

Short communication

Alveolar macrophage interleukin (IL)-10 and IL-12 production in atopic asthma

Magnan A, van Pee D, Bongrand P, Vervloet D. Alveolar macrophage interleukin (IL)-10 and IL-12 production in atopic asthma. *Allergy* 1998; 53: 1092–1095. © Munksgaard 1998.

Inflammation in asthma is characterized by a Th2 response. In many experimental systems, this response can be regulated by interleukin (IL)-10 and IL-12. IL-10 deactivates T cells, and IL-12 reorients the response toward a Th1 pattern. Alveolar macrophages (AM) can secrete both of these cytokines, and thus regulate T-cell behavior in asthma. They can enhance the Th2 response by turning off their secretion of IL-10 and IL-12, or tend to downregulate it by producing these cytokines. To elucidate that point, we assayed the AM IL-10 and IL-12 from 11 asthmatic patients and four controls. Six asthmatics were treated by inhaled corticosteroids. AM were recovered by bronchoalveolar lavage (BAL). They were isolated and cultured for 24 h without stimulation or in the presence of lipopolysaccharide (LPS). IL-10 and the p40 subunit of IL-12 were assayed in the BAL fluid and in AM culture supernatants by ELISA. Spontaneous AM IL-10 production was higher in asthmatics, particularly in the treated group. The AM IL-10 production after stimulation by LPS was also elevated in asthmatics, but was mainly so in untreated patients. IL-12 levels were higher in BAL fluids from untreated patients than from controls. The IL-12 production of LPS-stimulated-AM from these patients was increased. These results show that AM are at least primed for the production of IL-10 and IL-12 in asthma, and suggest that these cells could be involved in the resolution of the asthmatic inflammation.

A. Magnan¹, D. van Pee¹, P. Bongrand¹, D. Vervloet²

¹Service de Pneumo-Allergologie, UPRES 2050 Groupe de Recherche Clinique "Pathologie respiratoire et cutanée liée à l'environnement", Centre d'Investigations Cliniques de l'Hôpital Ste Marguerite, Marseille; ²INSERM U 387, Marseille Cedex 09, France

Key words: allergy; alveolar macrophage; asthma; atopy; interleukin-10; interleukin-12; Th2.

Dr A. Magnan
Service de Pneumo-Allergologie
Hôpital Ste-Marguerite
270 Bd de Ste Marguerite
13274 Marseille Cedex 09
France

Accepted for publication 29 June 1998

The asthmatic inflammatory process is characterized by the activation of Th2 lymphocytes, which induce the synthesis of IgE via the secretion of interleukin (IL)-4, and stimulate the attraction and activation of eosinophils upon IL-5 production (1). Therefore, the mechanisms involved in the Th1/Th2 commitment of T cells are probably of major importance in the triggering of asthma. Among these, the actions of cytokines produced by non-T cells seem of major importance: IL-12, a cytokine produced by cells of the monocyte/macrophage lineage, is a powerful Th1 inducer (2), and IL-10, synthesized by B cells and also monocytes and macrophages, is known to deactivate T cells (3). Thus, both cytokines are expected to reduce asthmatic inflammation. In support of this hypothesis, it was shown in animal models of allergic airway inflammation that IL-10 and IL-12 inhibit bronchial and alveolar inflammation (4, 5).

Alveolar macrophages (AM) can produce these regulating cytokines, and were shown to be activated in asthma, producing notably IL-1 β , IL-6, and tumor necrosis factor- α (TNF- α) (6). Therefore, in order to approach the potential role of AM in the T-cell activation in asthma, we studied *ex vivo* the production of IL-10 and IL-12 by AM recovered from bronchoalveolar lavage (BAL) performed in asthmatics.

Material and methods

Patients

Eleven patients with atopic asthma, median age 52, range 22–78 years, were included in this study. Atopy was evaluated by skin prick tests and the presence of specific IgE to relevant allergens. All the asthmatic patients were nonsmokers and had

