Serum Interleukin-13 Levels Are Elevated In Mild And Moderate Persistent Asthma

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Citation

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Abstract

Background: Interleukin-13 is thought to play a key role in modulating airway inflammation in asthma. Elevated levels of serum interleukin-13 (IL-13) have been documented in acute severe asthma. However, there are no reports of serum IL-13 levels in mild and moderate asthmatics who form the majority of patients.

Methods: In a cross sectional survey, fifty-four asthmatics and 54 age and sex matched controls were recruited prospectively from an outpatient department. Information on asthma severity and use of inhaled steroids was gathered by a questionnaire.

Results: There were 32 atopic and 22 non-atopic mild-to-moderate asthmatics. The median serum IL-13 level in the whole group of asthmatics (6.67 pg/ml) was significantly higher than in normal controls (3.09 pg/ml) (p=0.001). The median serum IL-13 levels in moderate persistent asthmatics (7.72 pg/ml) and mild persistent asthmatics (5.11pg/ml) were significantly higher than in normal controls (p<0.01and p=0.03). Whereas there was a tendency for serum IL-13 levels among moderate persistent asthmatics (7.72pg/ml) to be higher than in mild persistent (5.11 pg/ml) asthmatics, the difference was not statistically significant (p=0.9). The median serum IL-13 levels were not different among patients using regular inhaled glucocorticoids (6.72 pg/ml) and those using only an inhaled beta-2 agonist (3.36pg/ml) (p=0.8).

Conclusion: Serum IL-13 levels are elevated in mild and moderate persistent atopic and non-atopic asthmatics. These data suggest the presence of a systemic Th2 inflammatory response even in mild and moderate asthmatics, which is not abrogated by inhaled glucocorticoids.

INTRODUCTION

Asthma is a common respiratory disorder worldwide and the vast majority of patients exhibit mild to moderate states of clinical severity ([1]). IL-13 has been recognized as a key cytokine mediating allergic airway inflammation and airway remodeling in asthma which is characterized by mucous hypersecretion, airway hyper-responsiveness and sub-epithelial fibrosis ([2,3]). IL-13 is an immunoregulatory cytokine generated predominately by activated TH2 cells and it shares many functional properties with IL-4 ([4]). Animal studies have shown that the administration of either IL-13 or IL-4 confers an asthma-like phenotype to nonimmunized T cell-deficient mice by an IL-4 receptor alpha chain-dependent pathway ([5]). Lung fibroblasts activated by IL-4 and IL-13, may act as effector cells not only in the pathogenesis of asthma but also in lung remodeling processes ([6]). The role of mast cells in the expression and modulation of airways inflammation through IL-13 transcription has been highlighted by Masuda et. al. ([7]). Also, Brightling and associates demonstrated that in asthmatics, IL-4+ and IL-13+ cells present within the airway smooth muscle were predominantly expressed by mast cells, suggesting that IL-4 and IL-13 may play an important role in mast cell-airway smooth muscle interactions ([8]). Lee and associates have demonstrated elevated serum IL-13 levels in patients with acute severe asthma ([9]). Recently, we reported that levels of circulating serum interleukin-5, a Th-2 cytokine, were elevated in patients with mild and moderate

persistent asthma([10]).

The genes encoding the cytokines IL-4, IL-5, IL-13, and GM-CSF are located in close proximity on human chromosome 5 and a motif in the promoters of these genes with a common palindromic sequence has been described([11]). Therefore, increased expression IL-5 in asthmatics is likely to activate overproduction of IL-13 by sensitized CD4 T cells. To this effect, Jenmalm and associates have documented an increased expression of IL-4, IL-5, IL-9 and IL-13 by peripheral blood mononuclear cells (PBMC's) isolated from sensitized children after stimulation with the specific allergens([12]). Therefore, it is possible that serum IL-13 levels, also a cytokine derived from Th2 cells, may be elevated in this cohort of patients with mild to moderate persistent asthma. To test this hypothesis, we measured serum IL-13 levels in an adult population with mild and moderate persistent asthma some of whom were atopic and others non-atopic.

