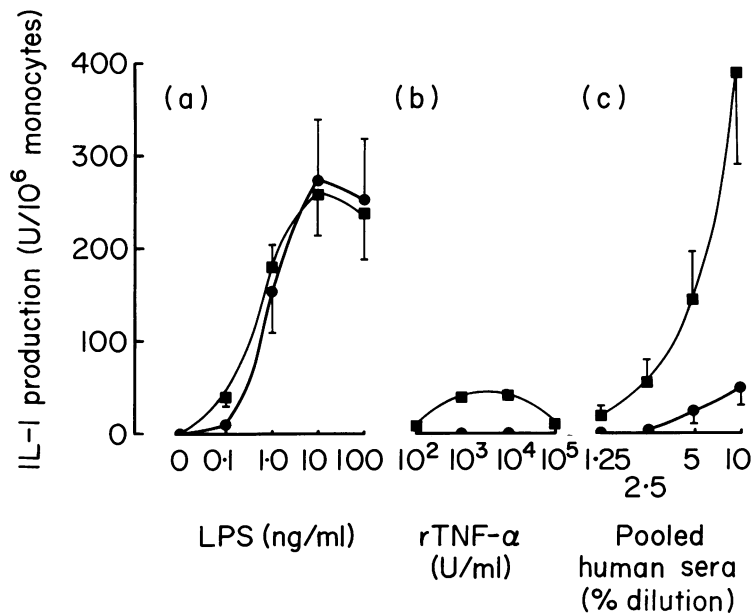

Fig. 2. Dose-dependent induction of monocyte IL-1 production by LPS (a), rTNF-α (b) and human serum (5% pooled, rheumatoid factor positive) (c). ●, Secreted IL-1; ■, cell-associated IL-1. Data from 12, six and six healthy subjects, respectively.

serum was used because it was thought to contain factors such as immune complexes that would stimulate IL-1 production. However, control studies of normal human serum or plasma, and various batches of FCS, indicated that the stimulatory activity of the pathological serum was not different from that of normal serum. There was variation in the stimulatory activity of individual serum samples (not related to any LPS contamination) as well as variation in the response of monocytes from different individuals (data not shown). The pooled pathological serum was used in these studies because its stimulatory effect was relatively consistent. TNF-α induced low levels of cell-

associated IL-1 activity in a dose-dependent manner (Fig. 2b). Serum (pooled, rheumatoid factor positive) stimulated a small amount of secreted IL-1 production but a large amount of cell-associated IL-1 similar to that produced under LPS stimulation (Fig. 2c).

Crude lymphokine (0·01%) as well as IFN-γ (10 U/ml) and TNF-α (1000 U/ml) augmented the production of IL-1 induced by LPS (see Fig 3). The effect of TNF-α on LPS-induced IL-1 production was restricted to the cell-associated component. TNF-α also potentiated the production of IL-1 (both secreted and cell-associated) in response to serum (5% pooled, rheuma-
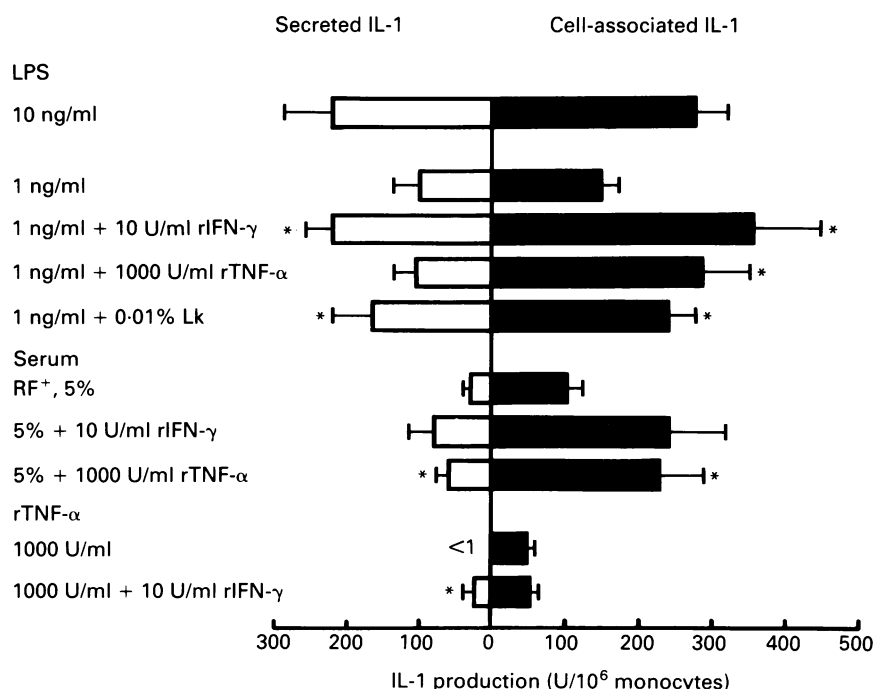
Secreted IL-1   Cell-associated IL-1

