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Interleukin-4 as a potent down-regulator for human alveolar macrophages capable of producing tumour necrosis factor-α and interleukin-1

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ABSTRACT: The effects of recombinant human interleukin-4 (IL-4) on the production of interleukin-1 (IL-1) and tumour necrosis factor-α (TNFα) by human alveolar macrophages (AM) and autologous peripheral blood monocytes (PBM) in response to lipopolysaccharide (LPS) were examined. AM and PBM were obtained by bronchoalveolar lavage and centrifugal elutriation, respectively, from healthy donors. The production of IL-1 (α and β) and TNFα by human AM and PBM were quantitated by enzyme immunoassays (EIA). When activated with LPS, AM secreted much more TNFα, but less IL-1β than PBM. The production of IL-1 (α and β) by activated AM and autologous PBM was suppressed dose-dependently by IL-4. The inhibitory effect of IL-4 was greatest when it was added to AM or PBM simultaneously with LPS or within 3 h after LPS. The suppressive effect of IL-4 was completely neutralized by pretreatment with rabbit anti-IL-4 antiserum. IL-4 also suppressed the production of IL-1 and TNFα by monocyte-derived macrophages. As measured by thymocyte co-stimulation assay, the production of cell-associated IL-1 was inhibited by coculture of AM plus LPS with IL-4. Northern blot analysis showed suppression by IL-4 of expression of messenger ribonucleic acid (mRNA) for IL-1 and TNFα in LPS-stimulated AM.

We conclude that IL-4 is a potent down-regulator for human alveolar macrophages capable of producing IL-1 and TNFα.

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Cells of the macrophage-histiocyte series are important in regulation of the immune response through production of monokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNFα) [1–4]. Moreover, the production of IL-1 (α, β) and TNFα were found to be regulated differentially depending on the maturation and/or activation states of the cells [5–7]. For example, human alveolar macrophages (AM) and monocyte-derived macrophages of healthy donors have a greater ability to produce more cell-associated IL-1α and TNFα, but less extracellular IL-1β than autologous peripheral blood monocytes (PBM) [6–9]. Recently, much attention has been paid to the contribution of cytokines to the development of lung diseases [10]. Accumulating evidence suggests the critical roles of monokines such as TNFα and IL-1 in the pathogenesis of various pulmonary diseases [11, 12]. For example, immune complex-induced alveolitis and bleomycin-induced pulmonary fibrosis were found to be mediated by TNFα produced by lung macrophages [10, 13]. On the other hand, IL-1 was found to be involved in the pathogenesis of chronic pulmonary granuloma formation [12]. Thus, it is important to elucidate the mechanism of regulation of monokine production by AM.

Recently, IL-4, originally defined as a B-cell growth factor [14–16], has been shown to have a variety of biological activities [17–32]. Monocyte-macrophages are known to express IL-4 receptor (IL-4R) [16]. In murine systems, IL-4 regulates *in vivo* immunoglobulin E (IgE) response [33] and stimulates the expression of class II antigen and adherence and antigen presenting ability of macrophages [21, 22], and activates these cells to the tumoricidal state [20]. In contrast, IL-4 inhibits both the ability of human PBM to produce monokines (IL-1 and TNFα) [22–25] and monocyte-mediated tumour cell killing [26]. IL-4 has also been found to suppress various monocyte functions such as their secretion of H_2O_2 and prostaglandin E_2, production of colony-stimulating factor, macrophage colony formation and human leucoyte antigen DR (HLA-DR) expression [27–32]. Little is known, however, about whether IL-4 affects the production of monokines (IL-1 and TNFα) by human AM in response to activation stimuli. In the present study we demonstrated that

IL-4 down-regulated the abilities of activated human AM and autologous PBM to produce IL-1 (α and β) and TNFα at the protein and messenger ribonucleic acid (mRNA) levels.

Materials and methods

Subjects

Cells were obtained from 16 healthy male donors (20–28 yrs old). These volunteers had no evidence by history or physical examination of lung disease, were nonsmokers, were not taking medications, gave normal results in pulmonary function tests and their chest roentogenograms were normal. They all gave informed consent to participate in the experiment.

Reagents

Fetal bovine serum (FBS) was purchased from M.A. Bioproducts (Walkerville, MD, USA). Recombinant human IL-4 (lot 801; specific activity 10^6 U·mg^{-1} protein) and polyclonal rabbit antisera to recombinant human IL-4 were gifts from Ono Pharmaceutical Co., Osaka, Japan. Monoclonal antibodies against recombinant human IL-1α, IL-1β and TNFα were provided by Otsuka Pharmaceutical Co. Ltd, Tokushima, Japan. Recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF; specific activity 6.7×10^6 U·mg^{-1} glycoprotein) was kindly supplied by the Genetics Institute (Cambridge, MA, USA). None of these materials contained endotoxins, as judged by *Limulus* amoebocyte assay (Seikagaku Kogyo Co., Tokyo, Japan). Lipopolysaccharide (LPS; *Escherichia coli* 055:B5) was obtained from Difco Laboratories (Detroit, MI, USA).

