Serum Soluble Interleukins-2 Receptors in Bronchial Asthmatic Children

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Abstract: Background: Bronchial asthma is an inflammatory airway disease characterized by infiltration of inflammatory cells into bronchial tree and increased airway hyper-reactivity to various physical and chemical stimuli. The aim of this study was to detect soluble interleukin-2 receptors (sIL-2) serum levels, as marker of T lymphocyte activation in *vivo,* among bronchial asthmatic children in relation to infection, atopy status and disease severity. **Methods:** Sixty bronchial asthmatic children (30 with acute and 30 with stable asthma); and 17 apparently healthy children as controls were recruited. History taking and clinical examinations were performed among all studied groups. Venous blood sample was withdrawn for measuring of sIL-2R using ELISA technique. Pharyngeal swabs were taken for detecting organism causes the disease.**Results:** The predominant infection was viral with total 40% of examined cases; *respiratory syncitial virus* and *Adenovirus* were prevalent virus pathogens in asthmatic children. While *Haemophilus influenza* and *Candida albicans* were most common causes of bacterial infections. sIL-2R serum level was significantly elevated in acute and chronic asthmatic children versus controls and in acute versus chronic patients. Meanwhile, in acute asthmatic children, insignificant differences were recorded between different degrees disease severity or allergic status. **Conclusion:** sIL-2R is an important interleukin associated with bronchial asthma in children; this interleukin can indicate disease activity. In addition it can't be used as indicator for severity or atopy of the disease.
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1. Introduction

Asthma is a chronic inflammatory lung disease that leads to significant morbidity, mortality, and financial burden (1) Bronchial asthma is characterized by episodic reversible narrowing of the airway, with associated bronchial hyper reactivity. Inflammation is responsible for airway obstruction and hyper responsiveness (2). Persistent inflammation of the respiratory mucosa, characterized by an eosinophilic infiltrate, and also involving other cell types (lymphocytes, mast cells, basophils and neutrophils) is thought to be important in the pathogenesis of asthma and is associated with airway hyper-responsiveness, a hallmark of the disease (3).

T-cells may orchestrate inflammatory responses to inhaled antigen and other stimuli in asthma by producing several cytokines (4).

Activation of T lymphocytes results in expression of interleukin-2 receptor (IL-2R) on the cell surface and releases of soluble interleukin 2 receptor (sIL-2R), a subunit of the IL-2 cell surface receptor molecules into the circulation (5). Various studies have confirmed the strong association between serum sIL-2R levels and the activation of T lymphocytes *in vitro* and have indicated that sIL-2R production is directly proportional to cellular IL-2R expression (6).

There is a worldwide trend of increasing asthma prevalence, with large international variations. A number of studies have been undertaken to try to explain the variations and to detect risk factors for the development of childhood asthma. The risk factors are several; including allergy, eczema, antibiotic use, reactions to food, early respiratory syncytial virus infection and parental asthma. Viruses are the most common causative agents of lower respiratory tract infections in young infants' worldwide (7). Among them, *respiratory syncytial virus* (RSV) and *adenovirus* (ADV) are responsible for 30 and 5% of hospitalized cases, respectively (8; 9).

To our knowledge, there are no studies that have examined the activation status of T cells in stable and acute asthma in children in relation to causative organisms. It is very important to measure peripheral blood markers (minimally invasive method) to assess airway inflammation in children with asthma. So, this cross sectional study aimed to evaluate the serum levels of soluble marker of T-cell activation in *vivo* sIL-2R level in the serum in acute and stable asthmatic children in relation to causative organisms, allergic status and disease severity.

2. Material and Methods:

This cross sectional study was conducted on 60 children suffering from bronchial asthma with duration of at least 6 months before attending the Pediatric Clinic at King Abdulaziz University Hospital from January 2011 to December 2011 and 17 apparently healthy non-asthmatic children as control group. All patients were diagnosed by a respiratory medicine specialist and diagnosis was confirmed by lung function tests on older children. The patients were subdivided into acute asthmatic group which consisted of 30 children suffering from acute attacks of bronchial asthma (16

boys, 14 girls; mean ±SD of age 5.33±1.70 years, range 2–8 years); chronic stable asthmatic group consisted of 30 children (17 boys, 13 girls; mean ±SD of age 5.46±1.30 years, range 2–9 years); control group consists of 17 children (9 boys, 8 girls; mean ±SD of age 4.75±2.20 years, range 2–9 years).

Written informed consent was obtained by all patients' caregivers for interviewing and blood sampling. The study was conducted according to Declaration of Helsinki and approved by the local ethics committee.

