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Increased Concentrations of Soluble Intracellular Adhesion Molecule-1 in Bronchial Asthmatics Caused by Inhaled Interleukin-5

支气管哮喘患者吸入白细胞介素-5所致细胞间粘附因子增高的研究

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Abstract In a randomized double-blind, placebo-controlled study design, eight nonsmoking patients with allergic asthma and six non-asthmatic allergic subjects were administered recombinant human interleukin (IL) -5 by nebulization, and the concentrations of soluble (s) ICAM-1 in both induced sputum and serum from each subject were determined before and at 2, 24, 48, 72 h after inhalation, respectively. Our results showed that sICAM-1 levels neither in sputum nor serum within the control group had no change from baseline at any time throughout the study. In allergic asthmatics, vehicle challenge was not able to cause any changes in sICAM 1 levels in both sputum and serum. However, there was an increase in sputum sICAM-1 levels after IL-5 inhalation, which increased with time was significantly greater than baseline value, respectively, reaching a maximum at 48 h, and lasted no more than 72 h. And a similar elevation in serum sICAM-1 levels after IL-5 inhalation was observed in asthmatics. Our results indicated that IL-5 was capable of inducing elevations of both sputum and serum sICAM-1 concentrations in allergic asthmatics, but not in non-asthmatic allergic subjects.

Key words Interleukin-5, intracellular adhesionmolecule-1, pathogenesis asthma

摘要 采用随机、双盲及设置对照组的办法进行研究。在吸入IL-5之前，对8例不吸烟的哮喘患者及6例非哮喘过敏性疾病患者测定其痰及血清的sICAM-1；吸入IL-5后，于2 h、24 h、48 h及72 h取上述标本分别测定其sICAM-1的水平。结果示对照组患者的sICAM-1在整个研究过程中都没有明显的改变。但吸入IL-5后支气管哮喘患者于痰中sICAM-1出现明显的增高，于48 h达最高峰，但持续不超过72 h。同样的结果亦出现在血清中。研究表明IL-5可能是导致支气管哮喘患者痰及血的sICAM-1增高的原因，对非哮喘过敏疾病患者则无此作用。

关键词 白细胞介素-5 细胞间粘附因子 支气管哮喘
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It has become common parlance that inflammation is an important component of the acute, subacute, and chronic phases of asthma in humans[1,2]. Th-2 T lymphocyte-derived interleukin 5 (IL-5) is of particular interest in the pathophysiology of asthma as it is associated with eosinophilic inflammation. Increased expression of IL-5 mRNA has been found in sensitized animals and asthmatic patients[3~5].

It has also been demonstrated[6] that as compared with normal controls, the numbers of IL-5 immunopositive cells as well as eosinophils were increased significantly in asthmatic bronchial mucosa, and that

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the degree of airway eosinophilia is associated with an increased expression of IL-5 protein. More recently, our results strongly indicated that IL-5 was capable of inducing eosinophil infiltration into the asthmatic airways, as well as the activation of infiltrating eosinophils[7].

Intracellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and consists of a 90 to 114 single-chain glucoprotein with a polypeptide core containing five domains[8,9], and is a ligand for lymphocyte function-associated antigen-1 (LFA-1)[10]. ICAM-1 expressed on cell surfaces has been considered to play a critical role in mediating cell-cell adhesion during inflammatory responses and is important not only for trafficking of inflammatory cells through endothelium and epithelium[10], but also for cellular presentation of antigen to lymphocytes during allergic inflammatory processes in the airways[11].

Soluble (s) ICAM-1 contains most of the structure of the extracellular portion of membrane-bound ICAM-1 and can also bind to LFA-1[12,13]. In the previous studies we and others have demonstrated that elevated concentrations of sICAM-1 were observed in serum and sputum of asthmatic patients[14~16]. In the present study, we therefore investigated the effect of IL-5 on the changes of concentrations of sICAM-1 in allergic asthmatics by administering recombinant human (rh) IL-5 by nebulization to asthmatics, and measured concentrations of sICAM-1 in both serum and induced sputum in a blinded crossover study.

