Original Article

IL-2 and IL-10 levels in induced sputum and serum samples of asthmatics

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Summary. *Background:* There is consisting evidence that asthma is associated with airway inflammation. Originally IL-10 and IL-2 were described as lymphokines produced by T cells in mediating cellular infiltration into the airways and continue to be of interest in evaluating asthma pathogenesis. The aim of this study was to evaluate the serum and sputum levels of IL-2 and IL-10 in asthmatic subjects and healthy controls and to correlate disease activity and other clinical indices with concentrations of IL-2 and IL-10 in serum and sputum samples.

Methods: We evaluated cell profiles and IL-2 and IL-10 levels in induced sputum samples and in serum samples of 6 mild, 5 moderate, 7 severe asthmatic patients and 5 healthy controls by using ELISA.

Results: The mean IL-2 in sputum samples of asthmatics and controls were 35.3±13.2 pg/ml and 35.3±8.4 pg/ml, respectively. The mean IL-2 in serum samples of asthmatics and controls were 42.7±21.1 pg/ml and 30.3±2.4 pg/ml, respectively. Both levels did not result in any statistically significant difference between asthmatics and controls. There was no correlation between serum and sputum IL-2 levels, however sputum IL-2 levels correlated with percentage of sputum lymphocytes ($p<0.03$, $r=0.51$). The mean IL-10 levels in sputum samples of asthmatics and controls were 4.4±3.3 pg/ml and 3.9±5.9 pg/ml, respectively, the mean IL-10 level in serum of asthmatics and controls were 4.1±3.8pg/ml and 2.3±2.5 pg/ml, respectively. We could not find statistically significant difference of serum or sputum IL-10 levels between asthmatics and controls There was only correlation between serum and sputum IL-10 levels in asthmatics ($p<0.0008$, $r=0.73$). There was no difference between asthmatic subgroups regarding sputum and serum levels of IL-2 and IL-10. No correlation could be demonstrated between sputum or serum IL-2 and IL-10 levels and clinical severity.

Conclusions: We have demonstrated the presence of detectable concentrations of the IL-2 and IL-10 in serums and induced sputum samples of asthmatics, however, they have no predictive value for asthma since their levels are not increased in asthmatic patients over controls. Moreover, IL-2 level positively correlated with lymphocyte percentage in induced sputum. The results suggest that measurement of IL-2 and IL-10 concentrations in serum and sputum will not be of diagnostic use in asthma and a reflection of the severity of asthmatic airway inflammation.

Keywords:IL-2, IL-10, induced sputum, asthma.

Introduction

Asthma is defined by airway inflammation and hyperresponsiveness. Histopathological analysis of airway biopsies and BAL (bronchoalveolar lavage) to obtain the fluid lining of the airways were used to show airway inflammation (1,2). A noninvasive technique such as sputum analysis could provide an alternative method to BAL (bronchoalveolar lavage) and tissue sampling in studying the pathophysiology of asthma (3). Current evidence suggests that T cells may orchestrate inflammatory responses in asthma by production of several cytokines (4,5). The cytokine profile was incompatible with a pure Th1 and Th2 cell response in asthma (6). IL-10 is a potent regulatory cytokine that decreases inflammatory responses and protects airway from developing inflammatory responses to inhaled allergens (7,8). It is now appreciated that human IL-10 is produced by Th0, Th1 and Th2 lymphocytes, although monocytes and tissue macrophages are important major sources of IL-10 (9-11). It inhibits the production of proinflammatory cytokines and chemokines by monocytes, T cells, neutrophils, and eosinophils (10-15). IL-10 production is controlled at a transcriptional level and low concentrations of IL-10 mRNA have been released by alveolar macrophages of asthmatics compared with controls and protein level of IL-10 in BAL fluid of asthmatics confirmed these findings (16-18). In contrast, the number of IL-10 mRNA positive cells in BAL fluid obtained from asthmatic subjects was

shown to be increased compared to that in control subjects (19). Recently, an induced sputum study demonstrated that cells immunopositive for IL-10 and IL-10 level in sputum were lower in asthmatics compared with those in healthy nonsmokers (20). Following these conflicting studies, the IL-10 level in the asthmatic airway still remains to be evaluated and the aim of this study was to assess the role of IL-10 invivo in asthma using sputum induction method.