Isolation and culture of human PBM

Leucocyte concentrates were collected from peripheral blood (200 ml·person^{-1}) of healthy donors in an RS-6600 rotor of a Kubota KR-400 centrifuge, and mononuclear cells were separated from the leucocyte concentrates in lymphocyte separation medium (Litton Bionetics). Then PBM were separated from the mononuclear cell samples by centrifugal elutriation in a Beckman JE-6B elutriation rotor as described in detail previously [8]. A fraction containing more than 95% of the total monocyte population was obtained at a speed of 3,000 rpm and flow rate of 26 ml·min^{-1}. More than 90% of these cells were PBM as determined by nonspecific esterase staining and morphological examination, and more than 97% were viable, as judged by the trypan blue dye exclusion test. This fraction was washed twice with phosphate buffered saline, and resuspended at a concentration of 5×10^5 PBM per ml in RPMI 1640 supplemented with 5% FBS and gentamicin, designated as CRPMI 1640. These cells were then plated for 1 h in 96-well Microtest III plates (Falcon, Oxford, CA, USA), and then nonadherent cells were removed by washing with medium. At this point the purity of the PBM was >99% as judged by their morphology and nonspecific esterase staining.

Harvesting and preparation of human AM

Bronchoalveolar lavage was performed as described in detail elsewhere [8, 9]. Briefly, after anaesthetizing the oral cavity and the upper airway with lidocaine spray, the tip of an Olympus fibreoptic bronchoscope (Model BF-1T20; Olympus Co., Tokyo, Japan) was wedged into one of the segments of the right or left lobe. The lung was washed with 50 ml of sterilized saline (0.9% NaCl) prewarmed to 37°C, and the fluid was gently sucked out with a 50 ml syringe. This process was repeated three times. A total of 150 ml of saline was instilled, of which about 65% was recovered. The yield of human AM from normal volunteers was approximately 1.8×10^7 viable cells per wedge segment (>93% viable as determined by trypan blue dye exclusion). Differential counts established that >89% of the lavaged cells were AM (staining for nonspecific esterase). The remaining cells were either small mononuclear cells or neutrophils, which were eliminated during subsequent washing. The AM were plated into the 96 wells of Microtest III plates at a concentration of 5×10^4 AM·well^{-1}), unless otherwise stated. Nonadherent cells (<10%) were removed 1 h after plating by washing the plates, and more than 99% of the adherent cells were AM with esterase activity.

Preparation of monocyte-derived macrophages

Blood monocytes were cultured in medium with 100 U·ml^{-1} of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF). After incubation for five days, more than 70% of the cells were viable and had the typical macrophage-like appearance, showing enlargement, spreading and decrease in the nuclear/cytoplasmic ratio. These monocyte-derived macrophages were then washed thoroughly with medium.

In vitro *activation*

Monolayers of AM, PBM or monocyte-derived macrophages were incubated in triplicate cultures for 24 h at 37°C in serum-free medium with or without various amounts of activating agent (LPS) in the presence or absence of IL-4, and then the cell-free supernatants were collected by brief centrifugation at 50 ×g. Extra-cellular monokine activity in each sample of triplicate cultures was quantitated by the enzyme-linked immunosorbent assay (ELISA) as described below. In preliminary experiments, there was no difference in the viabilities of PBM, AM and monocyte-derived macrophages incubated in medium with LPS (1 μg·ml^{-1}) in the presence and absence of IL-4 (100 U·ml^{-1}) (data not shown).

Cell-associated IL-1 assay

We previously found that cell-associated IL-1 activity produced by LPS-stimulated AM was largely IL-1α

[8, 9]. To measure extracellular and cell-associated IL-1 activities, PBM or AM were plated for 1 h in CRPMI 1640, gently washed twice with RPMI-1640, incubated in CRPMI 1640 medium with or without LPS in the presence or absence of IL-4 for 16 h, and then washed three times with RPMI 1640 and incubated for 15 min at room temperature with paraformaldehyde (PFA) (1% w/v in RPMI-1640 [8]. The cells were then washed three times with RPMI-1640 (200 $\mu l \cdot wash^{-1}$), kept at 37°C for 24 h in RPMI-1640 supplemented with 10% FBS, and washed three times with RPMI-1640 before assay of cell-associated IL-1. Then, thymocytes obtained from C3H/HeJ mice, 4–8 wks old, (Jackson Laboratory, Bar Harbor, ME, USA) were incubated at a concentration of 1.5×10^6 cells·well^{-1} with control medium or test supernatants for measurement of extracellular IL-1 activity and with various numbers of fixed PBM for measurement of cell-associated IL-1. Proliferation was assessed with a suboptimal dose (0.5 $\mu l \cdot ml^{-1}$) of phytohemagglutin P (PHA-P) (Difco). The cultures were then incubated at 37°C under 5% CO_2 in air for 72 h. Tritiated thymidine (^{3}H-TdR) (6.7 $Ci \cdot mmol^{-1}$; Amersham, Arlington Heights, IL, USA) was added 18 h before the end of the incubation. After incubation, the cells were harvested on glass fibre in a cell harvester, MASH II, and cellular ^{3}H-TdR incorporation was assessed by measuring radioactivity in a scintillation counter. IL-1 activity was expressed as ^{3}H-TdR incorporation. Preliminary experiments showed that LPS (1 $\mu g \cdot ml^{-1}$) and/or IL-4 (100 $U \cdot ml^{-1}$) with PHA-P or with recombinant IL-1 did not directly induce proliferation of C3H/HeJ mouse thymocytes (data not shown).