All recruited children were subjected to complete history intake, physical examination**,** complete blood picture, X-rays chest. After clinical examination, the children with acute exacerbation were assigned as mild, moderate and severe acute asthma according to established guidelines (10). Patients with acute asthma were classified into atopic and non-atopic groups. Atopy was defined as a positive skin prick test (wheal diameter >4mm at 15 minutes) to extracts of three common aeroallergens (mixed grass pollen, cat dander, house dust mite: Soluprick; ALK, Horsholm, Denmark) and/or a serum IgE concentration > 150 IU/ml (PRIST; Pharmacia, Uppsala, Sweden) (11). The atopic subjects in acute asthmatics included 16 subjects who had also a history of seasonal nasal symptoms. Non-atopic asthmatic subjects had a history of post-infectious onset of asthma, negative reaction to skin prick tests, and negative specific IgE against house dust mite. All normal volunteers were non-atopic. All acute patients were treated with nebulized β2-agonists, oral or intravenous corticosteroids, and oxygen. Stable asthma was required no more than intermittent inhalation of β2-agonists and regular inhaled steroids.

All recruited children were investigated for bacterial and viral infections by taking pharyngeal swabs which immersed in thioglycolate broth. The pharyngeal specimens were Gram stained, streaked on nutrient, blood, chocolate, MacConkeys and Sabarauds agar plates. The bacteria were recognized by their colony morphology and Gram smears. Blood culture was done for each patient. The result of blood culture was reported as negative if there was no growth in the blood culture up to 10 days. any sample showed visualized colonies in solid phase it was subculture into blood, chocolate, MacConkey and Sabaurauds and samples were diluted with virus transport medium (0.5% gelatin hanks balance salt solution with penicillin, streptomycin) and supernatant were obtained and treated in the same way three times .

Venous blood samples were taken (5ml) from all patients before any medications, serum was obtained from each sample and kept frozen at -20ºC for viral and soluble interleukin-2 receptors detection. EIA rapid diagnosis of *Respiratory syncytial virus* was done for all samples by using Abbott Test Pack RSV enzyme immunoassay (EIA), and (Virotech) system diagnostic GmbH) for and *Parainfluenza. Adenovirus* detection was done using Adenocolone EIA diagnostic kit (Cambridge bioscience) (12). Soluble interleukin-2 receptors was measured by enzyme-linked immunosorbent assay using (Genzyme; Cambridge, MA, USA) (13). All methods were used according to instruction manufacturers.

Statistical analysis

The obtained data were expressed as mean+/- standard deviation (SD) and range or number (%) as appropriate. Two-sided unpaired Student's t-tests and one way ANOVA tests were performed for comparison for parametric and Chi square test for non-parametric parameters. Results were considered significant at P <0.05. All statistical analyses were performed using the SPSS statistical software package version 16.

3. Results

Table (1) showed the demographic and clinical characteristic of all the studied groups. sIL-2R showed significant increase in acute and chronic asthmatic patients versus controls (P <0.0001) and in acute versus chronic patients (P <0.0001) (Table 1).

Table (2) showed that viral and bacterial infections were (n=14 and n= 16) in acute asthmatics and (n=10 and n= 6) in chronic asthmatics. In both acute and chronic patients, viral infections were mostly due to *Respiratory syncytial virus* (23.33%, 13.30%) followed by *Adenovirus* (10.00%, 6.70%), then *Influenza virus* (6.70%, 6.70%), *Parainfluenza virus* (6.70%, 6.70%); meanwhile bacterial infections were mostly due to *Haemophilus influenza* (23.30%, 6.70%) followed by *Candida albicans* (16.70%, 13.30%). In acute asthmatics only bacterial infections were also due to by *Streptococcus pneumonia* (2.7%) *and Mixed infections* (2.7%).

Table (3) showed that the serum levels of sIL-2R in different viral and bacterial infections were significantly higher in acute versus stable asthmatic patients.

Tables (4a and 4b) showed that in acute asthmatic patients, there were no significant differences in the serum sIL-2R levels according to severity or atopic state of the disease.

4. Discussion

Athma is a chronic inflammatory disease of the airways characterized by fibrosis of the airways, hyperplasia and hypertrophy of smooth muscle cells and mucous secreting cells due to infiltration of activated eosinophils and activation of resident mast cells and lymphocytes. These chronic inflammatory changes are mediated by secretion of cytokines from inflammatory cells such as IL-2 , antigen activate T cells to express genes encoding IL-2 and its receptor; therefore, the rate of release of the soluble form of IL-2R appears to reflect T cell activation *in vivo* (14).