METHODS

Asthmatic patients attending the outpatient department of a university hospital between January 2002 and May 2003 were recruited into this prospective study. Fifty-six patients (15 males, 41 females, ages 18 - 55 years) with a clinical diagnosis of asthma i.e. any patient with a history of recurrent wheeze, cough and dyspnea in the previous 12 months and a spirometry reading showing an improvement in FEV1 of 12% or more following a reversibility test, were included as study subjects ([13]). Two patients, whose serum sample volume was insufficient to carry out the IL-13 measurement, were excluded. Patients were excluded if they were pregnant, smokers or had received oral glucocorticoids, antileukotreines, theophyllines or antihistamines in the 4 weeks prior to recruitment. Patients receiving medication for any other illness were also excluded. Of the 54 patients, 30 had not received inhaled glucocorticoids two weeks prior to recruitment to the study whereas 24 reported regular use of inhaled glucocorticoids. Of the twenty-four patients using regular inhaled glucocorticoids, 19 were on fluticasone 250 microgram twice daily and five were on budesonide 400 microgram twice daily. The institutional research ethics committee approved the study protocol. After obtaining informed written consent, details of asthma symptoms and treatment, especially data regarding the use of inhaled corticosteroids were collected using a pre-designed questionnaire.

Patients were categorized as atopic if one or more specific IgE test was positive to the common allergens prevalent locally (Dermatophagoides pteronyssinus, Dermatophagoides farinae, Bermuda grass, mesquite, cockroach and Aspergillus fumigatus). A positive test was identified when the specific antibody value was >0.35 KIU/ml. In addition, study subjects were classified into one of three asthma subgroups: mild intermittent, mild persistent or moderate persistent using modified clinical criteria proposed by the National Institute of Health since peak flow variability data were not available in this cross-sectional study ([13]).

Healthy adults between the ages 18 and 55 years, matched for age and sex were recruited as normal controls. The following were exclusion criteria for normal controls: history of childhood asthma, family history of asthma, a febrile illness or chest infection within the previous four weeks, or episodes of cough and wheezing in the past 12 months. Finally, subjects with a serum total IgE value of >120 IU/L were excluded from the control group. A forearm venous blood sample was drawn from all study subjects for complete blood count and cytokine estimation upon recruitment.

Serum total IgE and specific IgE measurement: Total serum IgE was measured by a Microparticle Enzyme Immunoassay (IMx Total IgE Assay, Abbott Laboratories, IL,USA). Serum-specific IgE to six common allergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, Bermuda grass, mesquite, cockroach and Aspergillus fumigatus) was measured using ImmunoCAP technology (Pharmacia Upjohn Diagnostics, Uppsala, Sweden). The complete blood count was documented using a Coulter counter.

MEASUREMENT OF SERUM INTERLEUKIN-13

IL-13 was measured using a specific enzyme linked immunoassay (BioSource, Camarillo, CA). This assay has been calibrated against the World Health Organization reference preparation 94/622 (NIBSC, Hertfordshire, UK). The IL-13 standard curve was constructed using serial doubling dilutions of standard IL-13 from 0 to 2500 pg/ml. A curve-fitting software program was used to quantitate IL-13 concentrations. The minimum level of detection of IL-13 with this method was 2 pg/ml. The intra-assay coefficient of variation was 5% and the inter-assay coefficient of variation was 7.4%.

STATISTICAL ANALYSIS

Data were analyzed using computer software SPSS. All continuous variables not normally distributed, were compared using the Kruskal-Wallis test and if found significant then the Mann-Whitney test was used for pair-wise comparisons. The final p value for multiple comparisons was adjusted using Bonferroni method. Normally distributed continuous variables within groups like age, duration of asthma and FEV1 were compared using the t-test. Associations between serum IL-13 and serum IgE levels were estimated using Pearson's correlation coefficient. A p value of <0.05 was considered statistically significant.

RESULTS

Table 1 shows the age, sex and pertinent laboratory data among study subjects. Of the fifty-four asthmatics, 32 were atopic and 22 non-atopic. Asthma severity was mild-intermittent in 4 patients, mild-persistent in 36 and moderate-persistent in 14. As there were only 4 patients in the mild-intermittent group, they were combined with the mild persistent group for statistical analysis. Table 2 provides the eosinophil count and serum cytokine profile in study subjects. As expected, the median serum total IgE level was significantly higher in atopic and non-atopic asthmatics compared to normal controls (both p<0.0001). Furthermore, the median serum total IgE level in atopic patients was significantly higher compared to non-atopic asthmatics (p<0.0001). The median serum IL-13 level was significantly higher in atopic (6.6pg/ml) and non-atopic asthmatics (7.03pg/ml) compared to normal controls (3pg/ml) (p=0.03 and p =0.01 respectively). However, the median serum IL-13 levels were not significantly different between atopic and non-atopic asthmatics.