IL-10 is able to inhibit the cytokine synthesis by both Th1 and Th2 human cell clones (10, 15, 21,22). In contrast, IL-2 is a growth and activating factor for Th1 and Th2 cells (23,24). The relationship and balance between IL-2 and IL-10 play a crucial role in operating cellular complex network in asthma and remain to be elucidated. Several studies have suggested a role for Th2 like subset of CD4+ T lymphocytes contributes to eosinophil influx and activation by demonstration of mRNA for IL-3, IL-4, IL-5, GMCSF, but not Th1 cytokines such as IFN-δ except IL-2 in BAL fluid of atopic asthma compared with normal control subjects (25). Previous findings suggested that IL-2 cytokine secretion related to the allergen challenge and particularly in symptomatic nonatopic asthmatics (25-32).

Since, it has been discovered that lymphocytes migrate from the blood into the BAL and lung parenchyma (33,34). To test whether this sputum cytokine profile could be extended to the peripheral blood, blood samples of asthmatics and controls were undertaken in this study. The aims of this study were to evaluate the feasibility of using this sputum induction model to study the inflammatory response to determine the pattern of inflammatory cells and fluid phase markers in the sputum, particularly, relative contribution of the IL-2 and IL-10 to airway inflammation in patients with asthma and to compare with those of controls and to correlate with clinical and functional characteristics of patients.

Methods

Subjects

Five control subjects and 6 mild, 5 moderate, and 6 severe asthmatic subjects were studied. Before entry, all subjects were screened with history, physical examination, chest X-Ray, skin prick tests, serum IgE levels, spirometry, bronchodilator response, methacholine challenge test (in mild and moderate group), symptom questionnaire and daily PEFR(Peak expiratory flow rate) measurements. Control subjects had no respiratory symptom, no positive skin prick test and had normal lung function test. Asthmatics were divided into 3 subgroups as mild, moderate, and severe according to general asthma guidelines (35) . All of the patients were nonsmokers and atopics. Methacholine challenge tests were performed in mild and moderate asthmatics. Informed written consent was obtained from all subjects.

Sputum Induction, Sputum Processing and Biochemical Assays

All subjects were premedicated with inhaled 200 μg salbutamol, and then PEFR, FEV_1 measurements of subjects were performed and repeated every 5 minutes throughout the procedure. Sputum induction was performed with an aerosol of hypertonic saline (5%) generated by an ultrasonic nebuliser (Devilbiss ultrasonic nebuliser, USA) for approximately 30 minutes and the patients rinsed their mouth to avoid the contamination with saliva. The volume of induced sputum sample was determined and an equal volume of dithiotrietol 10% (DTT) was added. The samples were then mixed gently by vortex mixer and placed in a shaking water bath at 37°C for 30 minutes to ensure complete homogenisation. Entire induced sputum (plugs plus fluid component) was collected. The microliters of the homogenised sputum were used to determine the total cell counts of the samples using a standard hemocytometer. The remainder of the homogenized sputum was centrifuged at 1800 rpm for 5 minutes. The supernatants were frozen at -70°C for further analysis. The cell pellets were resuspended in PBS (phosphate-buffer-saline) solution and centrifuged. The remaining cell pellets were cytocentrifuged and stained with Wright stain. On each sputum slide at least 500 nonsquamous cells were counted. IL-2 and IL-10 concentrations in induced sputum samples and serum samples were measured using enzyme-linked immunosorbant assay (IL-2 Quantikine, R&D Systems Inc, Minneapolis, MN,USA and IL-10 ELISA Bender Med Systems, Vienna, Austria).

Statistical Analysis

Data are expressed as the mean and the standard deviation. Mann-Whitney U test was used to assess differences between healthy and asthmatic subjects, Kruskal-Wallis test to assess differences within asthmatic subgroups. Spearman Rank correlation was calculated to assess the correlation between serum or sputum Il-2 and IL-10 levels and symptom scores, FEV_1 %predicted, PD_{20} values, eosinophilia, IgE level, and sputum cell profiles.

