

## Selective enhancement of GM-CSF, TNF- $\alpha$ , IL-1 $\beta$ and IL-8 production by monocytes and macrophages of asthmatic subjects

M.P. Hallsworth, C.P.C. Soh, S.J. Lane, J.P. Arm, T.H. Lee

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**ABSTRACT:** Previous work has demonstrated an increase in the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) by monocytes derived from asthmatic individuals. We have suggested that monocytes and macrophages enhance airways inflammation by augmented cytokine production.

We tested this hypothesis by measuring the production of GM-CSF and macrophage-derived cytokines, namely interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-8 (IL-8), from unstimulated and lipopolysaccharide (LPS)-stimulated peripheral blood monocytes and alveolar macrophages in 31 asthmatic and 11 normal, control subjects.

The basal production of GM-CSF was four fold higher in the monocytes of asthmatic individuals, but there was no significant difference in the basal production of TNF- $\alpha$ , IL-1 $\beta$  and IL-8. After stimulation with LPS, asthmatic monocytes produced twofold more GM-CSF and fourfold more IL-1 $\beta$  than the monocytes from control subjects. Unstimulated macrophages from asthmatic subjects produced significantly less GM-CSF and TNF- $\alpha$  than macrophages from controls, and there was no difference in either IL-1 $\beta$  or IL-8 production. When stimulated by LPS, macrophages from asthmatic subjects produced twofold more GM-CSF, threefold more TNF- $\alpha$  and fourfold more IL-8. The levels of IL-8 produced by both monocytes and macrophages were at least 20 fold higher than those of the other cytokines measured.

There is selectivity in the upregulation of cytokine production by monocytes and macrophages in asthma. The putative role of different cytokines secreted by the mononuclear phagocyte in the pathogenesis of airways inflammation depends both on the relative numbers of monocytes and macrophages and on their state of stimulation.

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Dept of Allergy and Allied Respiratory Disorders, UMDS, Guy's Hospital, London, UK.

Correspondence: T.H. Lee  
Dept of Allergy & Allied Respiratory Disorders  
4th Floor Hunt's House  
Guy's Hospital  
London SE1 9RT  
UK

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Increasing evidence implicates cells of the monocyte-macrophage lineage in the mechanisms of asthma. Increased numbers of circulating monocytes expressing the low affinity immunoglobulin E (IgE) receptor (Fc $\epsilon$ R2) have been observed in allergic disorders, such as asthma [1]. After allergen challenge, an increased recruitment of monocytes has been observed in the bronchoalveolar lavage (BAL) fluid of asthmatic subjects [2], and monocytes from asthmatic patients have been shown to have increased expression of complement receptors [3]. Macrophages also express the low affinity IgE receptor and can be stimulated to release mediators by allergen [4, 5]. Immunohistochemical studies of bronchial biopsies from asthmatic patients have demonstrated an increased infiltration of macrophages into the airways, compared to normal individuals [6]. Many of these macrophages had the characteristics of blood monocytes, suggesting the active

recruitment of monocytes into the airways from the circulation.

Since the pathology of bronchial asthma demonstrates a multicellular process [6, 7], with significant infiltration of the airways by activated T-cells, eosinophils and macrophages, attempts to understand the pathogenesis of asthma should account for the mechanisms of cellular interactions. We have suggested that cytokines derived from macrophages may act as the messengers for cellular communication and that they are increased in asthma. We have shown that the major activity released from peripheral blood monocytes and macrophages, with the capacity to augment eosinophilic survival and function, is granulocyte-macrophage colony-stimulating factor (GM-CSF) [8, 9]. GM-CSF enhances the survival of the eosinophils in culture and primes these cells for subsequent mediator generation [10]. It promotes the

maturation and differentiation of granulocytes and macrophages from bone marrow progenitors [11]. It facilitates antigen presentation by the increase in the expression of class II molecules [12]. Thus, GM-CSF may play a critical role in the amplification of the inflammatory response in asthmatic airways.

It is not known whether the upregulation of GM-CSF is part of a more general increase in cytokine production by macrophages or whether it is selective for GM-CSF alone. We have, therefore, extended our previous experiments to examine the concentrations of GM-CSF as well as a number of other macrophage-derived cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-8 (IL-8), which may play a role in the mechanisms of the airways inflammation characteristic of bronchial asthma.

## Methods

### Subjects

*Peripheral blood mononuclear cells.* Eleven normal subjects (8 males and 3 females; mean $\pm$ SEM age 23 $\pm$ 1 yrs; 6 atopic; 2 smokers) and 31 patients with bronchial asthma (19 males and 12 females; mean $\pm$ SEM age 41 $\pm$ 3 yrs; 29 atopic; 7 smokers) were studied. The resting forced expiratory volume in one second (FEV<sub>1</sub>) for the asthmatic subjects was 78 $\pm$ 3% predicted values.

