

## ORIGINAL ARTICLE

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# Association of the Expression of IL-4 and IL-13 Genes, IL-4 and IgE Serum Levels with Allergic Asthma

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## ABSTRACT

Immune and inflammatory responses mediated by cytokines, play important roles in the pathophysiology of asthma. These responses are associated with overexpression of Th2 cytokines such as IL-4 and IL-13. These two cytokines use common receptors for signaling that lead to identical immunological effects and regulation of the Th1/Th2 balance. The aim of this study was to determine whether patients with allergic asthma display overexpression of IL-4 and IL-13 genes.

Using RT-PCR, we examined the expression of IL-4 and IL-13 genes in twenty asthmatic cases and twenty normal individuals. Total levels of serum IgE and IL-4 were also determined by ELISA method.

Expression of IL-13 gene in 70% of patients with allergic asthma was higher than controls ( $P=0.01$ ). There was no correlation between the expression of IL-13 gene and total level of serum IgE ( $P=0.07$ ). Expression of IL-4 gene was detected in 30% of the patients and none of the normal individuals as determined by RT-PCR ( $P=0.01$ ). Mean of serum IgE levels in patients and controls were 84.9 IU/ml and 62.2 IU/ml, respectively. Level of serum IgE was more than 100 IU/ml in 30% of patients ( $P=0.03$ ). Mean of serum IL-4 levels in patients and controls were 15.73 pg/ml and 13.07 pg/ml, respectively. There was a relation between levels of serum IgE and IL-4 in 73% of cases.

The results showed that there was a correlation between the expression of IL-4 gene and the level of serum IL-4. Levels of serum IgE and IL-4 were considerably higher in asthmatics than non-asthmatic controls.

**Keywords:** Asthma; Genes; IL-4; IL-13; RT-PCR

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## INTRODUCTION

Asthma is a multifactorial disease, influenced by genetic and environmental factors, and is characterized

by bronchial hyperresponsiveness (BHR) and variable degrees of airways obstruction, presence of IgE antibodies to inhalant allergens and often also by enhanced total serum IgE levels. A switch recombination of antibodies to IgE requires two signals from activated T cells; the expression of the ligand for CD40 and the secretion of IL-4 or IL-13.<sup>1</sup> Since family and twin studies have demonstrated the involvement of genetic predisposition in the development of atopy and asthma, much work has been done to find the responsible genes. Several loci linked to atopy and asthma have been suggested through genome-wide linkage studies.<sup>2</sup>

A linkage between total serum IgE and markers on chromosome 5q21- 5q33, which contains the IL-3, IL-4, IL-5, IL-13 and granulocyte-macrophage colony stimulating factor (GM-CSF) genes has been shown.<sup>3,4</sup> Several studies have demonstrated a linkage or association of the atopic phenotype with T-cell cytokine genes involved in the regulation of Th1/Th2 balance (e.g. IL-4, IL-13, and their common receptors, IL4RA).<sup>5,6</sup> Antigen specific IgE responses are regulated by human major histocompatibility complex (MHC) HLA class II and T-cell receptor (TCR) genes and involve T-B cognate interactions. By contrast, non-antigen specific IgE responses (determined as total serum IgE levels) involve non-cognate interactions of mast cells, basophils and T cells with B cells.<sup>7</sup>

Interleukin4 (IL-4) and IL-13 are pleiotropic cytokines contributing to the maintenance of the Th2 lymphocyte profile that leads to the elevation of baseline IgE levels. Murine models have demonstrated the critical nature of IL-13 independent of IL-4.<sup>8</sup> IL-13 is produced predominantly by T cells; in addition, human basophils and mast cells are also able to secrete IL-13. In contrast to IL-4, which is produced by peripheral blood CD4+ T cells, IL-13 is produced by CD4+ as well as CD8+ T cells and by both naïve and memory T cells. The regulation of IL-13 production is totally different from that of IL-4. This is illustrated by the fact that stimulating T cells with anti-CD28 and phorbol myristate acetate (PMA) results in the optimal induction of IL-13, with almost no IL-4 secretion. The local tissue expression of IL-13 has been shown in nasal mucosa of patients with allergic rhinitis and in bronchoalveolar lavage fluid of patients with allergic asthma after pulmonary allergen challenge, whereas normal controls failed to express IL-13. In peripheral blood mononuclear cells (PBMC) from patients with

atopic dermatitis, increased spontaneous expression of IL-13, but not IL-4 mRNA has been found. Little is known about the contribution of IL-4 and IL-13 to the production of IgE in patients with allergic asthma.<sup>1</sup> In order to evaluate the role of IL-4 and IL-13 in modulating asthma responses, we studied the association between the expression of IL-4 and IL-13 genes and total levels of serum IgE and IL-4 in patients with allergic asthma.