Results

The clinical characteristics of the patients are summarized in Table I. In the asthmatic group, there were 17 patients (5 males, 12 females), and in the control group the number of subjects was 5 (2 males, 3 females). In asthmatics, the mean IgE level was 687±1305 IU/ml (N<94 IU/ml), the methacholine PD_{20} was 1.9±4.11 mg/

Table I. Clinical characteristics of patients

	Mild asthmatics	Moderate asthmatics	Severe asthmatics	Control Group	P value
Male	3	1	1	2	-
Female	3	4	5	3	-
Age	30±15	41±7.5	39±13	30±9	NS
FEV1 (L)	2.94±0.47	2.01±0.74	1.61±0.95	3.26±0.77	0.04
(%pred.)	(103.5±9.4)	(79.2±5.9)	(51.7±14.8)	(93±14)	(0.0008)
FEV1/FVC %	92±10	73±9	61±16	84±2	0.01
Methacholine PD20 (mg/ml)	3.45±6.2	2.26±3.32	-	-	-

Table II. Cell Profiles in Sputum Samples of Asthmatics and Controls

	Patient Group	Control Group	p
Total cell count (x10^6/ml)	31.9±31.6	4.9±4.8	P<0.05
Neutrophil (x10^6/ml) (%)	5.5±7.0 (16.3±15.2)	0.28±0.23 (10.8±7.1)	p<0.01 (NS)
Eosinophil (x10^6/ml) (%)	11.8±13.9 (40.6±25.9)	0.08±0.16 (1.3±1.9)	p<0.001 (p<0.001)
Lymphocyte (x10^6/ml) (%)	1.9±2.3 (7.9±5.0)	0.50±0.53 (14.5±7.5)	NS (NS)
Macrophage (x10^6/ml) (%)	6.8±4.8 (35.4±21.9)	2.3±2.5 (73.5±12.0)	p<0.05 (p<0.01)

X±SD
NS : Nonsignificant

Table III. IL-2 and IL-10 levels in sputum and serum samples of asthmatic patients and controls

	Mild asthmatics	Moderate asthmatics	Severe asthmatics	Control Group	P value
Serum IL-2 (pg/ml)	41.0±20.4	40.7±23.9	46.3±23.0	30.3±2.4	NS
Sputum IL-2 (pg/ml)	37.9±14.4	35.0±13.5	33.1±13.9	35.3±8.4	NS
Serum IL-10 (pg/ml)	3.4±2.9	3.7±3.9	5.0±4.8	2.3±2.5	NS
Sputum IL-10 (pg/ml)	4.3±4.3	4.6±3.4	4.3±2.5	3.9±5.9	NS

ml in mild and moderate group. Ten patients were on inhaled steroid, 1 patient on oral steroid, 1 patient on inhaled nedocromil sodium, and 5 patients on prn inhaled salbutamol treatment. The mean volume of sputum produced by the asthmatic subjects was not significantly different from the mean volume of sputum produced by the healthy subjects (15±3.2 ml versus 16.1±4.6 ml).

In induced sputum the absolute number and percentages of nonsquamous cells were given in Table II. The absolute number of total nonsquamous cells, mean percentage and absolute number of eosinophils were higher in the sputum samples of asthmatics ($p<0.05$, $p<0.001$, and $p<0.001$, respectively) and the mean percentage of macrophages raised significantly in the sputum samples of controls ($p<0.01$) (Table II).

Peripheral blood white blood cell count, percentage and absolute number of eosinophils in peripheral blood were higher in asthmatics than controls; 8976±3061/ml versus 6480±1103/ml ($p<0.05$); 6.9±5.9% versus 0.6±0.9% ($p<0.01$); 629±609/ml versus 38±60/ml ($p<0.01$), respectively.

