Modulatory effects of alveolar macrophages on CD4+ T-cell IL-5 responses correlate with IL-1β, IL-6, and IL-12 production


Modulatory effects of alveolar macrophages on CD4+ T-cell IL-5 responses correlate with IL-1β, IL-6, and IL-12 production. C. Tang, J.M. Rolland, C. Ward, X. Li, R. Bish, F. Thien, E.H. Walters. ©ERS Journals Ltd 1999.

ABSTRACT: Increasing evidence suggests that the pattern of T-cell cytokine production can be modulated by antigen presenting cell (APC)-derived factors during the cell interactions. Recently, it has been shown that alveolar macrophages (AMs) from atopic asthmatics (AA) but not atopic nonasthmatics (AN) enhance interleukin (IL)-5 production by CD4+ T-cells.

The present study compared AM production of IL-1β, IL-6, and IL-12, as well as their associated functional capacity to influence IL-5 production by allergen-specific CD4+ T-cells in 10 AA, 10 AN, and nine nonatopic control subjects (C). AMs from AA showed a relatively high production of IL-1β and IL-6 (p<0.05) and a relatively low secretion of IL-12 compared to C, whereas AMs from AN and C behaved similarly. This study confirmed previous findings that co-culture with AMs augments IL-5 production from allergen-stimulated CD4+ T-cells only in AA and not in nonatopics even if they are atopic. On the other hand, stimulation with allergen alone did not enhance IL-5 production by CD4+ T-cells in either AA nor AN. AM-induced changes in CD4+ T-cell IL-5 production upon allergen stimulation significantly correlated with their ability to produce IL-1β (r=0.59, p<0.01), IL-6 (r=0.56, p<0.01), and inversely with IL-12 (r=−0.64, p=0.002) in all atopic subjects, and even more closely with the ratio of IL-12/IL-1β (r=−0.75, p<0.001) and IL-12/IL-6 production (r=−0.81, p<0.001) in these subjects.

These findings suggest that the role of alveolar macrophages from atopic asthmatics in enhancing interleukin-5 production by allergen-specific CD4+ T-cells is due, at least partly, to their aberrant production of interleukin-1β, interleukin-6, and particularly of interleukin-12.


Interleukin (IL)-5 has been identified as a key cytokine in the development of airway eosinophilia, and is quantitatively related to the extent of clinical asthma expression [1–6]. Thus, the mechanisms for controlling IL-5 production by CD4+ T-cells (the major source of IL-5 in the airways) may be of crucial importance in the pathogenesis of asthma and in determining which atopic individuals develop allergic asthma. These mechanisms require urgent clarification [7].

It has become clear that CD4+ T-cells are heterogeneous and thus their cytokine profiles and function are not predetermined but depend on how the cells are stimulated with antigen [8–10]. The development of T-helper (Th)-1 cells (production of IL-2 and interferon (IFN)-γ) or Th-2 cells (production of IL-4 and IL-5) effector populations from common precursor cells are profoundly influenced by the antigen type, antigen-presenting cell (APC) type, cytokine microenvironment, and costimulatory signals. These factors direct the differentiation of naive or memory CD4+ Th-0 cells into either Th-1 or Th-2 effectors [11, 12], affect the clonal expansion of the memory cells already committed to a Th-1 or Th-2 phenotype [13], and even alter the pattern of cytokine production from the same effector population [14, 15].

It is therefore conceivable that the excessive IL-5 production by CD4+ T-cells in the airways of atopic asthmatics (AA), compared to well-matched atopic nonasthmatics (AN) [4, 5], is related to the profiles of cytokine production by the local APC. Several APC-derived cytokines have been implicated in the development of a particular polarized T-cell response. Of these cytokines, IL-12 is the most important cytokine in promoting the development of the Th-1 immune phenotype [16–18], while IL-1β and IL-6 may contribute to the onset of a Th-2 response in maturing human T-cells [12, 19]. Furthermore, a powerful suppressive effect of IL-12 upon IL-5 production has been reported in recent studies [9, 20], showing that administration of recombinant IL-12 markedly inhibited antigen-induced IL-5 production in sensitized mice with subsequent downregulation of eosinophil recruitment into the airways. On the other hand, work has also shown that alveolar macrophages (AMs) from AA [21, 22], but not from AN subjects, enhance IL-5 production by allergen-stimulated CD4+ T-cells, which is inhibited by the...
addition of monoclonal antibodies against IL-1β or IL-6 to the parallel cocultures [21].