Fig. 3. Augmentation of IL-1 production by the synergistic action of cytokines.
* Augmentation (*P* < 0·05) compared with the appropriate stimulus without cytokine (Student's paired *t*-test). Data from 10 healthy subjects. Lk, lymphokine; FR, rheumatoid factor.

toid factor positive). IL-1 secretion, but not cell-associated IL-1 production induced by TNF-α was enhanced by IFN-γ

Inhibition of IL-1 production by IFN-α
IFN-α inhibited IL-1 production (both secreted and cell-associated) in a dose-dependent manner; the inhibitory effect was most striking when a high concentration of LPS was used to induce IL-1 production (Fig. 4). IFN-α also inhibited IL-1 production induced by TNF-α, serum (5% pooled, rheumatoid factor positive) and combinations of LPS and cytokines or serum and cytokines (Fig. 5).

Enhancement of IL-1 production by IL-2 and IL-1
IL-1 production (detected by the modified thymocyte assay) induced by several cytokines and combinations of cytokines is compared with background levels in Fig. 6. IL-1 production induced by 1 ng/ml of LPS is also shown. The results confirmed that TNF-α could induce directly substantial amounts of cell-associated IL-1 as well as some secreted IL-1. Low levels of IL-1 (mainly cell-associated) were induced by IFN-γ (10 U/ml), IL-2 (20 U/ml), IL-α and IL-1β (each at 1 U/ml). Increasing the IL-2 concentration up to 80 U/ml did not increase significantly the amount of IL-1 that was induced (data not shown). Higher concentrations of IFN-γ or IL-1 were not studied. However, using these low concentrations of cytokines we were able to show that significant amounts of IL-1 were produced in response to combinations of IL-2 (20 U/ml) with IFN-γ, IL-1α or IL-1β (Fig. 6), and that the effect of these combinations was more than additive.

The effect of TNF-α in combination with other low concentrations of these cytokines was also generally more than additive. The exceptions were the combinations with IL-1α or IL-1β which resulted in increased cell-associated IL-1 produc-
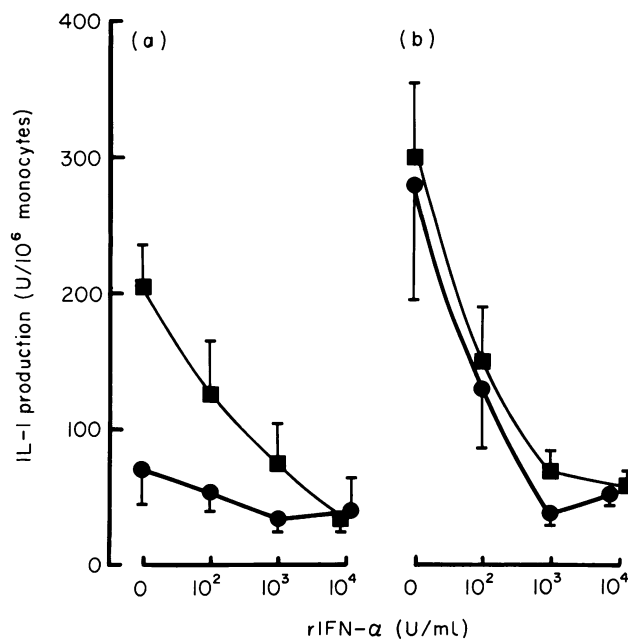

Fig. 4. The inhibitory effect of recombinant IFN-α on monocyte IL-1 production induced by LPS (a, 1 ng/ml; b, 10 ng/ml). Data from five and six healthy subjects for a and b, respectively. ●, secreted IL-1; ■, cell-associated IL-1.

tion but less secreted IL-1 production. As noted in the studies using the standard IL-1 assay, IFN-α inhibited both secreted and cell-associated IL-1 production induced by TNF-α.