Northern blot hybridization

Human AM were cultured in polypropyrene tubes (Falcon, Oxnard, CA, USA) for 4 h in CRPMI 1640 medium with or without LPS (0.1 $\mu g \cdot ml^{-1}$) in the presence or absence of IL-4 (100 $U \cdot ml^{-1}$). Cultured AM were washed briefly with ice-cold phosphate-buffered saline, frozen in liquid nitrogen, and stored at -70°C until use. Northern blot analysis of total RNA (10 μg) extracted from AM samples by the guanidine thiocyanate/cesium chloride method [34] was performed as described previously [35]. The complement deoxyribonucleic acid (cDNA) probes for IL-1α, IL-1β and TNFα were kindly provided by Dr Y. Hirai, Otsuka Pharmaceutical Co., Tokushima, Japan. A cDNA probe of β-actin was purchased from Wako Pure Chemical Industries (Osaka, Japan). RNA samples containing 10 $\mu g \cdot ml^{-1}$ of poly(A+)RNA were denatured and electrophoresed in agarose gel containing formaldehyde. After electrophoresis, the RNA was transferred to a Hybond-N+nylon membrane (Amersham) and hybridized with a ^{32}P-labelled probe. The membrane was then autoradiographed with Kodak XAR-5 film at -70°C with an intensifying screen. Film images were scanned with a transmittance/reflectance scanning densitometer (Bio-Rad model 1650; Bio-Rad Laboratories, Richmond, CA, USA) to determine quantitative variations in RNA.

Enzyme immunoassays (EIAs) of human IL-1β, IL-1α and TNFα

EIAs for human IL-1α, IL-1β and TNFα were performed essentially as described previously [8, 9]. Sensitivity limits of the EIAs for IL-1α, IL-1β and TNFα were 10 $pg \cdot ml^{-1}$, 20 $pg \cdot ml^{-1}$ and 20 $pg \cdot ml^{-1}$, respectively.

Statistical analysis

The statistical significance of differences between groups was analysed by Student t-test (two-tailed).

Results

Suppression by IL-4 of IL-1 and TNFα production by AM

Firstly, we examined whether IL-4 affected the ability of human AM and autologous PBM to produce IL-1 and TNFα in response to LPS. The results are shown in figure 1. In serum-free medium alone, AM or PBM did not secrete appreciable amounts or monokines (TNFα and IL-1β), but their treatment with LPS at concentrations of more than 10 $ng \cdot ml^{-1}$ resulted in dose-dependent production of extracellular IL-1 and TNFα. Activated PBM produced more IL-1β but much less TNFα than AM. Under these experimental conditions, the production of these monokines by AM and PBM were significantly suppressed by IL-4 ($p<0.05$), when they were activated with $\geq$10 $ng \cdot ml^{-1}$ and $\geq$1 $ng \cdot ml^{-1}$ of LPS, respectively.

We examined the effect of IL-4 concentration on monokine production by AM and PBM. Neither AM nor PBM incubated in medium alone secreted appreciable amounts of monokines (IL-1 and TNFα) (data not shown). The extracellular production of IL-1 (α and β) by PBM stimulated with LPS was suppressed by addition of more than 10 $U \cdot ml^{-1}$ of IL-4, whereas that by AM was only slightly affected by IL-4. In contrast, the production of TNFα by activated AM and PBM were both suppressed dose-dependently by IL-4, that by AM being suppressed very markedly (fig. 2).

In a parallel experiment, we examined the effect of the densities of AM and PBM on the suppression of the IL-1 (α and β) and TNFα production by IL-4. Results are shown in figure 3. At cell densities of more than 0.5×10^5 per well. LPS-stimulated AM produced much more TNFα, and less IL-1β than PBM, and IL-4 significantly suppressed the production of both IL-1 (α and β) and TNFα by both activated AM and PBM ($p<0.05$).

The variability in monokine production by AM ($5 \times 10^4 \cdot well^{-1}$) from nine different donors in the suppression of their production by IL-4 was examined. As shown in table 1, the ability of human AM from different healthy donors to produce monokines (IL-1β and TNFα) in response to LPS varied appreciably, but in almost all cases IL-4 suppressed their production of monokines.