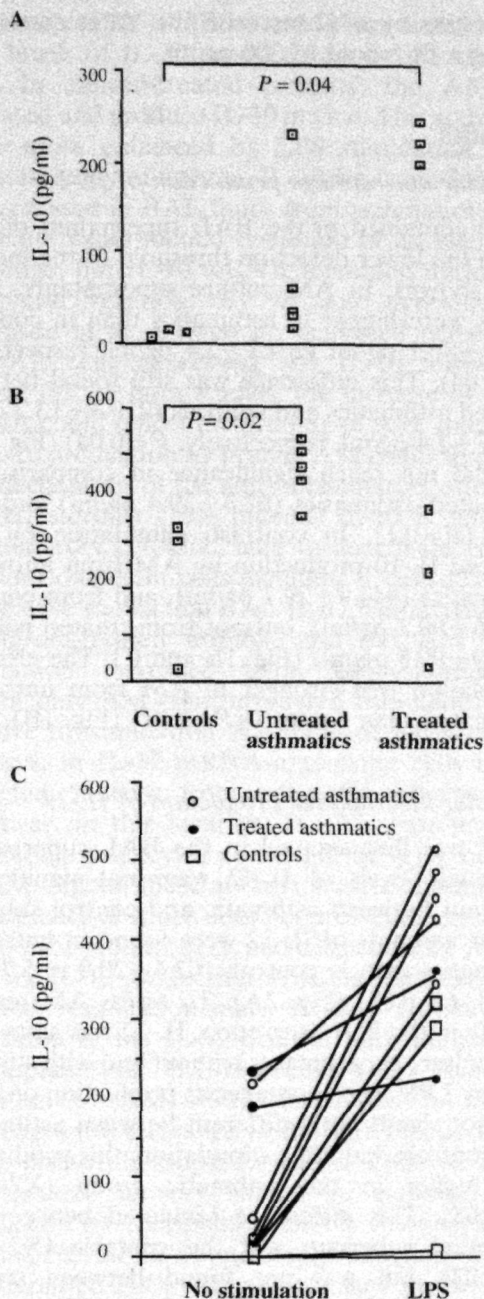


Fig. 1. Alveolar macrophage (AM) IL-10 production in asthma. AM were harvested by bronchoalveolar lavage (BAL) from untreated asthmatics ($n=5$), steroid-treated asthmatics ($n=3$), and control subjects ($n=3$). IL-10 was assayed by ELISA in 24-h culture supernatants of AM, without stimulation (A) or after stimulation with 20 $\mu\text{g/ml}$ of lipopolysaccharide (LPS) (B, C).

reversible airway obstruction characterized by 20% increase in FEV₁ after the inhalation of 200 μg of albuterol. Clinical severity was evaluated according to the Aas score, from score 1 for very mild forms to score 3 for a patient displaying prolonged periods with symptomatic bronchial obstruction (7). Eight patients displayed mild asthma; two,

grade 2 disease; and one, grade 3 asthma. Grade 2 and 3 asthmatics and two grade 1 patients required daily inhaled corticosteroids, whereas six were using only bronchodilators.

The control group comprised four nonasthmatic, nonatopic subjects referred to the asthma clinic for persistent cough. Their respiratory function was normal and they displayed a negative methacholine challenge. None of them were smokers.

Fiberoptic bronchoscopy and bronchoalveolar lavages

Fiberoptic bronchoscopy was performed when FEV₁ was more than 70% of the predicted value. A flexible bronchoscope was passed to a wedged position in the median lobe. Three aliquots of 50 ml of saline were introduced and gently aspirated. The recovery rate of BAL was 57 ± 5 ml on average, range 30–80 ml. The samples were immediately processed. An aliquot was used for cytologic examination, and the remainder was used for cytokine studies. In all cases, BAL contained more than 90% of macrophages.

Laboratory methods

Cell preparation and stimulation. Samples obtained by BAL were centrifuged (3000 rpm, 5 min). Supernatants were immediately aliquoted and stored at -70°C until they were assayed for cytokine content. Pellets were resuspended (2 million/ml) in HEPES-buffered RPMI 1640 medium supplemented with 10% calf serum, L-glutamine (2 mM), penicillin (50 U/ml), and streptomycin (50 $\mu\text{g/ml}$). Aliquots (500 μl) were incubated for 60 min at 37°C in flat-bottom, four-well culture plates (Nunc). The plates were then washed to remove nonadherent cells. Adherent cells were considered macrophages since they displayed macrophage structure under Wright's stain. More than 90% of cells were viable before culture, as assessed by trypan blue staining. Fresh medium was added without (control) or with (test) 20 mg/ml lipopolysaccharide (LPS) from *Escherichia coli* strain 026:B6 (Sigma). Supernatants were collected after 24 h at 37°C and stored at -70°C .

Cytokine assay. IL-10 and the p40 subunit of IL-12 were assayed with a sandwich ELISA technique (Quantikine, R&D system). The minimal detectable concentrations were 2 pg/ml for IL-10 and 5 pg/ml for IL-12.