We were unable to demonstrate any consistent effect of IL-2 on LPS-induced IL-1 production in 12 subjects tested (data not shown).
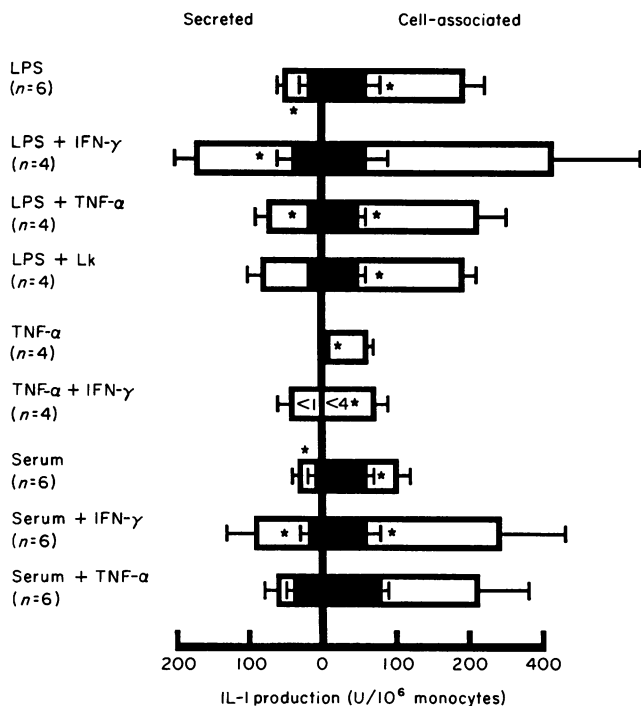
Secreted | Cell-associated

LPS (n=6)
LPS + IFN-γ (n=4)
LPS + TNF-α (n=4)
LPS + Lk (n=4)
TNF-α (n=4)
TNF-α + IFN-γ (n=4)
Serum (n=6)
Serum + IFN-γ (n=6)
Serum + TNF-α (n=6)

200 100 0 100 200 400

IL-I production (U/10⁶ monocytes)

Fig. 5. Inhibitory effect of IFN-α on monocyte IL-1 production induced by a variety of stimuli. Open bars, stimulus alone; solid bars, stimulus plus 100 U/ml of IFN-α.

* $P < 0.05$ compared with cultures not treated with IFN-α (Student's *t*-test). LPS 1 ng/ml; concentration of other stimuli as in Fig. 3.
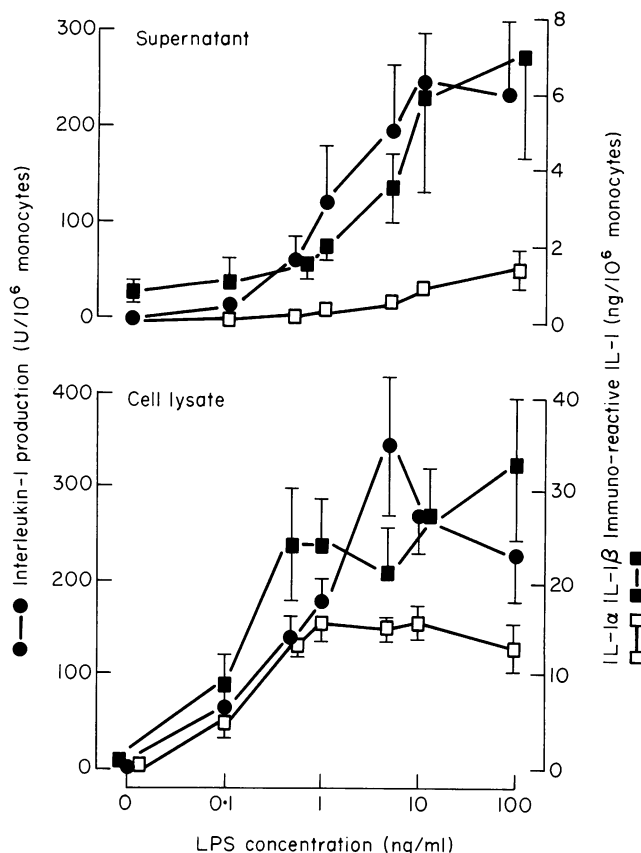