*Alveolar macrophages.* Nine normal subjects (6 males and 3 females; mean $\pm$ SEM age 23 $\pm$ 1 yrs; 5 atopic; 2 smokers) and 8 patients with bronchial asthma (5 males and 3 females; mean $\pm$ SEM age 23 $\pm$ 1 yrs; 7 atopic; 1 smoker) were studied. The resting FEV<sub>1</sub> for the asthmatic subjects was 91 $\pm$ 6% predicted values.

In some cases, peripheral blood mononuclear cells and alveolar macrophages were isolated from the same normal (n=9) and asthmatic (n=8) donors to determine if there was a relationship between the state of activation of the monocytes and macrophages in an individual.

Bronchial asthma was diagnosed on the basis of a history of episodic wheezing and 20% reversibility of airways obstruction after inhalation of 400  $\mu$ g salbutamol via a metered dose inhaler. Atopy was defined as the presence of a positive skin-prick test reaction for one of the common aeroallergens: grass pollen, silver birch pollen, cat fur, and *Dermatophagoides pteronyssinus*. The asthmatic subjects were only using salbutamol on an as-needed basis and were not taking any other medication. Each subject gave informed consent and the study was approved by the local Ethics committee.

### Preparation of monocyte-derived culture supernatants

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by centrifugation on Lymphoprep (Nycomed UK Ltd, Birmingham, UK) as described by BOYUM [13]. The cells were washed twice in Hank's

Balanced Salt Solution, without calcium and magnesium (HBSS) (Gibco, Paisley, UK). The cells were counted and assessed for viability in trypan blue; viability always exceeded 95%. PBMC were suspended at a concentration of 2 $\times$ 10<sup>6</sup> cells·ml<sup>-1</sup> in RPMI 1640 supplemented with 25 mM hydroxyethylpiperazine ethanesulphonic acid (HEPES) (Gibco), 2 mM L-glutamine (Gibco), penicillin and streptomycin (100 U·ml<sup>-1</sup> and 100  $\mu$ g·ml<sup>-1</sup>, respectively, Gibco) and 10% foetal bovine serum (FBS) (supplemented RPMI). Aliquots of the cell suspension (4 ml) were plated onto 60 mm plastic tissue culture dishes (Falcon, Marathon Laboratory Supplies, London, UK) and were incubated for 2 h at 37°C in 5% CO<sub>2</sub>. The culture medium was then removed and the plates were washed twice with supplemented RPMI (without FBS); the nonadherent cells were collected and counted. The adherent cells were >90% monocytes, as determined by immunostaining with anti-CD14. The remaining cells consisted of T-cells, B-cells and natural killer (NK) cells. The cells were cultured in supplemented RPMI (2 ml) for 24 h in the absence or presence of LPS (5  $\mu$ g·ml<sup>-1</sup>, Sigma Chemical Co. Ltd Poole, Dorset, UK). After 24 h the medium was aspirated, centrifuged at 400 $\times$ g for 10 min and the cell-free supernatants were stored at -20°C.

### Bronchoalveolar lavage (BAL)

Subjects had taken no medication for at least 12 h. Bronchoscopy was performed with a flexible fiberoptic instrument (Olympus BF-B3, London, UK) 15 min after premedication with atropine (600  $\mu$ g *i.v.*) and local anaesthesia with lignocaine (2 and 4%). BAL was performed, as described previously [14, 15], by instillation of 50 ml aliquots of 0.9% saline at 37°C into a subsegmental bronchus of the right middle lobe. Each instillation was followed by gentle aspiration, a maximum of 300 ml being instilled.

### Preparation of alveolar macrophage-derived culture supernatants

BAL fluid was centrifuged at 300 $\times$ g for 10 min at 4°C and the cell pellet was washed twice in HBSS as described above. The cells consisted of 73.4 $\pm$ 3.1% macrophages, 23.7 $\pm$ 4.3% mononuclear cells, 0.3 $\pm$ 0.2% polymorphonucleocytes (PMN), 0.2 $\pm$ 0.1% eosinophils, 2.2 $\pm$ 1.0% epithelial cells. Cells were suspended at a concentration of 1 $\times$ 10<sup>6</sup> cells·ml<sup>-1</sup> in supplemented RPMI. Aliquots of the cell suspension (3 ml) were plated onto 60 mm plastic tissue culture dishes and were incubated for 2 h at 37°C in 5% CO<sub>2</sub>. The culture medium was then removed and the plates were washed twice with supplemented RPMI (without FBS); the nonadherent cells were collected and counted. The adherent cells were >95% macrophages, and were cultured in supplemented RPMI (3 ml) for 24 h in the absence or presence of LPS (5  $\mu$ g·ml<sup>-1</sup>) under optimal conditions, which we had defined previously. After 24 h the medium was aspirated, centrifuged

at 400×g for 10 min and the cell-free supernatant was stored at -20°C.