Taken together, these observations suggest a potential imbalance between AM-derived IL-12 and pro-Th-2 cytokines, such as IL-1β and IL-6, in AA, which might contribute to the local overproduction of IL-5 by CD4+ T-cells upon allergen exposure and hence development of disease. The objective of this study was to test this hypothesis by comparing the capacity of AMs to produce IL-1β, IL-6, and IL-12 in vitro upon allergen stimulation in AA, AN, and nonatopic control subjects, and the relation of these cytokines to allergen-driven CD4+ T-cell IL-5 production.

Methods

Subjects

Ten AA, 10 AN and nine C subjects were studied (table 1). These group sizes have previously been shown, in a similar study, to reveal significant differences in cytokine production [21]. Atopy was defined by skin prick tests. All AA and AN subjects had two or more positive skin weal responses to a panel of common environmental allergens including a positive skin test reaction to either rye grass pollen or house dust mite (HDM) (Greer Laboratories, Lenoir, NC, USA) (> 3 mm weal diameter).

AA had a history of periodic wheeze, cough, or breathlessness accompanied by documented reversible airflow obstruction (20% improvement in forced expiratory volume in one second (FEV1) either spontaneously or in response to inhaled β2-agonist). All of them had a measurable provocative dose causing a 20% fall in FEV1 (PD20) methacholine when challenged with a standard protocol [23]. None had received inhaled or oral corticosteroid therapy or any other atopic condition and were skin prick test negative to the same panel of allergens. None of the subjects in any group were current smokers (or ex-smokers).

Sex M/F 1/8 3/7 1/9
Age yrs 39 38 39
Subjects n 9 10 10

Methods

Preparation of alveolar macrophages

AMs were obtained by bronchoalveolar lavage (BAL) according to the guidelines of the American Thoracic Society [24]. Subjects were premedicated with nebulized albuterol (2.5 mg), intravenous atropine (0.6 mg), and intravenous midazolam (5–10 mg). They received supplemental oxygen at 4 L·min⁻¹ throughout and were monitored by continuous pulse oximetry. Lignocaine was used for local anaesthesia. BAL was performed using three aliquots of 60 mL of phosphate buffered saline (PBS) at 37°C by gentle hand pressure into a subsegment of the right middle lobe and recovered by gentle suction.

With minimal delay, the BAL fluid was centrifuged (at 1,500 rpm, 4°C for 10 min) and the cell pellet was suspended in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% heat-inactivated foetal calf serum (FCS), 2 mM l-glutamine, 100 IU·mL⁻¹ penicillin, and 125 μg·mL⁻¹ gentamicin. The resuspended cells were then plated in plastic dishes for 1 h at 37°C in a 5% CO₂ humidified atmosphere. The nonadherent cells were discarded, and the adherent cells were removed by gently scraping with a plastic scraper and resuspended. After two repetitions of the protocol, with an average cell recovery of 50%, the harvested cells were >95% AM and >90% viable as judged by Quick Dip (Histo Labs, New South Wales, Australia) and trypan blue staining.

Isolation of peripheral blood CD4+ T-cells

Forty millilitres of venous blood were drawn into a heparinized container just before the bronchoscopy. To obtain CD4+ T-cells, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation on Ficoll-Paque™ (Pharmacia, Uppsala, Sweden) and incubated with magnetic beads coated with monoclonal antibody against CD4 (Dynal A. S, Oslo, Norway) at a ratio of 4:1, at 4°C for 1 h with gentle tilting and rotation. The rosetted CD4+ T-cells were isolated by placing the test tube on a magnet for 2–3 min and then washed three times with PBS containing 2% FCS. The CD4+ T-cells were then harvested using DETACHaBEAD™ (Dynal A. S) to remove the bound beads. This procedure normally yielded populations of >99% CD4+ T-cells with >99% viability, as determined by fluorescence-activated cell sorter (FACS) analysis and trypan blue dye exclusion.

Cell cultures

Peripheral blood CD4+ T-cells were cultured alone (2×10⁶ cells·2 mL-well⁻¹) with or without allergen stimulation, and cocultured for 5 days with AMs (1×10⁶ cells·2 mL-well⁻¹) plus allergen in the same RPMI 1640 medium as described above. AMs were incubated alone with or without allergen (1×10⁶ cells·2 mL-well⁻¹) for 5 days, and the cultures with allergen stimulation were also conducted for 3 days to test the kinetics of AM cytokine production.

This study was approved by the Alfred Hospital Ethics Committee and informed consent was given by all patients and control subjects.