## PATIENTS AND METHODS

### Patients and Control Subjects

Twenty patients with allergic asthma, twelve males and eight females (mean age 37 years, range 26-54) were recruited from the asthma clinic of Ghaem medical centre in the city of Mashhad, Iran.

Diagnosis of asthma was based on the guidelines proposed by the American Thoracic Society.<sup>9</sup> Each patient showed airway reversibility as determined by an inhalant bronchodilator-induced improvement of more than 15% of forced expiratory volume in one second (FEV1). There was no patient with the history of any other systemic disease or corticosteroid taking which could affect gene expression. Twenty sex/age-matched normal non-asthmatic volunteers took part in this study as controls. This study was approved by the local medical ethics committee, and written consents were taken from all participants.

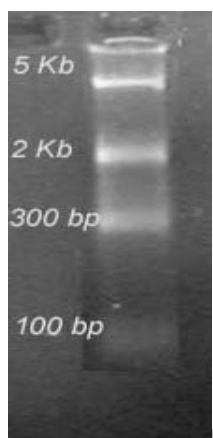
### Cell Isolation and Cultures

Mononuclear cells from patients and controls were isolated from peripheral blood by a density separation over Ficoll-paque (Pharmacia Biotech, Uppsala, Sweden). We used pooled PBMCs of normal subjects cultured in Iscove's modified Dulbecco's medium (IMDM) (Gibco, Life Technologies, Paisley, United Kingdom) at 5000 cells/ 200 µl/ well, with 10% FCS supplemented with penicillin 100 IU/ml and streptomycin 100 µg/ml as the control for determining the spontaneous expression of IL-13 and IL-4 mRNAs. Cells were kept at 37 °C in a 5% CO<sub>2</sub> incubator and processed for RNA extraction and subsequent mRNA analysis.

### RNA Isolation and cDNA Synthesis

Cellular RNA was extracted using a standard Trizol reagent (Life Technologies, Rockville, MD). In brief, cells were lysed in Trizol, homogenates were then

transferred to microcentrifuge tubes, and chloroform was added (at room temperature for 5 minutes). After centrifugation (14000 RPM for 5 minutes at 4 °C), the aqueous phase was transferred to a new tube and isopropanol was added (10 minutes at room temperature). Tubes were centrifuged (14000 RPM for 10 minutes at 4 °C), supernatants were removed, and the pellets were washed in 75% ethanol. 1 microgram of total RNA, M-MuLV Reverse Transcriptase and oligo(dt) (Fermentas, Germany) were used for the synthesis of cDNA. All RNAs were examined by agarose gel electrophoresis for obtaining an optimal RT-PCR. RNAs were intact and exhibited clear bands (Figure 1).



**Figure 1.** RNA bands after electrophoresis; 5 kb, 2 kb, 300 bp, and 100 bp for 28s, 18s, 5s, tRNA bands, respectively.



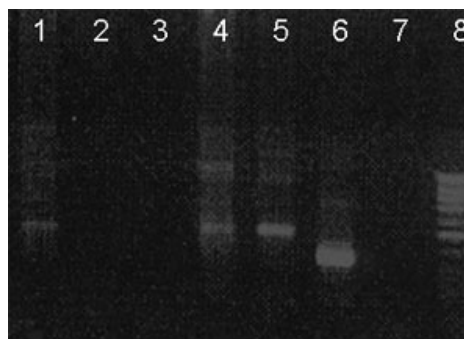
**Figure 2.** Analysis of the expression of IL-13 mRNA. Lane 1:  $\beta$ -actin as 215 bp band; Lane 2: Ladder (100 bp); Lane 3: Expression of IL-13 mRNA as 450 bp band.