### Cytokine measurements

The levels of GM-CSF, TNF- $\alpha$ , IL-1 $\beta$  and IL-8 were measured in the same macrophage and PBMC supernatants with commercially available enzyme-linked immunosorbent assay (ELISA) kits (British Biotechnology Products Ltd, Abingdon, Oxon, UK). The manufacturer's instructions were followed throughout. The lower limits of sensitivity for each of the assays were: GM-CSF 1.5 pg·ml<sup>-1</sup>; TNF $\alpha$  4.8 pg·ml<sup>-1</sup>; IL-1 $\beta$  0.3 pg·ml<sup>-1</sup>; and IL-8 4.7 pg·ml<sup>-1</sup>. Cytokine concentrations were expressed as ng·10<sup>-6</sup> cells (final cell numbers).

### Statistical analyses

The data were analysed using the Mann-Whitney U-test and Spearman's rank correlation. A p-value of <0.05 was considered to be significant and ns denotes that the p-value was not significant at 5% level. All analyses were carried out using the Minitab statistical software package. Data are expressed as median values with >95%

confidence intervals (CI) and are presented in the form of a "box and whisker plot", where the Y-axis displays the spread of the data. The shaded box represents the interquartile range, and the solid line across the box marks the median value. The "whiskers" extending from the top and bottom of the box represent the range of the data. All plots were generated using the Kaleidagraph data analysis program for the Apple Macintosh computer.

## Results

### Cytokine generation by peripheral blood monocytes

In the absence of LPS, the PBMC from asthmatic individuals produced significantly more GM-CSF ( $p<0.05$ ) (fig. 1a and table 1) than those of control subjects. There were no statistically significant differences between the two groups in either TNF- $\alpha$  ( $p<0.06$ ) (fig. 1b), IL-1 $\beta$  ( $p<0.17$ ) (fig. 1c) or IL-8 ( $p<0.12$ ) (fig. 1d) production by unstimulated PBMC. Table 1 shows the levels of cytokine generation by PBMC in the absence of LPS.

In the presence of LPS, the PBMC from asthmatic individuals produced significantly more GM-CSF ( $p<0.04$ )