Figure 2

Table 2: Laboratory Results in Study Subjects

Variable	Control n=54	Atopic n=32	Non-atopic n=22	P value
Age(mean)	27.5	28.5	27	* 0.8
Sex (m/f)	19/35	10/22	9/13	**0.33
Duration of asthma (months)	--	11(4.2-15)	7(1-10)	# 0.3
FEV1 (liters)	--	2 (1.7-2.5) (69.7%)	2.5 (2-2.8) (71.2%)	# 0.2
Severity of asthma *Mild persistent*		22	18	***0.35
Moderate persistent		10	4	

* by Kruskal Wallis test
# by Man-Whitney test
** by Pearson's Chi-square test
*** by Fishers Exact test

Figure 3

Figure 1: Serum IL-13 levels according to the severity of asthma

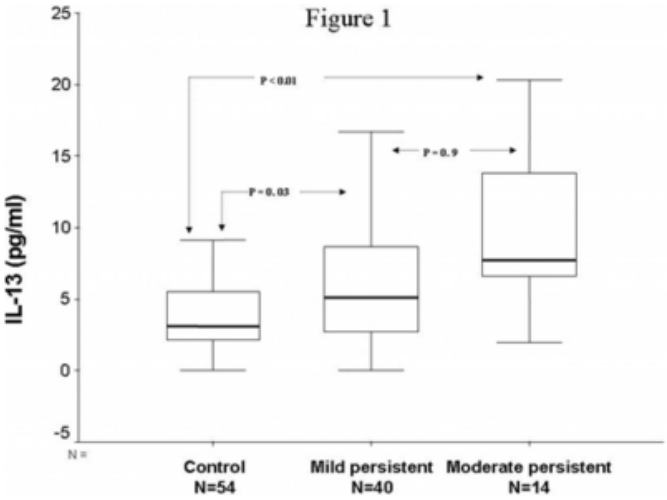
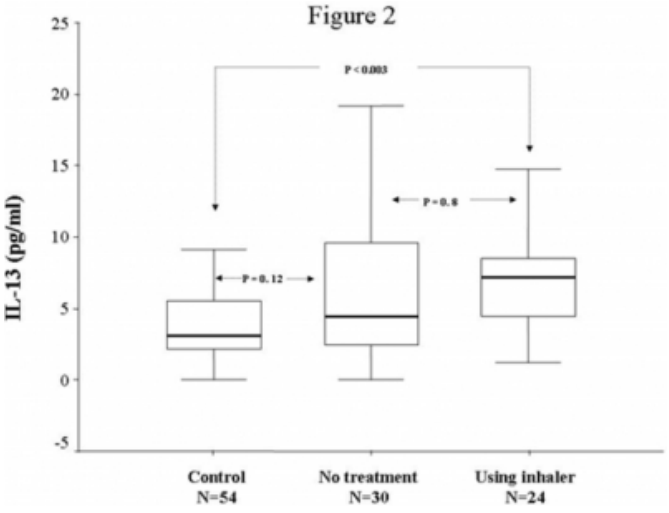


Figure-1 shows serum IL-13 levels in the mild and moderate persistent asthmatics. Of the 54 asthmatics, 24 reported regular use of inhaled glucocorticoids. The median serum IL-13 level was significantly higher in mild persistent asthmatics (5.11pg/ml) and moderate persistent asthmatics (7.72pg/ml) compared to normal controls (p=0.03 and p<0.01). However, there was no significant difference between median serum IL-13 levels in moderate persistent asthmatics (7.72pg/ml) compared to mild persistent asthmatics (5.11 pg/ml) (P= 0.9). Fig 2 shows median serum levels of IL-13 in asthmatics using regular inhaled glucocorticoids, in those not using inhaled glucocorticoids and in normal controls. The median serum IL-13 level was significantly higher in patients using regular inhaled glucocorticoids (6.72pg/ml) compared with normal controls (3.09pg/ml, p<0.001). However, there was no difference between the median serum IL-13 in normal controls and those not on regular inhaled glucocorticoids (p=0.12). There was no significant correlation between serum IL-13 level and IgE level in asthmatics (p=0.24) or normal controls (p=0.76). Also, there was no correlation between serum IL-13 levels and the peripheral eosinophil count in atopic (p=0.7) and non-atopic (0.55)) asthmatics.

Figure 4

Figure 2: Serum IL-13 levels according to treatment status.