Table 1. – Characteristics of patients and control subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Nonatopic control subjects</th>
<th>Atopic asthmatics</th>
<th>Atopic nonasthmatics</th>
</tr>
</thead>
<tbody>
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<td>Subjects n</td>
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<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age yrs</td>
<td>39 (21–61)</td>
<td>38 (24–70)</td>
<td>39 (21–49)</td>
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<tr>
<td>Sex M/F</td>
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<td>3/7</td>
<td>1/9</td>
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<td>112</td>
<td>86.6</td>
<td>110.5</td>
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<tr>
<td>% pred</td>
<td>(99–120)</td>
<td>(62.3–102)*</td>
<td>(96–132)</td>
</tr>
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<td>PD20 meth</td>
<td>&gt;2 mg</td>
<td>0.075</td>
<td>&gt;2 (0.002–0.364)</td>
</tr>
</tbody>
</table>

Data are presented as median with range in parentheses. M: male; F: female; FEVI: forced expiratory volume in one second; PD20 meth: provocative dose of methacholine causing a 20% fall in FEV1. *: p=0.004 compared to atopic nonasthmatics; †: p=0.0006 compared to control subjects.
For allergen stimulation, rye grass pollen (20 µg·mL⁻¹) or HDM (10 µg·mL⁻¹) was used for atopic subjects according to their skin test sensitivity (if sensitive to both, the one with stronger skin reaction was chosen) and for control subjects at random. The same batch of rye grass pollen and HDM was used for all of the experiments.

**Enzyme-linked immunosorbent assay for interleukin-1β, -5, -6 and -12**

Measurements of the cytokine levels in the supernatants of cell cultures were performed by an enzyme-linked immunosorbent assay (ELISA) using paired antibodies according to the procedures recommended by the manufacturers (IL-1β: R & D Systems, Minneapolis, MN, USA; IL-5, IL-6 and IL-12: PharMingen, San Diego, CA, USA): IL-5 in either CD4⁺ T-cell cultures or CD4⁺ T-cell-AM cocultures, IL-1β and IL-12 p40 (IL-12 is a heterodimeric cytokine consisting of p40 and p35 subunits) in the cocultures or cultures of AM alone, as well as IL-6 in AM-alone cultures (IL-6 is also produced by Th-2-type cells, thus this cytokine was not measured in the cocultures). Recombinant human IL-1β, IL-5, IL-6, and IL-12 p40 were used as standards. The limits of detection were 8 pg·mL⁻¹ for IL-1β, 31 pg·mL⁻¹ for IL-5 and IL-6, and 78 pg·mL⁻¹ for IL-12 p40.

**Statistics**

Statistical analyses were performed by means of nonparametric tests. The Mann-Whitney U-test and the Wilcoxon signed-rank test were used for inter- and intra-group comparisons, respectively. Fifty per cent of the limits of detectability was arbitrarily assigned to the undetectable cytokine levels in the cell culture supernatants. Relationships were tested using Spearman’s rank correlation. A p-value ≤0.05 was taken as significant.

**Results**

**Different profiles of cytokine production by alveolar macrophages from the three groups**

Comparisons of the levels of IL-1β, IL-6, and IL-12 in allergen-stimulated AM-alone cultures in the three groups are represented in figure 1. AA demonstrated a significantly higher median level for both IL-1β and IL-6 than control subjects (p<0.05). In the AN group, the levels of IL-1β and IL-6 were similar to those in control subjects, but the differences between AA and AN subjects did not reach significance.

In contrast to the results for IL-1β and IL-6, the lowest median level of IL-12 from the AM cultures was demonstrated in AA, whereas control subjects showed the highest, with AN being intermediate. However, because of the large intra-group variation, these differences were not significant.

IL-1β and IL-12 production by AMs in allergen-stimulated CD4⁺ T-cell-AM cocultures were also examined in the AA group. The median (range) level of IL-1β in the cocultures was similar (164 (4–424) pg·mL⁻¹) to that in the cultures of AM-alone. The median level of IL-12 (325 (39–1,617) pg·mL⁻¹) in the cocultures was lower than that in the cultures of AM-alone, but the difference was not significant.