1 Methods

1.1 Subjects

Eight nonsmoking patients (5 males, 3 females; 19 to 58 yr of age) who met the criteria for a diagnosis of asthma defined by American Thoracic Society[17] were enrolled in this study. All patients had mild atopic asthma, with baseline forced expiratory volume at 1 *s* (FEV1) greater than 70% of predicted value (92 ±3% of predicted), requiring only intermittent use of inhaled β2-agonists. All patients had a provocative concentration of methacholine producing a 20% fall at FEV1 (PC20-Mch) < 8 mg/mL. Each patient had one or more documented positive skin prick test responses to aeroallergens, but none was received immunotherapy or corticosteroid therapy.

Six nonsmoking non-asthmatic allergic subjects (4 males, 2 females; 21 to 51 yr of age) were enrolled for this study. Each subject had one or more documented positive skin prick test responses to aeroallergens without experiencing an asthmatic attack. Each one had a negative history for lung, heart, liver, or kidney disease as well as a normal chest radiograph and pulmonary function tests. No acute respiratory illness had occurred in the preceding 6 wk.

The study protocol was approved by the Ethics Committee of Guangxi Medical University, P. R. China, and all subjects provided written consent.

1.2 Study Design

A randomized double-blind, placebo-controlled study design was employed in which each subject acted as his or her own control.

At a preliminary visit, methacholine inhalation test was performed and skin prick tests to a panel of common aeroallergens were carried out. At the same time, serum and induced sputum samples (refer to the following sections) were obtained for determination of concentrations of sICAM-1. The following afternoon (24 h after baseline measurements), 10 μg of rhIL-5 (Genzyme Co., Boston, MA) in vehicle (0.1% bovine serum albumin in 0.9% saline) or vehicle only was inhaled in a 0.5 mL nebulized solution, the chamber was refilled twice with 0.5 mL vehicle, and the nebulization was kept on until any remaining IL-5 was scavenged. At least 4 wk were allowed to elapse between the two inhalations, and the order of inhalation of IL-5 or vehicle was randomized. The dose of IL-5 was based upon a preliminary study involving two asthmatic patients[18]. Sera and induced sputum were obtained at 2, 24, 48 and 72 h after the inhalation of rhIL-5 or vehicle.

1.3 Blood Samples

Venous blood samples were obtained from each subject. Samples were collected in tubes to obtain sera for determining sICAM-1 levels.

1.4 Sputum Induction and Examination

Sputum induction was performed by the method described previously[18].

Briefly, subjects inhaled nebulized 3.5% saline and lavage orally with water prior to voluntary cough-

ing every 2.5 min until 20 min had elapsed or until 5 mL of sputum had been expectorated. The induced sputum samples were added with an equal volume of 1 mmol/L dithiothreitol (Sigma Chemical Company, St. Luois, MO) in Hank's buffered salt solution, and then were mixed gently by votex mixer and incubated at 37 ℃ for 15 min to ensure complete homogenization. After incubation, the homogenized sputum was centrifugated at 2 000 r/m for 5 min. The supernatants were aspirated and stored at -70 ℃ for later detection of sICAM-1.

1.5 sICAM-1 Assay

Induced sputum and serum samples previously stored at -70 ℃ were thawed. Concentrations of sICAM-1 were determined with commercially available sICAM-1 test kits (Boehringer Mannheim, Germany). This is a sandwich enzyme-linked immunosorbent assay based on the binding of two monoclonal antibodies that recognize different epitopes of sICAM-1.

Both antibodies recognize natural, cell line derived sICAM-1 and recombinant sICAM-1 from E. coli at the same extent. All assays were done in duplicate.

1.6 Statistical Analysis

All data were presented as mean ±standard deviation (SD). Statistical analysis was done by repeated measures analysis of variance (ANOVA) for data conforming to a normal distribution, and by Friedman's test for those data with a nonparametric distribution (confirmed by the Shapiro-Wilk W test).