Secreted | Cell-associated

Medium only (n=12)
IFN-γ (n=6)
TNF-α (n=6)
IFN-α (n=6)
IL-2 (n=12)
IL-1α (n=7)
IL-1β (n=7)
IL-2
  + IFN-γ (n=11)
  + TNF-α (n=11)
  + IFN-α (n=11)
  + IL-1α (n=7)
  + IL-1β (n=7)
TNF-α
  + IFN-γ (n=6)
  + IFN-α (n=6)
  + IL-1α (n=6)
  + IL-1β (n=6)
LPS (n=12)

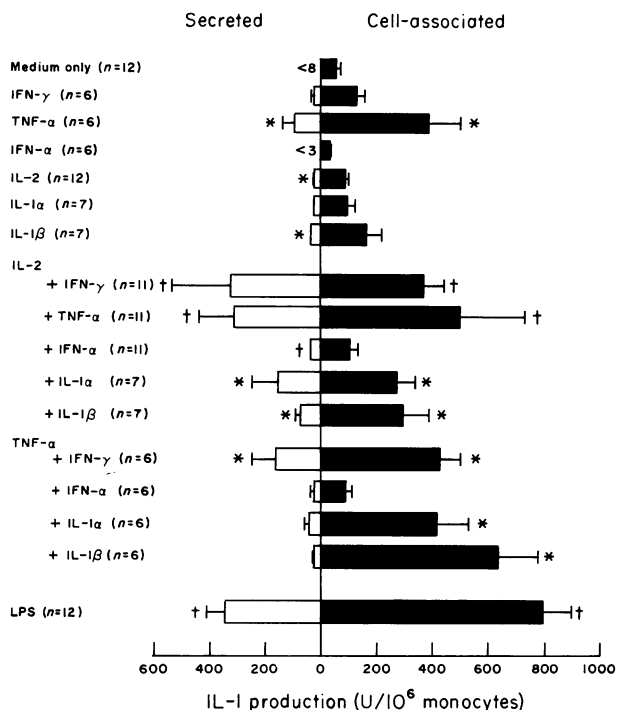600 400 200 0 200 400 600 800 1000

IL-I production (U/10⁶ monocytes)

Fig. 6. Induction of monocyte IL-1 production by cytokines. IFN-γ 10 U/ml; TNF-α 1000 U/ml; IFN-α 1000 U/ml; IL-2 20 U/ml; IL-1α and IL-1β 1 U/ml; LPS 1 ng/ml.

* $P < 0.05$, † $P < 0.01$ compared with the medium control (Wilcoxon signed rank test).

Fig. 7. Correlation between bioactive and immunoreactive IL-1 induced by increasing concentrations of LPS. Data from 15 normal subjects.

Production of immunoreactive IL-1

In order to confirm the results obtained with the thymocyte assays, specific immunoassays were developed. Figure 7 shows that IL-1 activity induced by LPS correlated with the production of immunoreactive IL-1β in supernatants and of IL-1α in cell lysates. The production of cell-associated immunoreactive IL-1 was maximally stimulated by 1 ng/ml of LPS; higher concentration of LPS, however, resulted in the expression of increased bioactivity in the lysates.

Induction of immunoreactive IL-1 production by a variety of stimuli are compared in Fig. 8. The results largely parallel those obtained with bioassays, with some differences that may reflect changes in specific activity rather than quantity of IL-1 produced. The essential similarities are that IFN-γ augmented LPS-induced IL-1 production; IFN-γ induced cell-associated IL-1 production; IL-1 production was augmented by combinations of cytokines (notably IL-2 and IFN-γ, TNF-α and IFN-α, and IFN-α and IL-1β); and IFN-α inhibited LPS-induced IL-1 production. Also, IL-2 had no effect on LPS-induced IL-1 production (data not shown).