#### RT-PCR for IL4 and IL-13

The synthesized cDNA was amplified by RT-PCR method. It was initially denatured at 94 °C for 5 min followed by 40 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min for IL-4. IL-4 primers were as follows: sense, 5'-CAG CTC GAA CAC TTT GAA-3', antisense, 5'-TCT CAC CTC CCA ACT GCT-3'; PCR amplification for IL-13 was performed by the same denaturing step as IL-4 and 35 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. IL-13 primers were as follows: sense, 5'-GCT CCT CAA TCC TCT CCT GTT-3'; antisense, 5'-GCA ACT TCA ATA GTC AGG TCC-3'. The PCR reaction mixture (final volume of 50  $\mu$ l) contained 1.0 U Taq DNA polymerase (Boehringer Mannheim, Bromma, Sweden) and 10  $\mu$ M (micromole) each of sense and antisense primers. PCR products were then analyzed by electrophoresis on a 1.5% agarose gel and IMAGO gel documentation system (B&L system, Germany). Validity of PCR was controlled by  $\beta$ -actin housekeeping gene ( $\beta$ -actin primers were: sense, 5'-AAG GCC AAC CGC GAG AAG ATG-3', antisense, 5'-ACA GGA CTC CAT GCC CAG GAA-3') (Figure 2).

#### Total IgE and IL-4

Blood samples were collected from all participants to determine total levels of serum IgE and IL-4. Measurements were done using commercial ELISA kits for IgE and IL-4 (Tahghighgostar, Iran and Bender MedSystem, Austria, respectively).



**Figure 3.** Analysis of the expression of IL-4 mRNA. Lane 1, 4, 5: patients with the expression of IL-4 mRNA as 438 bp; Lane 2, 3, 7: Lack of IL-4 mRNA expression in other patients; Lane 6:  $\beta$ -actin as 215 bp band; Lane 8: Ladder (100 bp).

**Table 1. Mean of IgE and IL-4 serum levels in patients and controls.**

Variable	Patients	controls	P value
IgE serum level (IU/ml)	84.90±40.33	62.20±20.18	0.03
IL-4 serum level (pg/ml)	15.73±2.30	13.07±2.33	0.01

## RESULTS

Expressions of IL-4 and IL-13 genes was determined by RT-PCR using specific primers in asthmatic patients and normal controls. Extracted RNA from PBMCs was run on agarose gel. Four bands were detected and proved to be 5kb, 2kb, 300bp and 100bp which represented 28s, 18s, 5s and tRNA bands, respectively (Figure 1). A band of 438bp was detected for IL-4 (Figure 3). Expression of IL-4 gene was observed in 30% of patients. In contrast, IL-4 gene was not expressed in controls.

Amplification of IL-13 gene was detected by a 450 bp band after gel electrophoresis (Figure 2). Moreover, the expression of IL-13 mRNA was higher in patients than controls. IL-13 gene was expressed in 70% of asthmatic cases; while, none of controls expressed this gene (P=0.07).

Level of serum IgE in asthmatic patients was also admittedly higher than controls (84.9 IU/ml and 62.2 IU/ml, respectively; P=0.03) (Table 1).

There was no correlation between the expression of IL-13 mRNA and total level of serum IgE. There was a relation between serum IgE and IL-4 in 73% of cases. However, there was no correlation between these two parameters in 27% of cases. In almost 30% of asthmatic patients, level of serum IgE was more than 100 IU/ml (P=0.03). Levels of serum IgE and IL-4 are shown in table 1.

## DISCUSSION

Asthma is characterized by BHR to a variety of specific and non-specific stimuli, chronic pulmonary eosinophilia, elevated serum IgE and excessive airway mucus production.<sup>10</sup> The pathogenesis of asthma is thought to be mediated by CD4+ T lymphocytes that produce a type 2 cytokine profile.<sup>11</sup> IL-4 and IL-13 appear to play key roles in the genetic susceptibility to asthma. Observations in experimental animals suggest that IL-13 may play a more important role than IL-4 in airway remodeling through its effects on BHR. In this