2 Results

2.1 Effect of IL-5 Inhalation on Levels of sICAM-1 in Sputum

All subjects tolerated the procedures without complication. The effects of rhIL-5 inhalation on changes of sputum sICAM-1 concentrations were shown in Table 1. Baseline values of sICAM-1 in induced sputum before both challenges showed no significant difference from asthmatics, and similar results were observed in non-asthmatic allergic subjects (all $P > 0.05$). In allergic asthmatics, vehicle challenge was not able to cause any changes in sputum sICAM-1 levels (all $P > 0.05$). However, there was an increase in sputum sICAM-1 levels after IL-5 inhalation, which increased with time was significantly greater than baseline value, respectively, reaching a maximum (48 h, and lasted no more than 72 h (all $P < 0.05$ or 0.01). We also noted that in non-asthmatic controls, neither rhIL-5 nor vehicle inhalation was able to cause any changes in sputum sICAM-1 levels at any time throughout the study (all $P > 0.05$).

2.2 Effect of IL-5 Inhalation on Levels of sICAM-1 in Serum

Baseline measurements of sICAM-1 before both IL-5 and vehicle challenge showed no significant difference in serum from allergic asthmatics or control subjects, respectively (all $P > 0.05$) (Table 2). In allergic asthmatics, concentrations of serum sICAM-1 within vehicle inhalation experiments had no change from baseline at any time throughout the study (all $P > 0.05$). It meant vehicle inhalation of asthmatics did not lead to elevations of sICAM-1 levels in circulation. After IL-5 inhalation, the concentrations of serum sICAM-1 increased with time, reaching a maximum at 48 h, and this significant elevations of serum sICAM-1 lasted at least 72 h (all $P < 0.01$). In control subjects, there was no any difference among the initial values of serum sICAM-1 levels measured at the beginning of each arm of the experiment and at subsequent time points (all $P > 0.05$). These results suggested that the inhalation of rhIL-5 did not affect serum sICAM-1 levels in non-asthmatic allergic subjects.

3 Discussion

In recent years, the role of airway inflammation in asthma has received much attention. The development of airway inflammation in asthmatic subjects is accompanied by a highly complex sequence of events at both cellular and hormonal levels in response to various stimuli against airway epithelium.

The course of any inflammatory response depends upon an efficient antigen presentation to relevant leukocytes, the mobilization of these cells to the inflammatory site, and their attachment to target cell, all of these mechanisms being dependent on cell-cell contacts. One of the mechanisms allowing this close cell-cell contact is dependent upon the expression of

Table 1 Significance of sICAM-1 levels in sputum from asthmatics and normal controls challenged with IL-5 ($\bar{x} \pm s$)

Group		sICAM-1 (μg/L)				
		Baseline	2 h	24 h	48 h	72 h
Asthmatics	IL-5	7.0±2.4	8.2±2.5*	10.5±2.6**	11.9±2.5**	10.7±2.4**
	Vehicle	6.9±2.2	7.0±2.3	6.7±2.1	7.3±2.5	6.9±2.3
Controls	IL-5	1.3±0.5	1.3±0.5	1.2±0.5	1.2±0.5	1.0±0.5
	Vehicle	1.5±0.6	1.6±0.6	1.6±0.6	1.6±0.6	1.5±0.6

Compared with baseline measurement, * $P < 0.05$, ** $P < 0.01$.

Table 2 Significance of sICAM-1 levels in sera from asthmatics and normal controls challenged with IL-5 ($\bar{x} \pm s$)

Group		sICAM-1 (μg/L)				
		Baseline	2 h	24 h	48 h	72 h
Asthmatics	IL-5	247.0±47.8	303.3±43.3*	325.1±42.9*	336.9±40.1*	326.9±38.5**
	Vehicle	243.8±46.6	240.6±44.4	249.4±45.2	245.6±48.2	253.1±47.4
Controls	IL-5	100.0±12.9	96.8±15.3	103.0±16.5	108.3±15.6	107.5±9.2
	Vehicle	102.5±12.2	108.0±12.8	104.2±12.3	106.7±10.8	103.3±9.9

Compared with baseline measurement, * $P < 0.05$, ** $P < 0.01$.

cell adhesion proteins, such as ICAM-1, which is a sign of cell activation[19]. It has been reported that increased expression of ICAM-1 in bronchial mucosa and sputum obtained from allergic asthmatics, and this was associated with a significant airway eosinophilia and a significant airway hyperresponsiveness[11,20,21]. When sensitized Brown-Norway rats were administrated with anti-ICAM 1 monoclonal antibody (mAb), the infiltration of eosinophils into the airways as well as airway reactivity were all inhibited[22]. All these data suggest that ICAM-1 is involved in the pathogenesis of bronchial asthma.