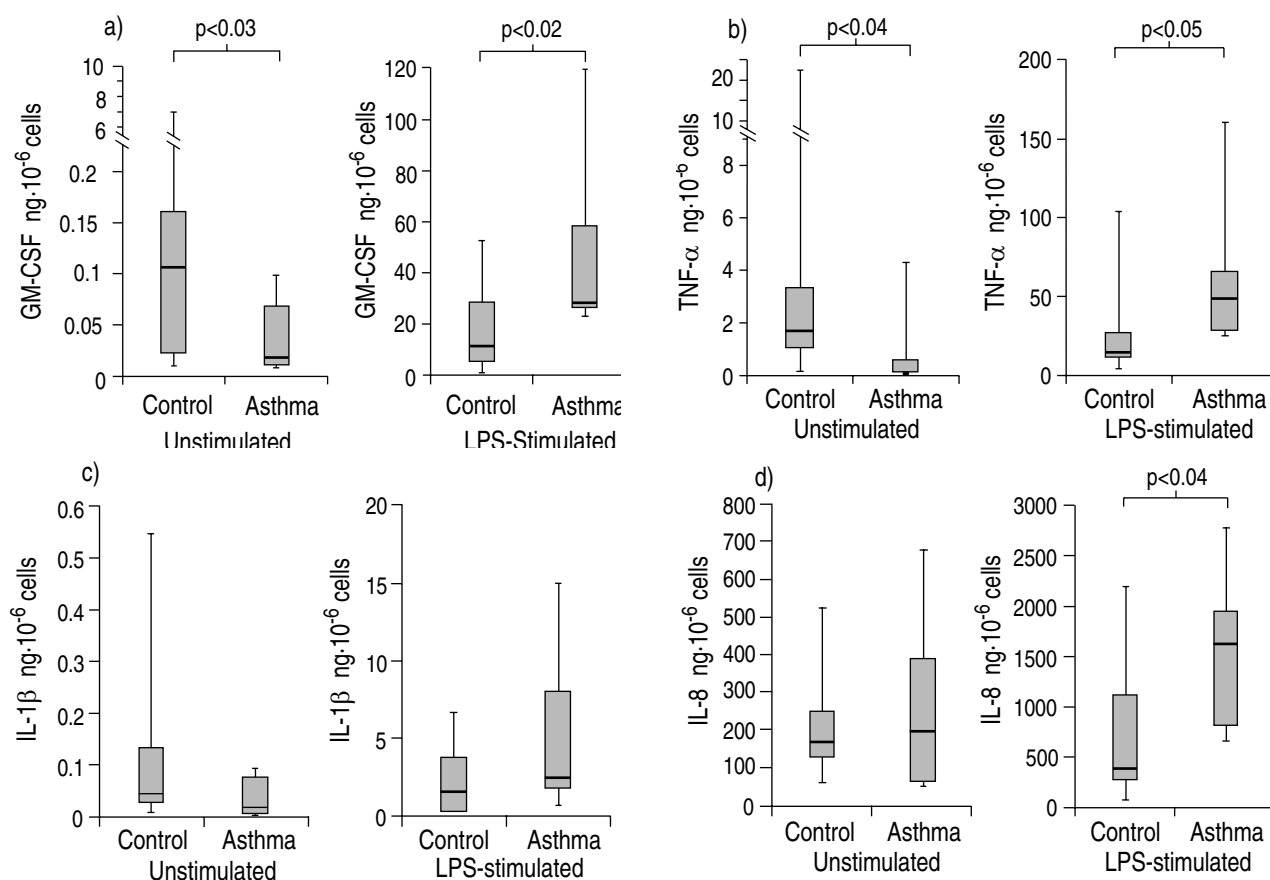


Fig. 1. — The generation of: a) GM-CSF; b) TNF- $\alpha$ ; c) IL-1 $\beta$ ; and d) IL-8 by PBMC, in the absence and presence of LPS; comparison between control and asthmatic groups. Data are presented in the form of a "box and whisker plot", where the Y-axis displays the spread of the data. The shaded box represents the interquartile range and the solid line across the box marks the median value. The "whiskers" extending from the top and bottom of the box represent the data. GM-CSF: granulocyte-macrophage colony-stimulating factor; TNF- $\alpha$ : tumour necrosis factor- $\alpha$ ; IL-1 $\beta$ : interleukin-1 $\beta$ ; IL-8: interleukin-8; PBMC: peripheral blood mononuclear cells; LPS: lipopolysaccharide.

Table 1. – Cytokine generation by peripheral blood monocytes in the absence of LPS

Cytokine	Control group n=11	Asthmatic group n=31	p-value
GM-CSF ng·10 <sup>-6</sup> cells	0.003 (0.001–0.293)	0.014 (0.004–0.326)	<0.05*
TNF- $\alpha$ ng·10 <sup>-6</sup> cells	0.028 (0.004–0.379)	0.106 (0.023–0.596)	<0.06
IL-1 $\beta$ ng·10 <sup>-6</sup> cells	0.009 (0.001–1.148)	0.078 (0.011–0.515)	<0.17
IL-8 ng·10 <sup>-6</sup> cells	32.5 (6.25–155.0)	110.0 (35.0–325.0)	<0.12

Data are presented as median, and 95% confidence interval in parenthesis. GM-CSF: granulocyte-macrophage colony-stimulating factor; TNF- $\alpha$ : tumour necrosis factor- $\alpha$ ; IL-1 $\beta$ : interleukin-1 $\beta$ ; IL-8: interleukin-8; LPS: lipopolysaccharide. \*: p-value <0.05 considered significant.

(fig. 1a and table 2) and significantly more IL-1 $\beta$  ( $p<0.03$ ) (fig. 1c) than those of control subjects. There were no significant differences between the two groups in either TNF- $\alpha$  ( $p<0.71$ ) (fig. 1b) or IL-8 ( $p<0.57$ ) (fig. 1d) production by LPS-stimulated PBMC. Table 2 shows the levels of cytokines generated by PBMC in the presence of LPS.

#### Cytokine generation by macrophages

In the absence of LPS, the macrophages from the control subjects produced significantly higher levels of GM-CSF ( $p<0.03$ ) (fig. 2a and table 3) and TNF- $\alpha$  ( $p<0.04$ ) (fig. 2b) than those from asthmatic individuals. No significant differences were observed between the two groups for IL-1 $\beta$  ( $p<0.15$ ) (fig. 2c) and IL-8 ( $p<1.0$ ) (fig. 2d). Table 3 shows the levels of cytokines generated by macrophages in the absence of LPS.