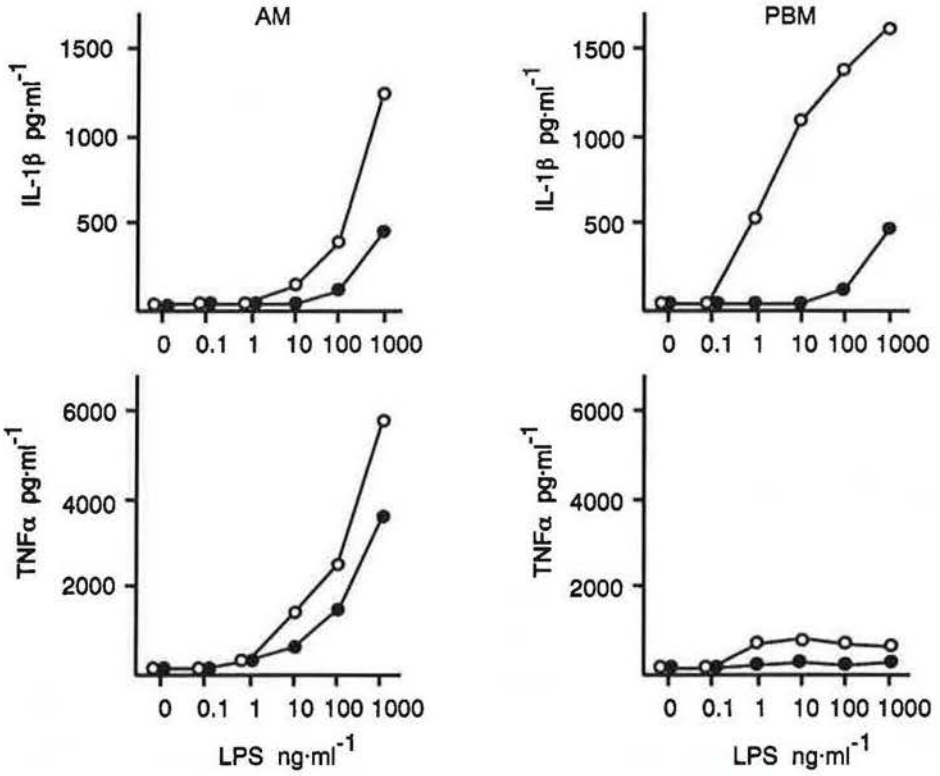

Fig. 1. – Suppression by IL-4 of IL-1β and TNFα production by AM activated with LPS. Samples of 5×10^4 AM or PBM were incubated in monolayer for 24 h in serum-free medium with various concentrations of LPS in the presence (●) or absence (○) of IL-4 (100 U·ml^{-1}). Then, the supernatants were harvested and IL-1β and TNFα were measured quantitatively by EIA as described in Materials and Methods. Data are representative of two separate experiments. SDs were consistently <10% of means. IL-4: interleukin-4; IL-1β: interleukin 1β; TNFα: tumour necrosis factor α; AM: alveolar macrophages; LPS: lipopolysaccharide; PBM: peripheral blood monocyte; EIA: enzyme immunoassay.

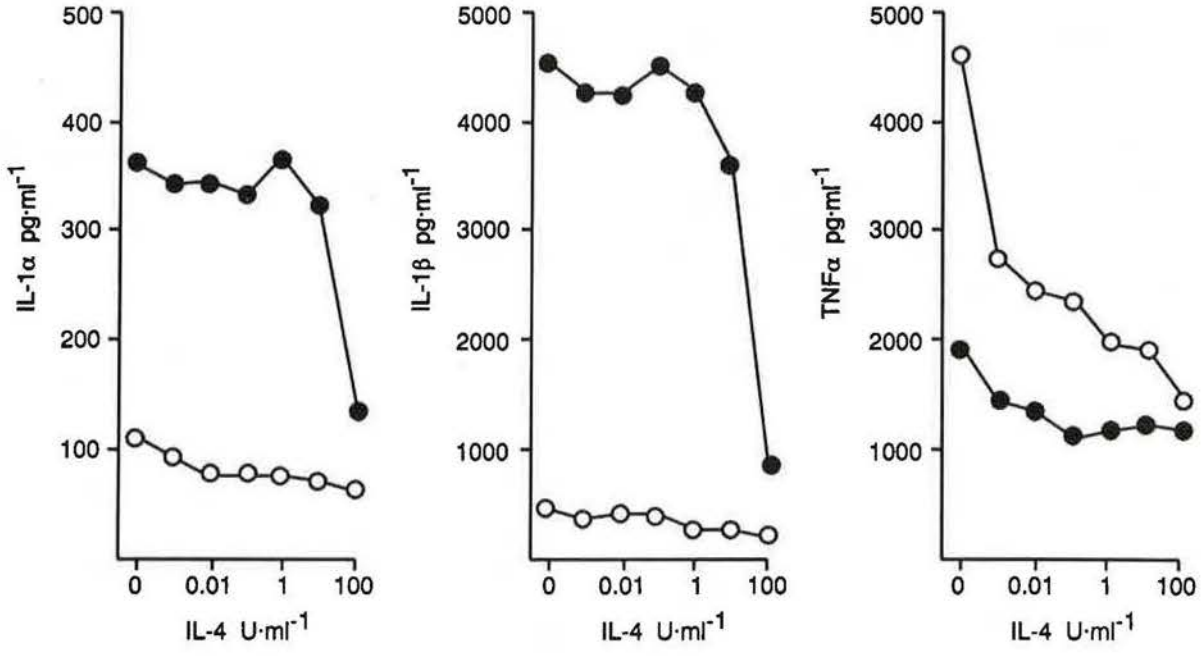

Fig. 2. – Dose-dependent suppression by IL-4 of monokine production by AM and PBM. Samples of 5×10^4 PBM (●) or AM (○) were incubated for 24 h in medium with 1 μg·ml^{-1} of LPS in presence or absence of IL-4 at the indicated concentrations. Then the supernatants were harvested and IL-1α, IL-1β and TNFα were measured quantitatively as described in Materials and Methods. Data are representative of three separate experiments. SDs were consistently <10%. For abbreviation see legend to figure 1.