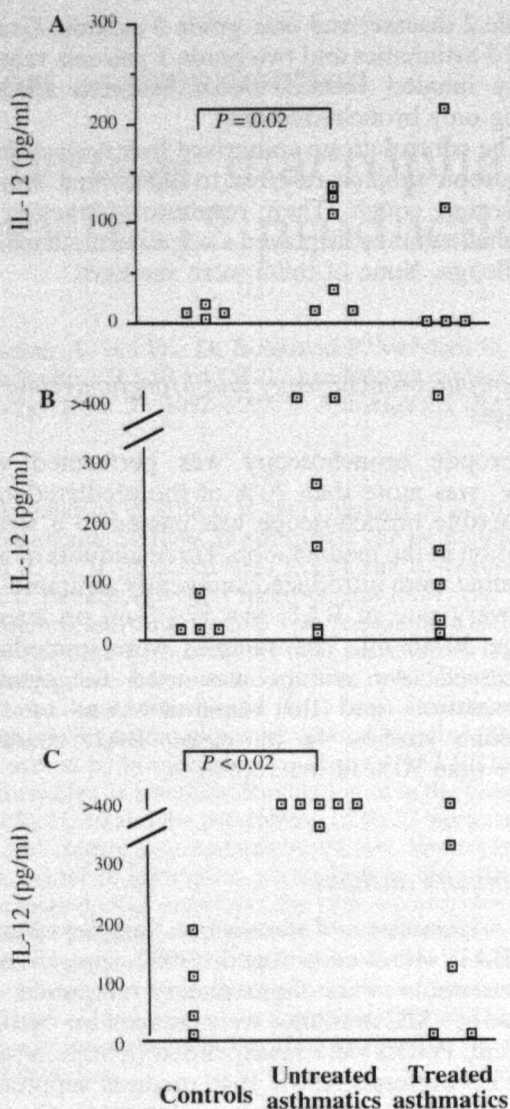


Fig. 2. Alveolar macrophage (AM) IL-12 production in asthma. AM were harvested by bronchoalveolar lavage (BAL) from untreated asthmatics ($n=6$), steroid-treated asthmatics ($n=5$), and control subjects ($n=4$). After centrifugation, IL-12 was assayed by ELISA in BAL supernatants (A). IL-12 was assayed by ELISA in 24-h culture supernatants of AM, without stimulation (B) or after stimulation with 20 μ g/ml of lipopolysaccharide (LPS) (C).

Statistical analysis

As some patients were treated by inhaled corticosteroids, the asthmatic group was analyzed as a whole, and then divided into steroid-treated and nonsteroid-treated patients, and both groups were analyzed separately. Levels of IL-10 between treated asthmatic, untreated asthmatic, and control patients were analyzed with the nonparametric Mann-Whitney test. As the higher detection threshold for IL-12 was 400 pg/ml, we analyzed

the results by a X2 test with the Yates correction, taking a threshold of 200 pg/ml.

Results

Alveolar macrophage production of IL-10

IL-10 contained in the BAL supernatant did not reach the lower detection threshold of the method (not shown). In AM culture supernatants, IL-10 levels were higher in asthmatics than in controls, 117.8 ± 36.1 pg/ml vs 4.7 ± 2.4 pg/ml, respectively, $P=0.04$). This difference was still found between treated asthmatics and controls (210.3 ± 13.2 pg/ml vs 4.7 ± 2.4 pg/ml, respectively, $P=0.04$) (Fig. 1A), but did not reach significance in comparison of untreated asthmatics (62.3 ± 39.4 pg/ml) and controls ($P=0.1$). In contrast, stimulation by LPS induced IL-10 production by AM from untreated asthmatics (444.9 ± 29.7 pg/ml), and from controls (205.6 ± 99.2 pg/ml), but not from treated patients (200.9 ± 99.5 pg/ml) (Fig. 1B and C). The effect of stimulation was stronger in AM from untreated asthmatics than controls ($P=0.02$) (Fig. 1B).

Alveolar macrophage production of IL-12

IL-12 was first assayed in the BAL supernatant. Although levels of IL-12 were not significantly different between asthmatic and control subjects, higher amounts of IL-12 were found in untreated asthmatics than in controls (62.4 ± 20.9 vs 5.7 ± 2.4 pg/ml, $P=0.02$) (Fig. 2A). To assess AM responsibility in the IL-12 secretion, IL-12 was assayed in AM culture supernatants without and with stimulation by LPS. The spontaneous production of IL-12 was not significantly different between asthmatics and controls, but after stimulation, this production was higher in the asthmatic group ($X^2=3.8$, $P<0.05$). This difference remained between the untreated subgroup and the controls ($X^2=6.2$, $P<0.02$), but was not found between treated asthmatics and controls (Fig. 2B and C).