In the presence of LPS, the macrophages of asthmatic individuals produced significantly more GM-CSF ( $p<0.02$ ) (fig. 2a and table 4), TNF- $\alpha$  ( $p<0.05$ ) (fig. 2b) and IL-8 ( $p<0.04$ ) (fig. 2d) than those of control subjects. There

Table 2. – Cytokine generation by peripheral blood monocytes in the presence of LPS

Cytokine	Control group n=11	Asthmatic group n=31	p-value
GM-CSF ng·10 <sup>-6</sup> cells	0.98 (0.31–3.19)	2.37 (1.42–3.92)	<0.04*
TNF- $\alpha$ ng·10 <sup>-6</sup> cells	3.27 (1.90–6.65)	4.17 (2.71–7.50)	<0.71
IL-1 $\beta$ ng·10 <sup>-6</sup> cells	1.41 (0.60–9.46)	6.37 (4.13–9.79)	<0.03*
IL-8 ng·10 <sup>-6</sup> cells	193.1 (96.3–805.0)	311.0 (191.3–361.2)	<0.57

Data are presented as median, with 95% confidence interval in parenthesis. For abbreviations see legend to table 1. \*: p-value <0.05 considered significant.

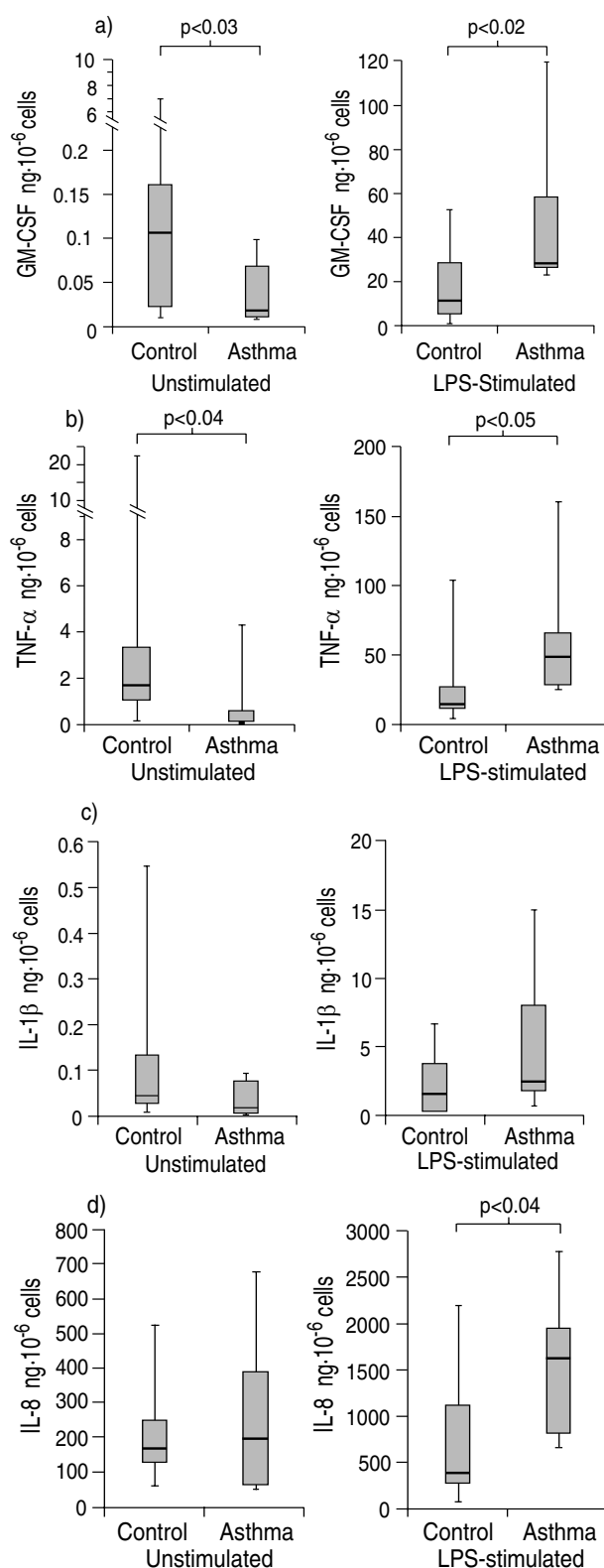


Fig. 2. – The generation of: a) GM-CSF; b) TNF- $\alpha$ ; c) IL-1 $\beta$ ; and d) IL-8 by alveolar macrophages, in the absence and presence of LPS; comparison between control and asthmatic groups. Data are presented in the form of a "box and whisker plot", where the Y-axis displays the spread of the data. The shaded box represents the interquartile range and the solid line across the box marks the median value. The "whiskers" extending from the top and bottom of the box represent the range of the data. For abbreviations see legend to figure 1.