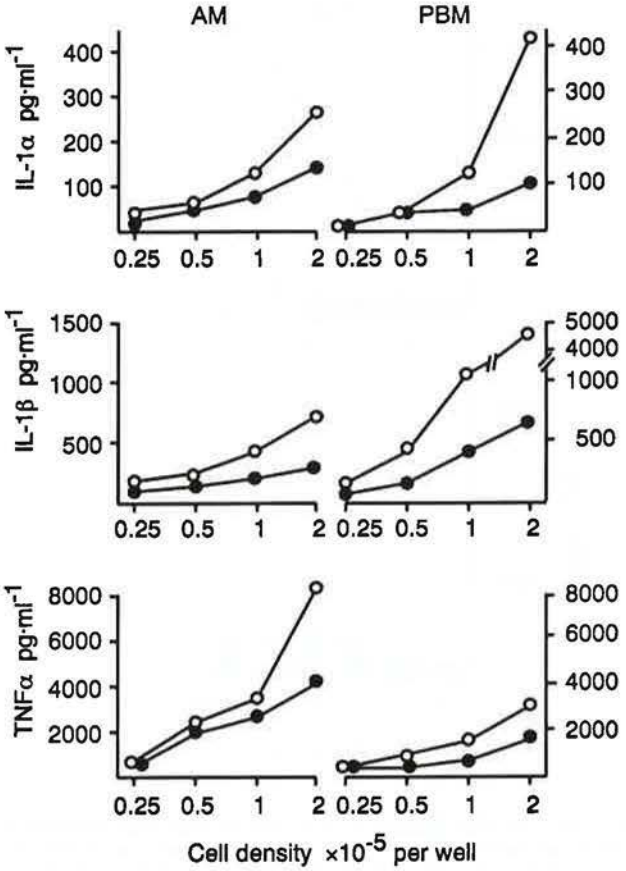

Fig. 3. – Effects of densities of AM or PBM on suppression by IL-4 of monokine production. AM or PBM were plated for 2 h in CRPMI 1640 at the indicated densities washed and incubated for 24 h in medium with 1 μg·ml⁻¹ of LPS in presence (●) or absence (○) of IL-4 (100 U·ml⁻¹). Then the supernatants were harvested and monokines were measured as described in Materials and Methods. Data are representative of two separate experiments. For abbreviations see legend to figure 1.

Kinetics of suppression by IL-4 of TNFα production by AM and PBM

The effect of the time of IL-4 addition on suppression of monokine production was examined. TNFα production was affected stepwise by addition of IL-4 to cultures of PBM at different times after treatment with LPS: the production of TNFα by AM and PBM was inhibited about 47% and 56%, respectively, by addition of IL-4 immediately after LPS activation (fig. 4).

Effects of pretreatment with anti-IL-4 antiserum on suppression of IL-1β and TNFα production by AM or PBM

We determined whether IL-4 itself or some contaminant in the recombinant human IL-4 preparation suppressed the production of monokines by AM or PBM. The results in table 2 show that pretreatment for 16 h with anti-IL-4 antiserum almost completely neutralized the suppressive effects of IL-4 (100 U·ml⁻¹) on the production of IL-1β and TNFα by AM and PBM.

Table 1. – Suppression by recombinant human IL-4 of production of TNFα and IL-1β by human AM

No. of expt	Addition of IL-4 100 U·ml⁻¹	Monokine production pg·ml⁻¹	
		TNFα	IL-1β
1	-	8074±11	676±3
	+	4449±496*	257±4*
2	-	4950±21	87±1
	+	2255±23*	<20*
3	-	4620±378	348±5
	+	1452±311*	150±2*
4	-	1230±74	131±6
	+	1048±79	75±1*
5	-	1342±124	<20
	+	287±23*	<20
6	-	2233±62	844±34
	+	1551±311*	456±4*
7	-	17170±1214	3057±86
	+	11357±925*	617±20*
8	-	10494±132	2508±83
	+	6105±116*	628±10*
9	-	8470±692	303±4
	+	5082±490*	88±14*

Values are the mean (±SD) for triplicate cultures. *: $p<0.05$ *vs* value for AM treated with LPS alone. IL-4: interleukin-4; TNFα: tumour necrosis factor-α; IL-1β: interleukin-1β; AM: alveolar macrophage; LPS: lipopolysaccharide.

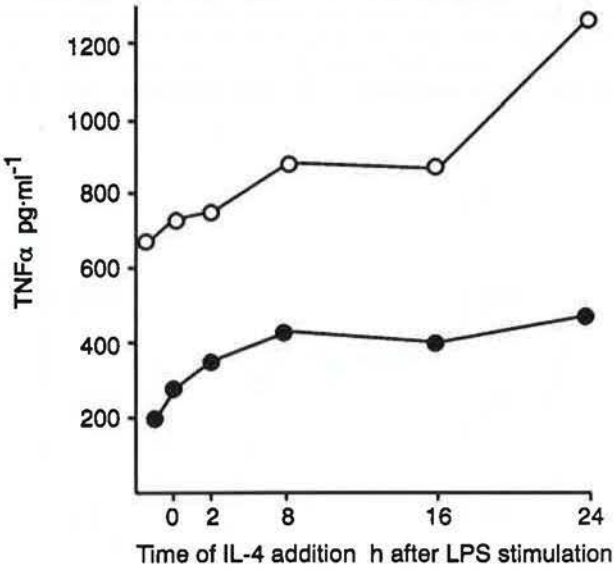

Fig. 4. – Kinetics of IL-4-mediated suppression of TNFα production by AM or PBM. Samples of 5×10^4 AM (○) or PBM (●) were incubated for 24 h in medium with 1 μg·ml⁻¹ of LPS, and then at the indicated times IL-4 (100 U·ml⁻¹) was added to cultures of AM plus LPS. The supernatants were harvested, and TNF production was assessed as described in Materials and Methods. Data are representative of two separate experiments. For abbreviations see legend to figure 1.

Suppression by IL-4 of extracellularly-secreted and cell-associated IL-1 production by activated AM and PBM

We examined whether IL-4 suppressed the ability of activated AM to produce cell-associated IL-1. As shown

in table 3, human AM produced more cell-associated IL-1 activity, but less extracellular IL-1 activity than PBM. Addition of IL-4 to the cultures of AM and PBM with LPS resulted in significant suppression of the production of extracellular and cell-associated IL-1 activities by LPS-activated AM as well as PBM.

Table 2. – Effect of pretreatment of IL-4 with anti-IL-4 antiserum on its suppression of monokine production by AM and PBM

Treatment of AM or PBM	Anti-IL-4 antiserum	Il-1β pg·ml^{-1}		TNFα pg·ml^{-1}	
		Medium	IL-4	Medium	IL-4
PBM					
Medium	-	<20	<20	<20	<20
	+	104	123	29	<20
LPS 1 μg·ml^{-1}	-	3307	483	1617	935
	+	3369	3923*	1122	1529*
AM					
Medium	-	<20	<20	<20	<20
	+	95	115	<20	<20
LPS 1 μg·ml^{-1}	-	449	115	16211	11726
	+	429	463*	15049	17373*

Values are the mean for triplicate cultures. The SDS were consistently <10% of the means. *: $p<0.05$ *vs* value for PBM or AM treated with LPS and IL-4 without anti-IL-4 antiserum. PBM: peripheral blood monocytes. For further abbreviations see legend to table 1.