There was no statistically significant difference between sputum IL-2 levels of asthmatics and controls (35.3±13.2 pg/ml and 35.3±8.4 pg/ml, respectively) and between serum IL-2 levels of asthmatics and controls (42.7±21.1pg/ml in serums of asthmatics and 30.3±2.4 pg/ml in those of controls). We could not find any significant change while we compared each asthmatic subgroups with controls and between them regarding serum and sputum IL-2 levels (Table III). Sputum IL-2 levels correlated with percentage of sputum lymphocytes ($p<0.03$, $r=0.51$). There was no correlation between serum and sputum IL-2 levels.

The mean IL-10 levels in sputum samples of asthmatics and controls were 4.4±3.3 pg/ml and 3.9±5.9 pg/ml, respectively and the mean IL-10 level in serum of asthmatics and controls were 4.1±3.8pg/ml and 2.3±2.5 pg/ml, respectively. Both levels did not result in any significant change between asthmatics and controls. Moreover, there was no difference between subgroups and between each subgroups and controls (Table III). There was only correlation between serum IL-10 and sputum IL-10 levels in asthmatics ($p<0.0008$, $r=0.73$) (Fig 1).

A correlation did exist between neither IL-2 nor IL-10 levels of serum and sputum samples and lung function indices, symptom scores, methacholine PD_{20} level, and eosinophilia. The steroid therapy did not influence the IL-2 and IL-10 levels of patients.

We did not see any adverse reaction during or after the sputum induction procedure in any patient.

Discussion

In this study, besides characteristic eosinophilic infiltrate, neither sputum IL-2 nor IL-10 did show striking difference between asthmatics and controls. Furthermore, there was significant correlation of sputum IL-2 with the percentage of lymphocytes in sputum, but not with the severity of asthma or other clinical indices. Although serum IL-10 correlated with sputum IL-10 in asthmatics, both of these cytokines in serum revealed no further difference between asthmatics and controls, and between mild, moderate, and severe asthmatics.

It has been speculated that reduced IL-10 may favor activation and proliferation of allergen specific T cells in atopic patients (10-12,15). Thus, IL-10 protein level has been measured in the BAL samples of asthmatics and it was characterized by diminished concentration of IL-10 compared to controls (9±18 pg/ml versus 130±61 pg/ml) (18). Takanashi et al supported these findings in first sputum study of IL-10 and IL-10 level in sputum was significantly lower in asthma, COPD patients and healthy smokers compared with that in healthy nonsmokers, furthermore, the percentage of IL-10 positive cells in the sputum was also significantly lower (20). Similarly, Gauvreau et al found that sputum cells immunopositive for IL-10 decreased significantly following allergen challenge (36). In contrast to these findings, increased number of IL-10 mRNA positive cells in BAL and higher IL-10 secretion by macrophages have been shown in asthmatics when compared with controls (19,37). Recently, it has been shown that the numbers of IL-10 mRNA cells were slightly increased in sputum samples of allergic asthmatics when compared with nonatopic asthmatics and controls (38). Furthermore, Hamzaoui et al showed higher production of IL-10 in induced sputum samples of asthmatics during acute exacerbation (39). The authors suggested that IL-10 production may contribute to the resolution of acute response. After these conflicting results, the findings of this study did not reveal different IL-10 levels in asthmatics over controls, also between mild, moderate, and severe asthmatics. There was also no effect of IL-10 level on clinical severity, and no correlation with cellular profile in sputum, however our patients were not on acute attack, all of them were stable asthmatics. This inability to demonstrate significant lower levels of IL-10 in asthmatics might be a result of the DTT treatment of the sputum despite Takanashi et al did not detect the role of DTT and sputum handling on the sputum level of IL-10 (20).