Table 3. – Cytokine generation by macrophages in the absence of LPS

Cytokine	Control group n=9	Asthmatic group n=8	p-value
GM-CSF ng·10 <sup>-6</sup> cells	0.12 (0.023–0.202)	0.018 (0.009–0.098)	<0.03*
TNF- $\alpha$ ng·10 <sup>-6</sup> cells	1.72 (0.25–8.17)	0.15 (0.057–4.328)	<0.04*
IL-1 $\beta$ ng·10 <sup>-6</sup> cells	0.045 (0.011–0.148)	0.017 (0.002–0.093)	<0.15
IL-8 ng·10 <sup>-6</sup> cells	169 (116–259)	193 (54–683)	<1.0

Data are presented as median, with 95% confidence interval in parenthesis. For abbreviations see legend to table 1. \*: p-value <0.05 considered significant.

Table 4. – Cytokine generation by macrophages in the presence of LPS

Cytokine	Control group n=9	Asthmatic group n=8	p-value
GM-CSF ng·10 <sup>-6</sup> cells	10.9 (5.5–28.3)	27.5 (23.0–119.1)	<0.02*
TNF- $\alpha$ ng·10 <sup>-6</sup> cells	15.1 (7.1–67.2)	49.1 (25.2–161.3)	<0.05*
IL-1 $\beta$ ng·10 <sup>-6</sup> cells	1.56 (0.34–5.89)	2.44 (0.64–15.01)	<0.27
IL-8 ng·10 <sup>-6</sup> cells	377 (190–1848)	1630 (667–2789)	<0.04*

Data are presented as median, with 95% confidence interval in parenthesis. For abbreviations see legend to table 1. \*: p-value <0.05 considered significant.

was no difference in IL-1 $\beta$  (p<0.27) (fig. 2c) produced by the two groups. Table 4 shows the levels of cytokines generated by macrophages in the presence of LPS.

#### Comparison of monocyte and macrophage cytokine generation

Unstimulated macrophages from the control subjects produced significantly more GM-CSF (p<0.02) and TNF- $\alpha$  (p<0.001) than unstimulated monocytes from the same group, and, after LPS stimulation, these macrophages produced significantly more GM-CSF (p<0.001) and TNF- $\alpha$  (p<0.0002) than the corresponding monocytes. In contrast, the macrophages from asthmatic individuals produced basal levels of GM-CSF and TNF- $\alpha$  similar to those of the monocytes; however, these macrophages were able to produce more GM-CSF (p<0.0001) and more TNF- $\alpha$  (p<0.0001) than the monocytes, following LPS stimulation. No significant differences were observed in either IL-1 $\beta$  or IL-8 production between monocytes and macrophages.

There was poor correlation between the amounts of each cytokine produced by monocytes and their respective macrophages, indicating that there was no relationship between the state of activation of monocytes and macrophages in either the control or asthmatic group.

The correlation coefficient "r" for the four cytokines are as follows: GM-CSF control group r=-0.079 (NS), asthmatic group r=0.084 (NS); TNF- $\alpha$  control r=-0.23 (NS), asthmatic r=0.29 (NS); IL-1 $\beta$  control r=-0.051 (NS), asthmatic r=0.006 (NS); IL-8 control r=0.35 (NS), asthmatic r=0.3 (NS).

## Discussion

We have shown that unstimulated monocytes from asthmatic subjects produce more GM-CSF than those from nonasthmatic subjects, with no significant difference in the production of TNF- $\alpha$ , IL-1 $\beta$  and IL-8. The increased production of GM-CSF by monocytes from asthmatic patients is consistent with previous findings [9]. Although there was no significant difference between asthmatic and normal subjects in the production of IL-8 by peripheral blood monocytes, the quantities of IL-8 produced were substantially greater than those of the other three cytokines detected. The levels of IL-8 measured were similar to those reported by other workers [16, 17]. We have measured cytokines in this study using commercially available ELISA techniques. The use of *in situ* hybridization to detect messenger ribonucleic acid (mRNA) would have provided additional information, but the presence of mRNA does not necessarily equate to secreted product.

Following stimulation of peripheral blood monocytes by LPS, there was a significant increase in the production of each of the cytokines measured. LPS was used in these studies to provide a global nonspecific activation signal, in an attempt to evaluate the full potential of the cells. LPS-activated monocytes from asthmatic subjects secreted more GM-CSF and IL-1 $\beta$  than those from normal individuals, whilst TNF- $\alpha$  and IL-8 were produced in similar quantities by both groups of subjects. If the elevated expression of certain cytokines by monocytes from asthmatic subjects is due to *in vivo* priming, then the priming mechanism may be selective for certain cytokines.