Table 3. – Suppression by IL-4 of cell-associated IL-1 production by activated AM and PBM

IL-4	Thymocyte blastogenesis to PHA-P PBM		AM	
U·ml^{-1}	Medium	LPS	Medium	LPS
Extracellularly-secreted IL-1				
0	2476±2	28310±58	1631±3	20631±9
1	2198±3	4195±9*	1813±8	6128±8*
10	2052±8	2828±3*	1990±6	2409±3*
100	2998±5	3059±1*	1993±4	2214±9*
Cell-associated IL-1				
0	1230±6	12408±14	1651±9	31809±68
1	1099±71	2192±38*	1422±29	12875±88*
10	1608±12	2210±5*	1234±20	7283±5*
100	1520±2	2408±6*	1117±66	2161±3*

Values are the mean cpm (±SD) for triplicate cultures. The background ^{3}H-TdR incorporation was 1757±123. The ^{3}H-TdR uptakes by thymocytes incubated with PHA-P and recombinant IL-1α (5 U·ml^{-1}) and IL-1β (5 U·ml^{-1}) were 27842±328 and 16231±522, respectively. *: $p<0.05$ *vs* value for PBM or AM treated with LPS alone. ^{3}H-TdR: tritiated thymidine. For further abbreviations see legends to table 1 and table 2.

Suppression by IL-4 of mRNA expression for IL-1α, IL-1β and TNFα in AM

Next, we examined whether IL-4 affected monokine production by altering the expression of mRNA for IL-1 and TNFα. IL-4 did not cause reduction in expression of β-actin mRNA (data not shown). Addition of IL-4 to the cultures of AM with LPS resulted in 33%, 29% and 25% reductions in their expressions of IL-1α, IL-1β and TNFα mRNA, respectively, as compared to AM treated with LPS alone (fig. 5).

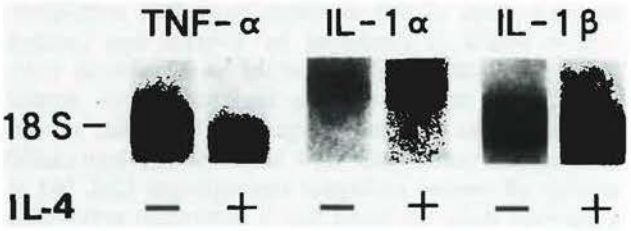

Fig. 5. – Suppressive effect of IL-4 on LPS-induced expressions of mRNA for TNFα, IL-1α and IL-1β in AM. AM were cultured for 4 h in medium with LPS (0.1 μg·ml^{-1}) in the presence or absence of IL-4 (100 U·ml^{-1}). Then total ribonucleic acid was isolated and analysed as described in Materials and Methods. Data are representative of two independent experiments. For further abbreviations see legend to figure 1.

Suppression by IL-4 of IL-1β and TNFα production by monocyte-derived macrophages

We examined whether IL-4 affected the ability of monocyte-derived macrophages to produce monokines in response to LPS. As shown in table 4, monocyte-derived macrophages produced a small amount of TNFα spontaneously in the absence of LPS, but no monokines were detected in the supernatants of fresh PBM or AM. On stimulation with LPS, monocyte-

derived macrophages showed a significantly greater ability than fresh PBM to produce TNFα, although their IL-1β production was less than that by fresh PBM. Addition of IL-4 simultaneously with LPS to the cultures significantly suppressed the production of both monokines (IL-1β and TNFα) by monocyte-derived macrophages as well as fresh PBM and AM.

Table 4. – Monokine production by monocyte-derived macrophages and its suppression by IL-4

Treatment	Addition of IL-4 100 U·ml^{-1}	Monokine pg·ml^{-1} Fresh PBM	Monocyte-derived macrophages	AM
Set 1: IL-1β production				
Medium	-	<20	<20	<20
	+	<20	<20	<20
LPS 1 μg·ml^{-1}	-	4382±190	189±3	676±1
	+	685±12*	48±1*	267±4*
Set 2: TNFα production				
Medium	-	<20	95±4	<20
	+	<20	44±2	<20
LPS 1 μg·ml^{-1}	-	2496±89	20503±1357	8074±10
	+	1176±13*	10703±435*	4409±4009*

Values are the mean (±SD) for triplicate cultures. *: $p<0.05$ *vs* value for those treated with LPS alone. For abbreviations see legend to table 1.

Discussion

The present study showed that on stimulation with bacterial endotoxin LPS, IL-4 suppressed the production of IL-1 (α and β) and TNFα by normal human AM at the protein and mRNA levels, and that IL-4 suppressed the early phase of AM activation to produce monokines.