The Box and Whisker plot shows the median and interquartile values of serum IL-13 levels. The median serum IL-13 values in mild persistent and moderate persistent groups were significantly higher compared to normal controls (both p = 0.03 and <0.01). There was a trend for the median serum IL-13 to be higher in moderate persistent asthma than in mild persistent type of asthma (p=0.9)

{image:4}

The Box and Whisker plot shows the median and interquartile values of serum IL-13 levels. The median serum IL-13 values in patients on regular inhaled glucocorticoids was significantly higher compared to normal controls (p<0.003) There was no significant difference in the median serum IL-13 levels between asthmatics using and not using inhaled glucocorticoids (p=0.8). The median serum IL-13 levels in controls and in asthmatics not using inhaled steroids were not significantly different (p=0.12)

DISCUSSION

Asthma is a chronic inflammatory disease of the airways characterized by mucus cell gland hyperplasia, basement membrane thickening and eosinophil infiltration ([14]). The role of IL-5 and IL-13 in this local inflammatory process is well established ([15,16,17,18]). We now report that serum IL-13 levels are also significantly elevated in the same cohort of patients with mild and moderate persistent asthma. As expected, the elevation of IL-13 levels was of similar magnitude for both atopic and non-atopic types of asthma compared to normal controls, thus pointing to a common inflammatory mediator in atopic and non-atopic types of asthma ([19]). Furthermore, there was a trend for serum IL13 levels to be higher in moderate persistent asthmatics compared to mild persistent asthmatics.

Therefore, elevation in serum levels of both IL-13 and IL-5 in mild and moderate persistent asthmatics may points towards an ongoing systemic Th2 inflammatory response. The significance of a systemic inflammatory response has been recognized in patients with Chronic Obstructive Pulmonary Disease([20,21]). Such a phenomenon is increasingly being recognized in asthma ([10,22,23,24,25]).

The source of elevated serum IL-13 levels in this cohort of asthmatics is not clarified by our cross-sectional study. However, there is evidence to suggest that the circulating PBMCs may be a major source for circulating IL-13 in asthmatics ([26]). In addition, the proportion of IL13 producing PBMC's has been shown to increase upon exposure to allergens ([27]). The contribution of Natural Killer T cells (NKT cells) ([28]) and mast cells ([7,8]) to the production of IL-13 is also increasingly recognized in asthmatics.

The serum IL-13 level was elevated in both atopic and non-atopic asthmatics in this cohort. Interestingly, increased expression of IL-13 has been documented in the bronchial mucosa of atopic and nonatopic asthmatics ([29]). Therefore, spillover of IL-13 from inflamed airways to peripheral blood cannot be discounted as a possibility for the raised serum IL-13 levels observed in this cohort of asthmatics.

Bronchial hyperresponsiveness and airway inflammation can be elicited through IgE independent mechanisms as documented in an experimental model of asthma ([30]). Furthermore, a human genetic study involving IL13 gene polymorphism has clearly shown a relationship between asthma susceptibility independent of serum IgE levels([31]). This may partly explain the discordant relationship between IL-13 and IgE concentrations in our study subjects.

While the significance of elevated airway IL-13 levels is being evaluated ([19,32,33]), the inability of inhaled glucocorticoids, even in high doses, to abolish the airway remodeling processes([34]), needs attention. Inhaled glucocorticoids reduce airway inflammation and can, under some circumstances suppress airway IL-13 production ([35]). However, there is a paucity of data concerning regular use of inhaled glucocorticoids and regulation of the antigen sensing processes by airway dendritic cells and its presentation to CD4 lymphocytes. Therefore, it is possible that in patients

with persistent asthma, the processes of airway allergen recognition and presentation of these allergens to the regional lymph nodes by dendritic cells continue despite suppression of airway inflammation by inhaled glucocorticoids. These sensitized CD4/NKT cells circulating in the peripheral blood may be expressing increased amounts of Th2 cytokines contributing towards the raised IL-13 levels observed in asthmatics.

The role of systemic administration of antibody targeted against mediators of inflammation in asthma including anti IgE is well established. However, the use of antibody against th2 cytokines such as IL-5, is still at an experimental stage. In this context, IL-13 blocking antibody has been tested in animal models of asthma with some success ([36,37]). Also, gene therapy directed against IL-4 receptors may be yet another new approach to controlling airway inflammation in asthma ([38]). Clearly, it remains to be seen whether the administration systemic antibody against IL-13 in asthmatics would be rewarding.

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