In the absence of LPS stimulation, macrophages from asthmatic subjects produced significantly lower levels of GM-CSF and TNF- $\alpha$  compared with the nonasthmatic individuals. Furthermore, the quantities of GM-CSF and TNF- $\alpha$  generated by macrophages and monocytes from asthmatic patients are similar, in contrast to those of normal subjects, where the production of these cytokines by macrophages is substantially higher than by monocytes. This implies that macrophages recovered from asthmatic subjects may be functionally different. This suggestion is supported by the data of POSTON *et al.* [6] who showed that macrophages infiltrating the airways in asthma are phenotypically different from those in normal airways. We found no statistical difference between monocyte and macrophage IL-1 $\beta$  production in the control group, in contrast to the findings of WEWERS *et al.* [18], who measured total IL-1 by bioassay.

In contrast to the data from unstimulated macrophages, LPS-activated macrophages from asthmatic subjects produced greater amounts of GM-CSF, TNF- $\alpha$  and IL-8

compared to cells from normal individuals. This suggests that macrophages recovered from asthmatic airways may already have been primed *in vivo* and have the potential for enhanced cytokine production.

Enhanced production of IL-1, TNF- $\alpha$  and IL-8 may contribute to airways inflammation. Interleukin-1 induces the proliferation and activation of T cells [19]. It increases expression of adhesion molecules on endothelial cells [20], and may play a critical role in initiating the leukocyte infiltration into asthmatic airways. IL-1 upregulates GM-CSF production in human bronchial cells [21], and may be critical in enhancing GM-CSF expression in asthmatic airway epithelium. TNF- $\alpha$  also increases endothelial cell adhesion molecule expression [22] and, in addition to cytotoxic actions [23], it increases production of inflammatory mediators, such as GM-CSF, prostaglandins and platelet-activating factor [24–26]. The recent finding that TNF- $\alpha$  secretion is increased in the allergen-induced late phase asthmatic response [27] supports the possible role of this cytokine in airways inflammation. IL-8 is a potent neutrophil- [28], and eosinophil-activating chemotactic peptide [29], and its expression can be stimulated by TNF- $\alpha$  and IL-1 $\beta$  [30]. This molecule is relatively resistant to proteases [28] compared to other cytokines, and may be involved in more prolonged inflammatory actions.

These experiments support the view that there is an increase in the production of cytokines by monocytes and macrophages of asthmatic individuals compared with those of normal subjects. The data presented here show a wide distribution of cytokine levels within each group; consequently, whilst there may appear to be differences between the groups, only large differences are statistically significant. There does, however, seem to be a degree of selectivity in the upregulation of cytokine production which is dependent not only on the maturity of the cell, but also on its state of activation. *In vivo*, monocytes and macrophages are exposed to a wide variety of cytokines and other mediators, which may prime the cells for enhanced function. The cytokine network in inflammation is complex and prediction of its effects cannot be based on the action of one cytokine; therefore, caution needs to be exercised in the interpretation of data based on the analysis of a single cytokine when considering the mechanisms of bronchial asthma.