Table (1). Demographic Characteristics and soluble interleukin-2 receptors (sIL-2) of different studied groups

Item	Control (n=17)	Patient	
		Acute (n=30)	Stable (n=30)
Age (years)			
mean	4.75±2.20	5.33±1.70	5.46±1.30
range	2.00-9.00	2.00-8.00	2.00-9.00
P value		*P* >0.05	*P* >0.05 **P* >0.05
Sex			
Number of male	9 (52.94%)	16 (53.33%)	17(56.66%)
Number of Female	8 (47.06%)	14 (46.67%)	13 (43.34%)
P-value		*P >0.05*	*P >0.05*
Height (cm)			
Mean	103.00±10.24	100±11.24	103.00±9.26
Range	90.00-130.00	87.00±120.00	97.00-118.00
P-value		*P* >0.05	*P* >0.05 **P* >0.05
Weight (kg)			
Mean	3.70±1.70	3.20±1.70	4.60±18.9.0
Range	14.00-2.50	13.00-2.60	15.00- 3.00
P-value		*P* >0.05	*P* >0.05 **P* >0.05
Temperature (ºC)			
Mean	37.00±0.25	37.00±0.45	37.00±0.12
range	36.70-37.50	36.70-37.30	37.00-37.20
P-value		*P* >0.05	*P* >0.05 **P* >0.05
sIL-2R (U/ml)			
mean	240.34 ±355.67	705.00±1550.00	308.00±570.24
range	154.00-1252.00	450.00-2854.00	155.00-1255.00
P-value		***P* <0.001**	***P* <0.001** **P* <0.001

P: significance versus controls; **P*: significant acute versus chronic asthmatic patients

Table (2). Type of respiratory tract infections among different studied groups

Type of infection	Patients		Significance
	Acute (n=30)	Stable (n=30)	
Viral infections	(n= 14, 46.67%)	(n= 10, 33.33%)	
Respiratory syncytial virus	7 (23.33%)	4 (13.30%)	***P* <0.05**
Adenovirus	3 (10.00%)	2 (6.70%)	*P* >0.05
Influenza virus	2 (6.70%)	2 (6.70%)	*P* >0.05
Parainfluenza virus	2 (6.70%)	2 (6.70%)	*P* >0.05
Bacterial infections	(n= 16, 53.33%)	(n= 6, 20.00%)	
Haemophilus influenza	7 (23.30%)	2 (6.70%)	*P* **<0.05**
Candida albicans	5 (16.70%)	4 (13.30%)	*P* >0.05
Streptococcus pneumonia	2 (6.70%)	-	*P* >0.05
Mixed infections	2 (6.70%)	-	*P* >0.05

Data are expressed as number (%), *P*: significant between acute and chronic groups.

Table (3): Serum levels of soluble interleukin-2 receptors (sIL-2R) U/ml among different studied asthmatic groups according to type of infections.

Items	Acute asthma	Stable asthma	Significance
Viral infections			
Respiratory syncytial virus (n=11)			
Mean	1790.00±250.00	550.00 ±230.6.00	***P*** **<0.001**
range	635.00-2555.00	154.00-955.00	
Adenovirus (n=5)		590.00±220.10	***P*** **<0.001**
Mean	1680.00±550.00	254.00 -1254.00	
range			
	452.00-2854.00		
Influenza virus (n=4)		620.00 ± 310.50	***P*** **<0.001**
Mean	1850.00±250.00	254.00-852.00	
range	605.00 -2452.00		
Parainfluenza virus (n=4)			***P*** **<0.001**
Mean	1690.00±320.00	599.00±300.80	
range	566.00-2225.00	152.00-955.00	
Bacterial infections			
Haemophilus influenza (n=9)			***P*** **<0.001**
Mean	1855.00±55.00	640.00 ±230.00	
Range	1425.0-2585.000	254.00- 850.00	
Candida albicans (n=9)			***P*** **<0.001**
Mean	1880.00±60.00	670.00±210.00	
Range	1251.00-2452.00	451.00-1252.00	
Streptococcus pneumonia (n=2)	0.00	0.00	-
Mixed infections (n=2)	0.00	0.00	-

Data are expressed as mean ±SD and range, P: significant between acute and chronic groups.

Table (4a): Serum levels of soluble interleukin-2 receptors (sIL-2R) U/ml among acute asthmatic patients' subgroups according to severity of the disease.

Items **Severity**	**Acute asthma** (n= 30)	**Significance**
Mild (n=12)	1574.40±714.00 455.00-2585.00	
Moderate (n=10)	1391.00±747.00 452.00-2854.00	**P* >0.05
Severe (n=8)	1594.00±699.00 455.00-2555.00	**P* >0.05 ***P* >0.05

Data are expressed as mean ±SD and range. **P*: significance versus mild asthmatic, ***P*: significance versus moderate asthmatic.