To discriminate the effect of allergen stimulation from the baseline macrophage cytokine production, the levels of the above three cytokines in unstimulated AM cultures were compared with those in allergen-stimulated AM cultures in four AA and three control subjects. The levels of IL-1β, IL-6, and IL-12 in unstimulated AM cultures in these subjects were very comparable to those in matched cocultures.
allergen-stimulated cult-ures (data not shown). In addition, no significant difference was found in AM cytokine production between allergen-stimulated cultures for 3 days and 5 days. The ratios of IL-12 production to either IL-1β or IL-6 production by allergen-stimulated AMs were evaluated in the three groups. The median ratio for the levels of IL-12/IL-1β was lower in AA (7.2 (0.2–75.5)) compared to both AN (134 (2–1,266), p=0.06) and control subjects (77 (0.1–20.8), p=0.01). This was also the case for comparing the median ratio of IL-12/IL-6 levels in AA subjects (0.26 (0.01–5.5)) with those in the two nonasthmatic groups (AN: median 1.4 (0.1–20.8), p=0.06; C: median 3.8 (0.1–14.9), p<0.05). There was no significant difference in concentration ratio of either IL-12/IL-1β or IL-12/IL-6 between the two nonasthmatic groups. Additionally, in the AA group, no difference was seen for the median ratio of IL-12/IL-1β levels between allergen-stimulated AM cultures with (2.5 (0.2–338.0)) and without CD4+ T-cells.

**Effect of allergen alone on CD4+ T-cell interleukin-5 production**

Before allergen stimulation, IL-5 production by CD4+ T-cells in AA subjects (380 (16–1042) pg·mL−1) was higher than that in AN subjects (120 (16–593) pg·mL−1), but the difference was not significant (fig. 2). After stimulation, there were no significant changes in IL-5 production in any group, but levels for the two atopic groups were now significantly higher than for control subjects (p<0.05).

**Effect of alveolar macrophages on CD4+ T-cell interleukin-5 production**

In agreement with previous observations [21], the biological effects of AMs on IL-5 production by allergen-stimulated CD4+ T-cells were markedly different between AA and AN subjects, whereas AMs from the two nonasthmatic groups behaved similarly (fig. 2). In the AA group, the median IL-5 level in allergen-stimulated CD4+ T-cell-AM cocultures was significantly enhanced to 702 (177–2,431) pg·mL−1 (p<0.01). In contrast, the increases in both AN (to 250 (80–930) pg·mL−1) and control subjects (to 90 (16–279) pg·mL−1) were not significant. As a result, the addition of AMs to allergen-stimulated CD4+ T-cell cultures significantly amplified the differences in the IL-5 production between the AA and AN (p<0.01) or control subjects (p>0.001).

**Correlation between changes in interleukin-5 and cytokine production**

AM-modulated changes in CD4+ T-cell IL-5 production were significantly related to AM-alone production of IL-1β (r=0.59, p<0.01), IL-6 (r=0.56, p<0.01), and IL-12 (r=0.64, p<0.002) when tested in all atopic subjects. However, when tested in just the AA group, a significant correlation was seen or approached only for the relationship with IL-12 production by allergen-stimulated AM with (r=0.64, p<0.05) and without CD4+ T-cell coculture (r=0.58, p=0.06) (fig. 3). For the relationship of AM-modulated changes in IL-5 production to the ratio of IL-12/IL-1β or IL-12/IL-6 production, the significances were striking both in the AA group alone (for IL-12/IL-1β: r=−0.71, p=0.02; for IL-12/IL-6: r=−0.75, p<0.01) and in all atopic subjects combined (for IL-12/IL-1β: r=−0.75, p<0.001; for IL-12/IL-6: r=−0.82, p<0.001).

**Discussion**

In the current study, AMs from AA, AN, and control subjects were analysed for IL-1β, IL-6, and IL-12 production, as well as their associated capacity to modulate IL-5 production by allergen-stimulated CD4+ T-cells. Consistent with findings in earlier studies [21, 22], the current data suggest contrasting effects of AMs on IL-5 production by CD4+ T-cells in response to allergen in AA versus AN patients. The relative effects of AMs on allergen-specific CD4+ T-cell IL-5 responses in atopic individuals were significantly associated with the respective production of IL-1β, IL-6, and IL-12 by these AMs. It has previously been demonstrated that IL-5 production in this coculture model can be partially blocked by monoclonal antibodies against IL-1β and IL-6 [21]. These observations suggest that the role of AMs in enhancing IL-5 production by allergen-specific CD4+ T-cells in AA subjects may be mediated, at least in part, through their relatively high production of IL-1β and IL-6 combined with a relatively low production of IL-12, at least in comparison with AN and control subjects.