study, we chose to analyze the expression of IL-4 and IL-13 genes by RT-PCR using specific primers in asthmatic and control groups. We did not use allergen-induced T cell differentiation, which is already known to lead to Th2-biased responses,<sup>12,13</sup> neither allergen-specific IgE-producing B cells which are no longer influenced by IL-4 or IL-13.<sup>6</sup> The expression of IL-4 and IL-13 genes in asthmatics and non-asthmatic controls were evaluated in PBMCs. In addition, levels of serum IgE and IL-4 were measured by ELISA method. Our results indicate that total level of serum IgE in patients is considerably higher than controls. IL-4 is one of the two cytokines known to cause switching in B-cells, a prerequisite for elevated IgE synthesis.<sup>14</sup> There is a correlation between the expression of IL-4 gene and IL-4 glycoprotein in serum as a gene product. However, there were some asthmatic cases who expressed IL-4 gene; while, they had normal level of serum IL-4. This might conceivably take place for the following reasons: First, RT-PCR is noticeably more accurate than ELISA and second, cytokines are transient and non-permanent products.<sup>15-17</sup> Humbert et al. demonstrated expression of IL-4 and IL-5 proteins in the bronchial mucosa of both atopic and non-atopic asthmatics, which strongly suggests the translation of IL-4 and IL-5 mRNAs, although the translation rate and efficiency for individual cytokines may vary.<sup>3</sup> Kabesch et al. genotyped a large cross-sectional population of 1120 children aged 9 to 11 years for 18 polymorphisms in the respective genes of the IL-4/IL-13 pathway. One polymorphism per gene was selected because of its putative functional role, and extended haplotypes were built in a stepwise procedure where gene-by-gene interactions were assessed using a Cordell model. Their data indicated that only the combined analyses of genetic alterations in the IL-4/IL-13 pathway revealed its actual significance in the development of atopy and childhood asthma.<sup>18</sup> Moreover, in most of the asthmatic patients there was a correlation between the expression of IL-4 gene and the level of serum IgE. This might not be take place in steroid-dependent patients because of gene suppression.

Our observed correlation between the expression of IL-4 gene in PBMCs and the level of serum IgE in asthmatics is compatible with the hypothesis that the maintenance of elevated IgE synthesis in these patients is at least partly IL-4 dependent. We found no correlation between IL-4 gene and its products in some non-allergic asthmatics. This could be due to the fact that some cytokines might not be detectable while their genes have been expressed.

Allergen provocation is a very useful way to study the inflammatory response in asthmatic patients. The expression of IL-4, IL-5, IL-13 and interferon (IFN)-gamma mRNAs was found in bronchoalveolar lavage (BAL) cells and peripheral blood CD4+ and CD8+ T cells following any of the two provocation regimens.<sup>19</sup>

In another research the data suggest that in an allergic environment, Th2 cytokines and allergens have the potential to sustain airway inflammation through a cooperative effect on cytokine release by the bronchial epithelium. The novel finding that IL-4, IL-13, and allergen enhance release of TNF-alpha, a ligand for the epidermal growth factor receptor that stimulates fibroblast proliferation and goblet cell differentiation, provides a potential link between allergen exposure, Th2 cytokines, and airway remodeling in asthma.<sup>20</sup>

Truyen et al. studied the airway T cell cytokine profiles including IL-4, IL-5, IL-13, IL-10 and IFN-gamma measured in sputum of 15 healthy and 39 asthmatic individuals at the mRNA level by real time RT-PCR. They found that the levels of expression of IL-4, IL-5, and IL-13 mRNAs in sputum were significantly correlated with the percentage of eosinophils and were higher in subjects with allergic asthma than in those with non-allergic asthma ( $p = 0.03, 0.02, 0.0002$ , respectively).<sup>21</sup>

Regarding the expression of IL-13 mRNA, our results were consistent with the other researchers that investigated the expression profile of IL-13 mRNA in other specimens such as bronchoalveolar lavage or bronchial biopsy specimens. The striking observation was that IgE production in the asthmatic group was more independent of the expression of IL-13 mRNA. Overexpression of IL-13 mRNA with normal levels of total IgE might happen in the non-allergic asthmatics or patients who have no expression of IL-13 gene; however, high total level of serum IgE may be due to other cytokines such as IL-4. Wills-Karp et al. showed that IL-13 was one of the cytokines that induced the

pathophysiological features of asthma in a manner that was independent of IL-4, IgE and eosinophils.<sup>22</sup>

This study provides additional evidence that the expression of Th2 cytokine (IL-4, IL-13) genes play key roles in the imbalance of Th1/Th2 in asthmatic patients; but the role of IL-13 is more prominent.

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