IL-4 which is produced by T-cells, has various biological effects of lymphoid and myeloid cells [15–32], but its up- or down-regulatory effects depend on the species of macrophages. For example, several investigators found that IL-4 enhances the tumoricidal activity of murine peritoneal macrophages [20], but in a previous study we found that it suppressed tumoricidal activation of human PBM [26]. Moreover, WOLPE *et al.* [36] reported that treatment of thioglycollate-induced murine macrophages with IL-4 increased their level of IL-1 mRNA, but not TNF mRNA [36]. IL-4 was also found to act synergistically with LPS in inducing murine peritoneal macrophages to produce TNF [37]. In contrast, treatment of human PBM with IL-4 decreased their production of IL-1 (α and β) and TNFα [20–22], suggesting regulatory roles of IL-4 on human PBM. These findings raise the question of whether IL-4 up- or down-regulate mature human macrophages to produce monokines. Little is known about the effect of IL-4 on the functions of human AM. The expressions of the monokines IL-1α, IL-1β and TNFα appear to be differentially regulated in human AM and PBM. Human AM obtained from healthy donors have been found to produce more TNFα, but less IL-1β than autologous blood monocytes [6, 7]. We found previously that the cell-associated IL-1 activity was IL-1α [8]. In the present study IL-4 inhibited the production of both extra-cellularly-secreted and cell-associated IL-1 activities by activated human AM and PBM (table 3). Moreover, the present study clearly showed that at both protein and mRNA levels IL-4 down-regulated the production of IL-1α, IL-1β and TNFα by human AM in response to stimuli with LPS.

The inhibitory effect of IL-4 was not due to direct toxicity of IL-4 or some contaminant of the IL-4 preparation. Moreover, pretreatment of the recombinant human IL-4 preparation used with anti-IL-4 serum resulted in complete loss of its ability of the preparation to suppress monokine production by AM or PBM (table 2). Therefore, the inhibitory effect of IL-4 appears to be functional, and not due to a cytotoxic effect.

As in previous studies on TNFα and IL-1α [8, 9], we found that after prolonged incubation of PBM in medium with GM-CSF, the resulting monocyte-derived mature macrophages had the ability to secrete more TNFα, but less IL-1β than freshly isolated PBM (table 4). Moreover, IL-4 also suppressed the ability of these monocyte-derived macrophages to produce TNFα and IL-1β. These findings indicate the ability of cells of human monocyte-macrophage lineage to produce TNFα and IL-1β.

TNF and IL-1 are important immunoregulatory monokines [1–4]. These extracellular signalling proteins are involved in various pathological lung diseases. Indeed, a recent report showed that in experimental immune complex-induced alveolitis, pathologically relevant concentrations of TNF are elaborated within the lung during development of acute alveolitis, and are involved in injury of the lung [9]. TNF was also found to be involved in bleomycin-induced pulmonary fibrosis [11]. Modulation at a local or systemic level of the production of IL-1 and TNFα by activated AM or newly-migrated PBM on a local or systemic level may be important for the abrogation of inflammatory and/or immunological processes in the lung.

As the present findings showed that IL-4 suppressed abilities of human monocyte-macrophages to produce IL-1 and TNFα, which play important roles in various immunopathological states such as pulmonary granulomatous diseases, IL-4 may be therapeutically useful in these pathological states.

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References

1. Unanue ER. – The regulatory role of macrophages in antigenic stimulation. Symbiotic relationship between lymphocytes and macrophages. *Adv Immunol,* 1981; 31: 1–136.
2. Mizel SB. – Interleukin-1 and T-cell activation. *Immunol Rev,* 1982; 63: 51–72.
3. Beutler B, Cerami A. – Cachectin: more than a tumor necrosis factor. *N Engl J Med,* 1987; 317: 379–385.
4. Schollmeier K. – Immunologic and pathophysiologic