Previous studies have shown that sICAM in human serum is structurally similar to cellular ICAM-1 and can participate in LFA-1-dependent adhesive reaction[12,13]. In consistent with the previous reports[14,16], we have also found in the present study that the concentrations of sICAM-1 could be detected in both serum and induced sputum from allergic asthmatics and non-asthmatic allergic subjects and that sICAM-1 levels from asthmatics were significant higher than those from controls. In view of these findings, it seems likely that sICAM-1 released into the serum of asthmatics in response to some stimuli might also retain a capacity to bind LFA-1.

The most important findings in this study were that rhIL-5 inhalation, not vehicle inhalation, of allergic asthmatics produced a marked increase in concentrations of sICAM-1 in induced sputum from patients with allergic bronchial asthma. The changes were time-course-related, in that the response was most marked at 48 h. Compared with baseline measurement before challenge, an increased sputum sICAM-1 levels were observed from 2 h after rhIL-5 challenge, and could last no more than 72 h. We also found that serum sICAM-1 levels were elevated in asthmatics by rhIL-5 inhalation in a similar manner. Our results demonstrated directly that inhalation of 10 mg rhIL-5 in patients with allergic asthma was able to induce an increase in concentrations of sICAM-1 not only in airways but also in circulation. On the other hand, we could not observed that inhaled rhIL-5 had any effects on the changes of sICAM-1 concentrations in non asthmatic allergic subjects.

The exact mechanism by which IL-5 contribute to elevations of sICAM-1 in both sputum and serum is still unknown. In atopic asthmatics, local endobronchial allergen instillation leads to an increased expression of ICAM-1 in bronchial mucosa, and this is accompanied by a significant recruitment of eosinophils into the airways[23]. IL-5 and tumor necrosis factor alpha have been reported to be able to upregulate the expression of ICAM-1 in human eosinophils[24]. These data suggest that IL-5 makes a contribution to the upregulation of ICAM-1 expression on eosinophils. Actually, stimulation of rhIL-5 was directly shown to in-

duce ICAM-1 gene expression in the upper airway mucosa of allergic subjects[25]. In a similar study design to the present study, we also found that there were increases in the eosinophil numbers and eosinophil cationic protein in induced sputum after rhIL-5 inhalation, which increased with time was significantly greater than in control inhalation, reaching a maximum at 24 h[18]. Taken with the previous results[7], it could be concluded that IL-5 not also induces eosinophil infiltration into the asthmatic airways, but also results in the activation of infiltrating eosinophils. Therefore, one mechanism by which IL-5 induced an elevation of sICAM-1 in induced sputum observed in this study might be that eosinophils recruited and activated by IL-5 in the asthmatic airways had an elevated IL-5 expression and thus produced an increased concentration of IL-5 protein into sputum. IL-5 inhalation can also induce an increase in number of activated eosinophils in atopic asthmatics[18,26]. Therefore, the production of sICAM-1 from increased blood eosinophils might be a explanation for the elevation of serum sICAM-1 concentrations caused by IL-5 inhalation in the present study. On the other hand, other source of sICAM-1 might also be a contributor to the IL-5 induced elevation of sputum sICAM-1 levels. In human, lymphocytes, mononuclear cells, granulocytes, fibroblasts[9,24], vascular endothelium[27], alveolar macrophages[28], lung dendritic cells[29], bronchial epithelial cells[30], and airwaysmooth muscle cells[31] are all potential candidates. It is possible that IL-5 stimulates these cells to secret more sICAM-1.

In summary, we have shown that rhIL-5 is functionally important in causing elevations of concentrations of sICAM-1 in both airways and circulation of patients with allergic asthma. Now that sICAM-1 is involved in the infiltration of eosinophils into the asthmatic airways, and recombinant sICAM-1 augments eosinophil oxidative metabolism[32], furthermore, signaling from ICAM-1 and its ligands might induce eosinophil activation and might be involved in degranulation of eosinophil granule proteins, e. g. ECP and eosinophil-derived neurotoxin[33], sICAM-1 might be an important effector in the mechanism by which IL-5 induces airway hyperresponsiveness and eosinophilic inflammationin allergic asthmatics[7,18].

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