Discussion

In this study, AM IL-10 production was increased in asthma. However, this elevation was mainly due to AM recovered from inhaled-steroid-treated patients. In addition, LPS-induced AM IL-10 production was twice as high in untreated patients as in controls, but not in treated subjects. These results suggest that, with regard to IL-10 production, AM are in a different state of activation according to diagnosis. In controls, AM are quiescent and produce IL-10 only upon stimulation. In untreated asthmatics, AM are primed to produce

high levels of IL-10. This priming is revealed by the high levels of IL-10 produced upon LPS stimulation. In steroid-treated patients, the AM are activated and produce IL-10 *in vivo*. This activation is no more enhanced by LPS stimulation. In a previous study of asthma, IL-10 was even found to be decreased in BAL fluids from asthmatics (8) in the acute experimental condition of an allergenic challenge, but IL-10 mRNA increased during the late-phase reaction. By *in situ* hybridization, Robinson et al. showed an increased percentage of BAL cells expressing the IL-10 mRNA in asthma (9). In both studies, the increase of IL-10 mRNA could reflect the priming of AM. Induction of IL-10 production by primed AM could be one of the mechanisms of the anti-inflammatory effects of inhaled steroids (10). Indeed, IL-10 is an anti-inflammatory cytokine, able to deactivate various immunocompetent cells including T cells (3).

We have shown that BAL IL-12 was increased in nonsteroid-treated asthma, and the AM IL-12 production increased upon LPS stimulation. Again, only AM from untreated asthmatics were reactive to stimulation. Naseer et al. have found a decrease in IL-12 mRNA-expressing cells in the bronchial mucosa from asthmatic subjects (11). However, in this location, IL-12 is produced by infiltrating monocytes and dendritic cells, but not by AM. Again, these authors studied the mRNA, the presence of which does not predict the secretion of the final product (12), and cells could be primed only for IL-12 production. IL-12 is responsible for the Th1 response in many models. Notably, the disruption of the IL-12 gene in mice impairs the development of Th1 responses (13). Thus, the elevation of AM IL-12 production in asthma challenges the relevance of the Th1/Th2 paradigm in this disease. In atopic dermatitis, a disease whose pathogenesis is very similar to asthma, IL-12 produced by peripheral blood monocytes is normal, but the IL-12-induced production of IFN- γ is impaired (14), suggesting a lack in the IL-12 signaling rather than a decrease in the cytokine itself. Supporting this hypothesis, Szabo et al. have demonstrated an extinction of IL-12 signaling in Th2 cells (15). The selective expression of the β_2 chain of the IL-12 receptor on Th1 cells, and not on Th2 cells, could account for these data (16).

Taken together, our results give new insights into the activation of AM in asthma, which appears more as a consequence than as an initiating factor of the T cell Th2 commitment, and could play a role more in the resolution of asthmatic inflammation than in its triggering.

References

1. Robinson DS, Hamid Q, Ying S, et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;**326**:298–304.
2. Manetti R, Parronchi P, Giudizi MG, et al. Natural killer cell stimulatory factor (interleukin-12) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993;**177**:1199–1204.
3. Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper and type 2 helper T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993;**150**:353–60.
4. Zuani-Amorim C, Hallé S, Leduc D, et al. Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J Immunol* 1995;**95**:2644–51.
5. Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin-12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995;**182**:1527–36.
6. Gosset P, Tsicopoulos A, Wallaert B, Joseph M, Capron A, Tonnel AB. Tumor necrosis factor- α and interleukin-6 production by human mononuclear phagocytes from allergic asthmatics after IgE-dependent stimulation. *Am Rev Respir Dis* 1992;**146**:768–74.
7. Aas K. Heterogeneity of bronchial asthma. *Allergy* 1981;**36**:3–10.
8. Borish L, Aarons A, Rumblyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 1996;**97**:1288–96.
9. Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am J Respir Cell Mol Biol* 1996;**14**:113–17.
10. Tabardel Y, Duchateau J, Schmartz D, et al. Corticosteroids increase blood interleukin-10 levels during cardiopulmonary bypass in men. *Surgery* 1996;**119**:76–80.
11. Naseer T, Minshall EM, Leung DYM, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Respir Crit Care Med* 1997;**155**:845–51.
12. Schoenhaut DS, Chua AO, Wolitzky AG, et al. Cloning and expression of murine IL-12. *J Immunol* 1992;**148**:3433–40.
13. Magram J, Connaughton SE, Warrier RJ, et al. IL-12-deficient mice are defective in IFN- γ production and type 1 cytokine responses. *Immunity* 1996;**4**:471–81.
14. Lester MR, Hofer MF, Gately M, Trumble A, Leung DYM. Downregulating effects of IL-4 and IL-10 on the IFN- γ response in atopic dermatitis. *J Immunol* 1995;**154**:6174–81.
15. Szabo SJ, Jacobson NG, Dighe AS, Gubler U, Murphy KM. Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. *Immunity* 1995;**2**:665–75.
16. Rogge L, Barberis-Maino L, Biffi M, et al. Selective expression of an IL-12 receptor component by human T helper 1 cells. *J Exp Med* 1997;**185**:825–31.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.