role of tumor necrosis factor. *Am J Respir Cell Mol Biol,* 1990; 3: 11–12.
5. Burchett SK, Weaver WM, Westall JA, Larsen A, Kronheim S, Wilson CB. – Regulation of tumor necrosis factor/cachectin and IL-1 secretion in human mononuclear phagocytes. *J Immunol,* 1988; 140: 3473–3481.
6. Wewers MD, Rennard SI, Hance AJ, Bitterman PB, Crystal RG. – Normal human alveolar macrophages obtained by bronchoalveolar lavage have a limited capacity to release interleukin-1. *J Clin Invest,* 1984; 74: 2208–2218.
7. Martinet Y, Yamauchi K, Crystal RG. – Differential expression of tumor necrosis factor/cachectin gene by blood and lung mononuclear phagocytes. *Am Rev Respir Dis,* 1988; 138: 659–665.
8. Sone S, Okubo A, Ogura T. – Normal human alveolar macrophages have more ability than blood monocytes to produce cell-associated interleukin-1-alpha. *Am J Respir Cell Mol Biol,* 1989; 1: 507–515.
9. Okubo A, Sone S, Tanaka M, Ogura T. – Membrane-associated interleukin-1α as a mediator of tumor cell killing by human blood monocytes fixed with paraformaldehyde. *Cancer Res,* 1989; 49: 265–270.
10. Kelley J. – Cytokines of the lung. *Am Rev Respir Dis,* 1990; 141: 765–788.
11. Warren JS, Yabroff KR, Remick DG, Kunkel SK, Chensue SW, Kunkel RG, Johnson KJ, Ward PA. – Tumor necrosis factor participates in the pathogenesis of acute immune complex alveolitis in the rat. *J Clin Invest,* 1989; 84: 1873–1882.
12. Kobayashi K, Allred C, Cohen S, Yoshida T. – Role of interleukin-1 in experimental pulmonary granuloma in mice. *J Immunol,* 1985; 1234: 358–364.
13. Piguet PF, Collart MA, Grau GE, Kapanci Y, Vassalli P. – Tumor necrosis factor/chachectin plays a key role in bleomycin-induced pneumopathy and fibrosis. *J Exp Med,* 1989; 170: 655–663.
14. Oliver KR, Noelle J, Urh JW, Kramer PH, Vitetta ES. – B-cell growth factor (B-cell growth factor 1 or B-cell stimulating factor, provisional 1) is a differentiation factor for resting B-cells and may not induce cell growth. *Proc Natl Acad Sci USA,* 1985; 82: 2465–2467.
15. Howard M, Farrar J, Hifiker M, Johnson B, Takatsu K, Hamaoka T, Paul WE. – Identification of a T-cell-derived B-cell growth factor distinct from interleukin-2. *J Exp Med,* 1982; 155: 914–923.
16. Park LS, Friend D, Sassenfeld HM, Urdal DL. – Characterization of the human B-cell stimulatory factor 1 receptor. *J Exp Med,* 1987; 166: 476–485.
17. Nagler A, Lanier LL, Phillips JH. – The effects of IL-4 on human natural killer cells. A potent regulator of IL-2 activation and proliferation. *J Immunol,* 1988; 141: 2349–2351.
18. Kawakami Y, Custer MC, Rosenberg SA, Lotze MT. – IL-4 regulates IL-2 induction of lymphokine-activated killer activity from human lymphocytes. *J Immunol,* 1989; 142: 3452–3461.
19. Mitchell LC, Davis LS, Lipsky PE. – Promotion of human T-lymphocyte proliferation by IL-4. *J Immunol,* 1989; 142: 1548–1557.
20. Grawford RM, Finbloom DS, Ohara J, Paul WE, Meltzer MS. – B-cell stimulatory factor-1 (interleukin-4) activates macrophages for increased tumoricidal activity and expression of Ia antigens. *J Immunol,* 1987; 139: 135–141.
21. Zlotnik A, Fischer M, Roehm N, Zipori D. – Evidence for effects of interleukin 4 (B-cell stimulatory factor 1) on macrophages: enhancement of antigen presenting ability of bone marrow-derived macrophages. *J Immunol,* 1987; 138: 4275–4279.
22. Weiss L, Haeffnet-Cavaillon N, Laude M, Cavaillon J, Kazatchkine MD. – Human T-cells and interleukin-4 inhibit the release of interleukin-1 induced by lipopolysaccharide in serum-free cultures of autologous monocytes. *Eur J Immunol,* 1989; 16: 1347–1350.
23. Hart PH, Fitti GF Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. – Potential anti-inflammatory effects of interleukin-4: suppression of human monocyte tumor necrosis factor-α, interleukin-1, and prostaglandin E_2. *Proc Natl Acad Sci USA,* 1989; 86: 3803–3807.
24. Donnelly RP, Fenton MJ, Finbloom DS, Gerrard TL. – Differential regulation of IL-1 production in human monocytes by IFN-γ and IL-4. *J Immunol,* 1990; 145: 569–575.
25. Essner R, Rhoades K, McBride WH, Morton DL, Economou JS. – IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J Immunol,* 1989; 142: 3857–3861.
26. Nishioka Y, Sone S, Okubo A, Ogura T. – Suppression by interleukin-4 of activation of human blood monocytes to the tumoricidal state. *Jpn J Cancer Res,* 1990; 81: 936–941.
27. TeVelde AJ, Klomp JP, Yard BA, De Vries JE, Figdor CG. – Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. *J Immunol,* 1988; 140: 1548–1554.
28. Wieser M, Bonifer MW, Oster W, Lindemann A, Mertelsman R, Herrman F. – Interleukin-4 induces secretion of CSF for granulocytes and CSF for macrophages by peripheral blood monocytes. *Blood,* 1989; 73: 1105–1108.
29. Vercelli D, Jabara HH, Lee B-W, Woodland N, Geha RS, Leun DYM. – Human recombinant interleukin-4 induces Fc R2/CD23 on normal human monocytes. *J Exp Med,* 1988; 167: 1406–1416.
30. Jansen JH, Wientjens GHM, Fibbe W, Willemze R, Kluin-Nelemans HC. – Inhibition of human macrophage colony formation by interleukin-4. *J Exp Med,* 1989; 170: 577–582.
31. Littman BH, Dastvan FR, Carlson PL, Sanders KM. – Regulation of monocyte/macrophage C2 production and HLA-DR expression by IL-4 (BSF-1) and IFN-γ. *J Immunol,* 1989; 142: 520–525.
32. Lehn M, Weiser WY, Engelhorn S, Gillis S, Remord HZ. – IL-4 inhibits H_2O_2 production and antileishmanial capacity of human cultured monocytes mediated by IFN-γ. *J Immunol,* 1989; 143: 3020–3024.
33. Finkelman FD, Katona IM, Urban JF, Homes J, Ohara J, Tung AS, Sample JVG, Paul WE. – IL-4 is required to generate and sustain *in vivo* IgE responses. *J Immunol,* 1988; 141: 2335–2341.
34. Chirgwin JM, Przybala AE, McDonald RJ, Rutter WJ. – Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry,* 1979; 18: 5294–5299.
35. Kumatori A, Tanaka K, Inamura N, Sone S, Ogura T, Matsumoto T, Tachikawa T, Shin S, Ichihara A. – Abnormally high expression of proteasomes in human leukemic cells. *Proc Natl Acad Sci USA,* 1990; 87: 7071–7075.
36. Wolpe SD, Davatelis G, Luedke C, Cerami A. – Differential regulation of cachectin/TNF and IL-1 by IL-4 (BSF-1). *J Leukocyte Biol,* 1987; 42: 359 (Abstract).
37. Somers SD, Erickson KL. – Comparison of macrophage activation with interleukin-4 produced by a T-cell hybridoma to gamma interferon. *Proc Am Assoc Cancer Res,* 1988; 29: 368 (Abstract).