There is increasing evidence about involvement of IL-2 in addition to Th2 cytokines in asthma particularly in nonatopic asthmatics (5,26). No significant differences in percentage of T cells generating IL-2 in BAL specimens and tissue samples were observed between atopic asthmatic patients and normal controls (27,40). Moreover no differences were found between symptomatic and asymptomatic asthmatics (41) or after allergen challenge regarding IL-2 expressing BAL cells (42). It is also of interest to note that higher IL-2 production during the late phase reaction after allergen exposure (28) and allergen segmental provocation supported the role of allergen exposure on IL-2 production. Taken together these conflicting results, we evaluated sputum samples of asthmatics in this study and we found no significant change of IL-2 levels of serum and sputum samples between asthmatics and controls and between subgroups of asthmatics. This may be due to our stable asthmatic population and we did not perform any allergen challenge in our study. However, in the present study symptomatic severe astmatic group did not have higher IL-2 when we

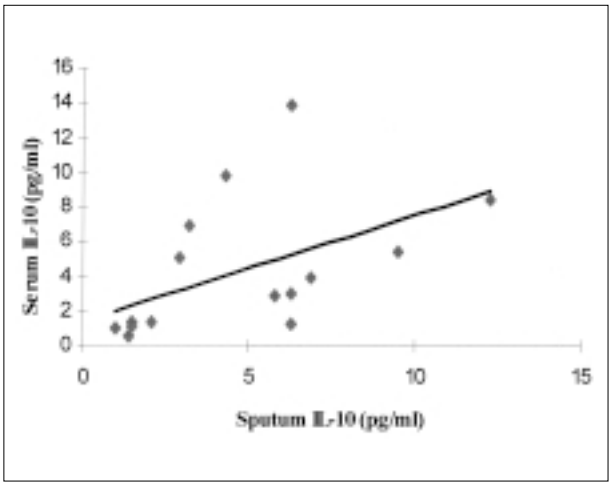

Figure 1. Correlation between serum and sputum IL-10 levels ($p<0.0008$, $r=0.73$)

compared with other mild and moderate asthmatic subgroups. In addition, we found positive correlation between sputum IL-2 levels and percentage of lymphocytes in sputum samples. Our data are in keeping with the evidence of previous study showing positive correlation between IL-2 levels and the percentage of lymphocytes (43) and in accordance with the knowledge of IL-2 being a chemotactic factor for T cells (23,24).

We found no difference between serum and sputum levels of IL-2 and IL-10 between those of asthmatics and controls, the serum IL-10 levels correlated with sputum IL-10 in asthmatics only. This may suggest this cytokine affects two compartments, lungs and peripheral blood. Sanchez et al found that serum IL-10 was significantly increased in nonallergic asthmatics compared with allergic asthmatics, we could not support this study since all of our patients were atopic (44). In addition, Borish et al reported that peripheral blood mononuclear cells of patients with asthma demonstrated decreased spontaneous and stimulated IL-10 production compared with normal subjects (18). We found no significant change of serum IL-2 levels between asthmatics and controls and reinforced contrast results published previously (27,28). Consequently, the contribution of IL-2 or IL-10 is most likely less important in peripheral circulation.

Asthma therapy can influence the cytokine profile in the airway. Our data confirm the observation by Takanashi et al showed that inhaled corticosteroids did not modify the level of IL-10 positive cells in sputum of asthmatics although there were positive and negative studies regarding the effects of steroid on IL-10 level in BAL (16,18,20). In our study, there was also no significant difference between serum and sputum levels of IL-2 in patients who had been taking steroid. Similarly, it has been shown that BAL T cells expressing IL-2 mRNA was not different between asthmatics and controls after corticosteroid therapy (45).

To the best knowledge of the authors, this is the first report determining concentrations of IL-2 and IL-10 together in serum and induced sputum samples of asthmatics. In a previous study, it was found that higher production of IL-10 in induced sputum mononuclear cell culture of asthmatic patients compared with sputum mononuclear cells from healthy controls and no alteration was found in the IL-2 levels, an explanation for the discrepancy between this study and ours, Hamzaiou et al investigated only asthmatics on acute exacerbation and our patients were stable (39). This might be consistent with the previous speculation that IL-10 production in asthma would increase to oppose the synthesis of proinflammatory cytokines in acute asthma. Finally, we found that IL-2 and IL-10 levels in serum and sputum levels have no predictive value since these are not increased in stable asthmatics over controls, but IL-2 levels correlate with lymphocyte percentage in sputum. Further studies are required to validate these interpretations.

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