There was a higher, although not significant, CD4+ T-cell IL-5 production at baseline in AA versus AN and control subjects, presumably reflecting a higher in vivo activation of these cells in the former. CD4+ T-cell IL-5 production upon stimulation with allergen alone only resulted in minor changes in IL-5 production in both atopic groups, excluding a potential concern that the markedly increased IL-5 level in allergen-stimulated CD4+ T-cell-AM cocultures in AA subjects was independent of the
A central finding in this study was that the excessive IL-5 production by allergen-reactive CD4+ T-cells in AA patients, compared to AN patients, seems to be related to the decreased IL-12 secretion by their AMs in the co-cultures. IL-12 has been well defined as an obligatory factor for inducing effective Th-1 responses by acting at three different levels: it induces IFN-γ gene expression and protein production in preactivated T-cells within a few hours of stimulation, favours the differentiation of a Th-1 phenotype in the early immune response, and promotes the proliferation of Th-1-like cells once differentiated [17, 18, 25, 26]. Consequently, the presence of IL-12 in the T-cell micromilieu can inhibit the production of the Th-2 phenotype [26]. The current data provide direct evidence for the relatively low production of IL-12 by AMs from AA subjects, and for endogenous production of IL-12 by AMs "negatively" controlling the IL-5 response. Thus, relative lack of IL-12 production by AMs may lead to the induction of the asthma phenotype. Compatible with this suggestion are recent studies showing that administration of recombinant (r)IL-12 potently inhibited IL-5 production in sensitized mice [20, 27], which leads to exciting therapeutic possibilities for human asthma management.

It was also demonstrated that AMs from AA subjects produced high levels of IL-1β and IL-6, and that in atopic subjects the production of these two cytokines was related to the levels of allergen-induced IL-5 production by CD4+ T-cells. This is consistent with a previous study showing that the addition of monoclonal antibodies against IL-1β and IL-6 to allergen or mitogen-stimulated cell cultures in atopic asthma significantly inhibited the IL-5 production by CD4+ T-cells. This observation is consistent with the idea that the addition of IL-1β and IL-6 to allergen-stimulated AM cultures in atopic asthmatics (n=20; r=0.64, p<0.02). The actual contribution of AM-derived IL-1β, IL-6, and IL-12 to CD4+ T-cell IL-5 responses probably depends on the balance of their concentrations, because these cytokines may coexist during antigen-specific CD4+ T-cell activation. Indeed, all three cytokines were produced in significant amounts in allergen-stimulated AM cultures. The potential relevance of the imbalance in AM cytokine production to the pathogenesis of asthma was further emphasized by the close relationship between the ratio of IL-12/IL-1β or IL-12/IL-6 production and AM-modulated changes in IL-5 production by allergen-stimulated CD4+ T-cells. Nevertheless, it appears that a decreased IL-12 production by local APC is more critical to the development of the asthma-phenotypic immune responses upon allergen exposure, because the Th-2-inhibitory role seems unique for IL-12. Consistent with this idea, was the finding that the levels of IL-12, rather than IL-1β or IL-6, were significantly inversely related to IL-5 production by allergen-reactive CD4+ T-cells in AA alone. These causative relationships need to be clarified further with more detailed investigations, including cytokine blocking and supplemental studies.

IL-12 is produced predominantly by APC populations such as monocytes, B-cells, macrophages, and dendritic cells [16]. IL-1β and IL-6 are produced by a rather
broad range of cell types including endothelial cells, and IL-6 is also produced by Th2-type cells [12, 19]. However, from the viewpoint of local immunoregulatory mechanisms, AMs probably represent a very important source for all of these cytokines, since they are such a quantitatively large cell population in the respiratory tract, and have an intimate relationship with T-cells through antigen presentation and/or contact-mediated costimulatory signals in the lungs.

It is not clear however, from the current study, what mechanisms were responsible for the dichotomy in the production of IL-12 and the production of IL-1β and IL-6 by AMs in atopic asthma. Previous studies have shown higher production of IL-1β and IL-6 by AMs from asthmatics compared to AMs from control subjects [28, 29], and this was thought to be attributable to the greater activation status because of their relatively low production of IL-12 under the same conditions. This phenomenon might reflect a different composition of the various cytokine profiles in AA versus AN or control subjects. Indeed, it has been shown that IL-12 production by macrophages is associated with specific phenotypic expression [30, 31]. Examining such mechanisms more closely should lead to a deeper understanding of asthma pathogenesis, and how to modify it.

In summary, the present study has demonstrated that the differential modulation of CD4+ T-cell interleukin-5 production by alveolar macrophages upon allergen stimulation between atopic asthmatics and atopic nonasthmatics was associated with the differential production of interleukin-1β, interleukin-6, and interleukin-12 by their respective alveolar macrophages. It was suggested that the concentration ratio of interleukin-12 to interleukin-1β or interleukin-6 is able to influence up- or down-regulation of interleukin-5 production by allergen-specific CD4+ T-cells in atopic subjects. Since the role of interleukin-12 seems to be unique in inhibiting the interleukin-5 phenotype, the mechanisms controlling antigen presenting cell interleukin-12 production in the lungs deserve further study.

References