Some of the points of difference are: TNF-α did not significantly augment the quantity of IL-1 induced by LPS; IFN-α did not inhibit the quantity of IL-1 secreted; IL-2 induced significant levels of cell-associated IL-1 (which unlike that for TNF-α was not correlated with increased biological activity); and combination of IL-2 and IL-1 had a less than additive effect on the generation of IL-1α. In addition, serum (rheumatoid factor positive, pooled) induced an enormous amount of
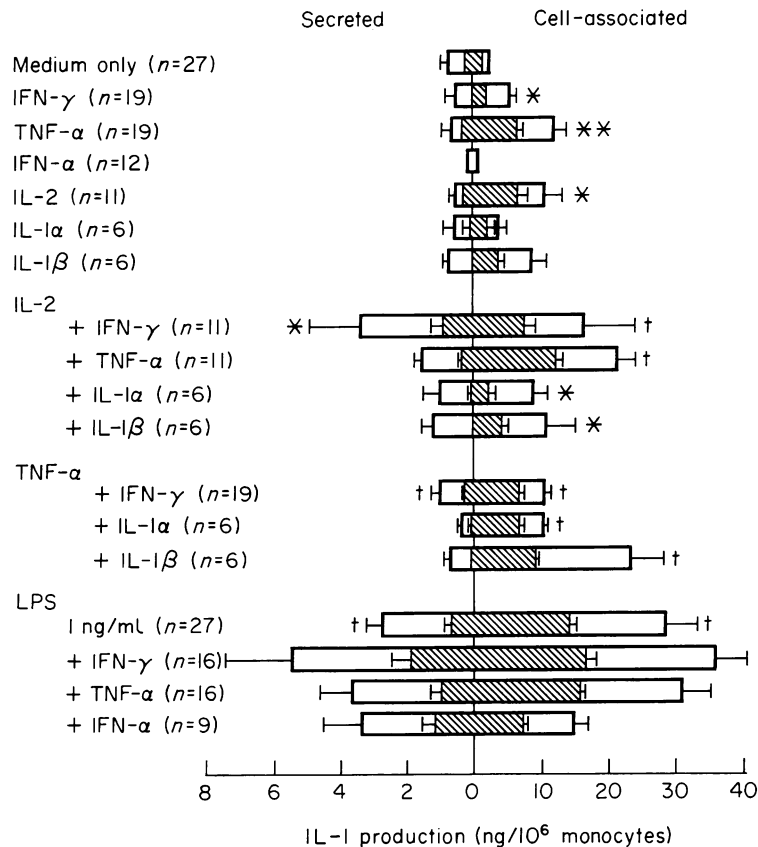
Fig. 8. Induction of immunoreactive IL-1 production by cytokines. Inner hatched area of the bars indicates the contribution of Il-1α to the total IL-1 production. Cytokine concentrations are as in Fig. 6, and LPS was at 1 ng/ml.

$* P < 0.05$; $† P < 0.001$ compared with medium only control (Student's paried t-test).

immunoreactive IL-1β production (18·0 ± 0·4 ng/ml and > 40 ng/ml for supernatants and cell lysates, respectively; $n = 19$). Bioactivity probably rested with the IL-1α components (1·3 ± 0·4 ng/ml and 14·6 ± 1·2 ng/ml for supernatants and cell lysates, respectively).

DISCUSSION

We report the effect of cytokines on IL-1 production by normal human monocytes *in vitro*. The specificity of the assays for IL-1 have been confirmed by the following procedures: duplication of results using two different thymocyte assays and specific immunoassays; specificity checks with neutralizing antibodies; and the exclusion of the possible confounding effects of IL-6. It was also necessary to minimize *in vitro* artifacts in the culture of monocytes.

Using a serum-free, non-adherent culture system we were able to reduce background IL-1 production to minimal levels. Adherence to plastic or the presence of serum stimulated IL-1 production. Although we have used mainly pathological human serum in these studies, control studies with normal human serum or plasma or bovine serum yielded similar results (data not shown). Adherence has been shown to result in the transcription of genes for several monokines including TNF-α (Haskill *et al.*, 1988) and IL-1 (Fuhlbrigge *et al.*, 1987).

Production of TNF-α after adherence is very rapid (Hofsli, Lamvik & Nissen-Meyer, 1988) and may serve to potentiate IL-1 production induced by adherence or by an exogenous stimulus such as LPS. The effect of adherence and serum on IL-1 production has been overlooked until recently (Van der Meer *et al.*, 1988). The fact that serum induced mainly cell-associated IL-1 activity, even though large amounts of IL-1β were produced and released from the cells, suggests that processing mechanisms for pro-IL-1β were not activated. Cell-associated IL-1α, which does not require processing for the expression of biological activity (Mosley *et al.*, 1987), was also produced and in amounts equivalent to those induced by 1 ng/ml of LPS.