## References

- Melewicz FM, Zeiger RS, Mellon MH, O'Connor RD, Spiegelberg HL. Increased peripheral blood monocytes with Fc receptors for IgE in patients with severe allergic disorders. *J Immunol* 1981; 126: 1592–1595.
- Metzger WJ, Zavala D, Richerson HB, *et al.* Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. Description of the model and local airway inflammation. *Am Rev Respir Dis* 1987; 135: 433–440.
- Carroll MP, Durham SR, Walsh GM, Kay AB. Activation of neutrophils and monocytes after allergen- and histamine-induced bronchoconstriction. *J Allergy Clin Immunol* 1985; 75: 290–296.
- Rankin JA, Hitchcock M, Merrill W, Bach MK, Brashler JR, Askenase PW. IgE-dependent release of leukotriene C<sub>4</sub> from alveolar macrophages. *Nature* 1982; 297: 329–331.
- Joseph M, Tonnel AB, Torpier G, Capron A, Arnoux B, Benveniste J. Involvement of immunoglobulin E in the secretory processes of alveolar macrophages from asthmatic patients. *J Clin Invest* 1983; 71: 221–230.
- Poston RN, Chanez P, Lacoste JY, Litchfield T, Lee TH, Bousquet J. Immunohistochemical characterization of the cellular infiltration in asthmatic bronchi. *Am Rev Respir Dis* 1992; 145: 918–921.
- Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139: 806–817.
- Howell CJ, Pujol J-L, Crea AEG, *et al.* Identification of an alveolar macrophage-derived activity in bronchial asthma that enhances leukotriene C<sub>4</sub> generation by human eosinophils stimulated by ionophore A23187 as a granulocyte-macrophage colony-stimulating factor. *Am Rev Respir Dis* 1989; 140: 1340–1347.
- Burke LA, Hallsworth MP, Litchfield TM, Davidson R, Lee TH. Identification of the major activity derived from cultured human peripheral blood mononuclear cells, which enhances eosinophil viability, as granulocyte-macrophage colony-stimulating factor. *J Allergy Clin Immunol* 1991; 88: 226–235.
- Owen WF, Rothenberg ME, Silberstein DS, Gasson JC, Stevens RL, Austen KF. Regulation of human eosinophil viability, density and function by granulocyte-macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987; 166: 129–141.
- Metcalf D. The granulocyte-macrophage colony-stimulating factors. *Science* 1985; 229: 16–22.
- Alvaro-Gracia JM, Zvaifler NJ, Firestein GS. Cytokines in chronic inflammatory arthritis. IV. Granulocyte-macrophage colony-stimulating factor-mediated induction of Class II MHC antigen on human monocytes, a possible role in rheumatoid arthritis. *J Exp Med* 1989; 170: 865–875.
- Boyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968; 21 (Suppl. 97): 7.
- Godard P, Clot J, Jonquet O, Bousquet J, Michel FB. Lymphocyte subpopulations in bronchoalveolar lavage of patients with sarcoidosis and hypersensitivity pneumonitis. *Chest* 1981; 80: 447–451.
- Godard P, Aubas P, Calvayrac P, Taib J, Michel FB. Endoscopie et lavage bronchiolo alveolaire chez l'asthmatique allergique. *Nouv Presse Med* 1981; 10: 3141–3148.
- Standiford TJ, Junkel SL, Rolfe MW, Evanoff HL, Allen RM, Strieter RM. Regulation of human alveolar macrophage- and blood monocyte-derived interleukin-8 by prostaglandin E<sub>2</sub> and dexamethasone. *Am J Respir Cell Mol Biol* 1992; 6: 75–81.
- Sylvester I, Rankin JA, Yoshimura T, Tanaka S, Leonard EJ. Secretion of neutrophil attractant/activation protein by lipopolysaccharide-stimulated lung macrophages determined by both enzyme-linked immunosorbent assay and N-terminal sequence analysis. *Am Rev Respir Dis* 1990; 141: 683–688.
- Wewers MD, Rennard SI, Hance AJ, Bitterman PB, Crystal RG. Normal human macrophages obtained by bronchoalveolar lavage have a limited capacity to release interleukin-1. *J Clin Invest* 1984; 74: 2208–2218.

19. Mattoli S, Miente S, Calabro F, Mezzetti M, Fasoli A, Allegra L. Bronchial epithelial cells exposed to isocyanates potentiate activation and proliferation of T-cells. *Am J Physiol* 1990; 259: L320–L327.
20. Bevilacqua MP, Poher JS, Wheeler ME, Cotran RS, Gimbrone A Jr. Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes and related leukocyte cell lines. *J Clin Invest* 1985; 76: 2003–2009.
21. Marini M, Soloperto M, Mezzetti M, Fasoli A, Mattoli S. Interleukin-1 binds to specific receptors on human bronchial epithelial cells and upregulates granulocyte-macrophage colony-stimulating factor synthesis and release. *Am J Respir Cell Mol Biol* 1991; 4: 519–524.
22. Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci* 1985; 82: 8667–8671.
23. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumours. *Proc Natl Acad Sci USA* 1975; 72: 3666–3671.
24. Broudy VC, Kaushansky K, Segal GM, Harlan JM, Adamson JW. Tumor necrosis factor type- $\alpha$  stimulates human endothelial cells to produce granulocyte-macrophage colony-stimulating factor. *Proc Natl Acad Sci* 1986; 83: 7467–7471.
25. Elias JA, Gustilo K, Baeder W, Freundlich B. Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin-1 and tumor necrosis factor. *J Immunol* 1987; 138: 3812–3816.
26. Camussi G, Bussolino F, Salvidio G, Baglioni C. Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. *J Exp Med* 1987; 166: 1390–1404.
27. Gossett P, Tsicopoulos A, Wallaert B, *et al.* Increased secretion of tumor necrosis factor- $\alpha$  and interleukin-6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. *J Allergy Clin Immunol* 1991; 88: 561–571.
28. Peveri P, Walz A, Dewald B, Baggiolini M. A novel neutrophil-activating factor produced by human mononuclear phagocytes. *J Exp Med* 1988; 167: 1547–1559.
29. Warringa RAJ, Mengelers HJJ, Raaijmakers JAM, Bruijnzeel PLB, Koenderman L. Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J Allergy Clin Immunol* 1993; 91: 1198–1205.
30. Strieter RM, Chensue SW, Basha MA, *et al.* Human alveolar macrophage gene expression of interleukin-8 by tumor necrosis factor- $\alpha$ , lipopolysaccharide, and interleukin-1- $\beta$ . *Am J Respir Cell Mol Biol* 1990; 2: 321–326.