The induction of cell-associated IL-1 activity was slightly more sensitive to LPS than was that of secreted IL-1, as has been reported by others (Lepe-Zuniga & Gery, 1984; Le Moal *et al.*, 1988). Cell-associated IL-1 production was maximally stimulated by 1 ng/ml of LPS, and higher concentrations of LPS or the addition of cytokines such as IFN-γ or TNF-α resulted in increased secretion of IL-1 as well as increased biological activity of IL-1 in both the secreted and cell-associated fractions. The ability of IFN-γ to augment IL-1 production is well documented (Newton, 1985; Gerrard *et al.*, 1986; Hart *et al.*, 1989). The enhancing effect of TNF-α, however, appeared to be restricted to the cell-associated component of IL-1 production and was related to enhanced expression of biological activity

rather than increased production of IL-1. Nevertheless, TNF-α could induce directly IL-1 production and this was mainly of the cell-associated form. IFN-γ could cause some secretion of IL-1 induced by TNF-α with resultant expression of biological activity in the supernatant. IFN-γ has recently been shown to cause secretion of IL-1 in monocytes stimulated with another agent that induced predominantly cell-associated IL-1, lipid A (Hurme & Serkkola, 1989).

A previous report of TNF-α induction of IL-1 secretion was in the context of mixed mononuclear cells in adherent cultures containing serum (Dinarello *et al.*, 1986). We have found that TNF-α induced only a small amount of IL-1 secretion directly, but could augment IL-1 secretion in serum-stimulated cultures. A recent study using the human monocytic cell line THP-1 has confirmed the fact that TNF-α could induce IL-1 production directly, and that this could be enhanced by co-stimulation with IFN-γ (Portillo *et al.*, 1989). Monocyte IL-1 production induced by immune complexes could also be augmented by IFN-γ or TNF-α (Chantry *et al.*, 1989).

Other cytokines (IFN-γ, IL-2, IL-1α and IL-1β) had only limited ability to induce IL-1 activity at the low concentrations employed. IL-2 induced cell-associated IL-1 production, as reported by others (Numerof *et al.*, 1988) although this did not correlate with increased biological activity. IL-1, particularly at high concentrations, is also known to induce directly IL-1 production (Dinarello *et al.*, 1987; Manson *et al.*, 1989). It is significant, however, that combinations of IL-2 at low concentration with low concentrations of other cytokines (IFN-γ or IL-1) resulted in the production of significant levels of IL-1 activity (both secreted and cell-associated) with increased production of immunoreactive IL-1, as reported (Numerof *et al.*, 1988; Herrmann *et al.*, 1989). IL-1 production induced by TNF-α was also enhanced by combination with IL-2 or IL-1. We were unable to demonstrate augmentation of LPS-induced monocyte IL-1 production by IL-2, although this has only been noted in cultures of the non-adherent fraction of mononuclear cells (Tilden & Dunlap. 1989).

TNF-α did not induce any significant IL-1 production over a wide concentration range. This is in conflict with a previous report (Gerrard *et al.*, 1986) probably due to the different culture conditions (40-h culture in serum-containing medium). We found it to be a potent inhibitor of IL-1 production induced by LPS or other stimuli. The fact that high (1000 U/ml) doses of purified human IFN-α could inhibit IL-1 induction has already been noted (Newton, 1985). Our results show that significant inhibition could be achieved with less than 100 U/ml of recombinant human IFN-α. IFN-α reduced the amount of immunoreactive cell-associated IL-1 production (IL-1α and IL-1β) without affecting the amount of IL-1 released from the cells, suggesting an effect at both pre- and post-translational levels. Defective IFN-α production has been associated with active disease in patients with rheumatoid arthritis (Seitz *et al.*, 1987; Hertzog *et al.*, 1988).

These results suggest that TNF-α may be an important inducer of IL-1 production in sites of inflammation. In addition, low concentrations of some cytokines (IFN-γ, IL-2, and IL-1) although having little effect on IL-1 production individually, may act in concert to stimulate IL-1 production and activity significantly. Other cytokines such as IFN-α may function to limit IL-1 production and activity.

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