Table (4b): Serum levels of soluble interleukin-2 receptors (sIL-2R) U/ml among atopic and non-atopic group in acute asthmatic patients.

Items	**Atopic** (n= 17)	**Non-atopic** (n= 13)
Mean	1546.60±794.00	1455.20±596.70
range	452.00-2854.00	455.00-2555.00
P-value		*P* >0.05

Data are expressed as mean ±SD and range. *P*: Significant between atopic and non-atopic groups.

Our study revealed in both acute and stable asthmatic patients that virus infections was predominant infection in both acute (46.67%) and stable asthma (33.33%) with total (24 children) 40.00% of examined cases. Viral infections were mostly due to *Respiratory syncytial virus* (23.33%, 13.30%) followed by *Adenovirus* (10.00%, 6.70%), then *Influenza virus* (6.70%, 6.70%), *Parainfluenza virus* (6.70%, 6.70%). These results agreement with others (15-18). reported that viral respiratory tract infections, predominantly those caused by *human rhinoviruses*, are associated with asthma exacerbations. Also, Malek-shahi *et al.* (19) reported that wheezing episodes early in life due to *human rhinoviruses* are a major risk factor for the later diagnosis of asthma at age 6 years. Recently mechanisms for virus-induced exacerbations of childhood asthma are beginning to be focused on and defined. Viruses cause systemic immune activation and also produce local inflammation. These factors are likely to affect airway pathogenesis leading to airway narrowing, an increase in mucus production, and eventually bronchospasm, and airway obstruction (20). These new insights related to the pathogenesis and disease activity are likely to provide new targets for the therapy and prevention of early asthma in childhood.

Our data declared bacterial infection in both acute and stable asthma with (53.33% and 20.00%) with total (22 children) 36.67%% of examined cases. In acute and stable asthmatic patients, *Haemophilus influenza* was the prevalent bacteria (23.30%, 6.70%) then *Candida albicans* (16.70%, 13.30%). In only acute asthmatics, bacterial infections were also due to by *Streptococcus pneumonia* (2.70%) *and Mixed infections* (2.70%). Our result were in partial agreement with others (21) who found that over 50% of patients have direct and/or indirect evidence of infection, most commonly bacterial, bacteria as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis,* or atypical organisms as *Chlamydia pneumoniae*, *Mycoplasma pneumonia*. Also, Hare *et al.* (22) reported that *Streptococcus pneumoniae, nontypable Haemophilus influenzae (NTHi), and Moraxella catarrhalis* were prevalent causes of lower airway infection.

In this study, serum sIL-2R levels in acute and stable asthmatics were found to be significantly elevated versus healthy controls and in acute versus chronic asthmatics. Also, in acute asthmatic children, serum sIL-2R levels were significantly elevated in different types of viral and bacterial infections than stable cases. Our data are in agreement with previous study (23) which indicate that the serum concentration of sIL-2R in asthmatic children remained significantly higher than in controls subjects. Although other study (12) have reported that no changes in serum sIL-2R concentration between patients with acute severe asthma and patients with stable asthma. Other study (24) indicate that serum sIL-2R concentration was elevated in asthmatic patients especially in patients during acute exacerbation. Apparently, from the above, elevated serum sIL-2R levels should be carefully interpreted in the presence of clinical, acute and stable asthma.

Whether the serum sIL-2R level can reflect the severity of asthma is a subject of considerable debate, and for paediatric asthma, there is a scarcity of data. In this study, in acute asthmatics, serum sIL-2R levels were not different according to severity of the cases. On contrary, Park *et al.* (25) reported that circulating sIL-2R was the reflection of local inflammatory activity within lung involved, suggesting that sIL-2R test might clinically be useful in the evaluation of patients with bronchial asthma with respect to the severity. Tang and Chen (23) reported that the serum level of sIL-2R in asthmatic children correlated positively with the severity of exacerbation, significantly higher than at clinical remission and could be a potential index of asthma severity.

It is well established that CD4+ Th2 lymphocytes and Th2-associated cytokines (IL-4, IL-5, IL-13) play a crucial role in orchestrating the chronic inflammatory response in atopic asthma (26). However; The immunological mechanisms and the role of T-cell activation occurring in patients with non-atopic asthma are less well characterized, particularly in children. In

this study, we examined T-cell activation in vivo by measuring sIL-2R concentration in serum of acute atopic asthma and non-atopic asthma subjects. We found that serum sIL-2R levels were not different in non-atopic and atopic acute asthmatic patients. However, the data of elevated concentrations of IL-2 and IL-5, and elevated numbers of IL-2R on CD3, CD4, and CD8+ lymphocytes in the bronchial tree, as well as sIL-2R and interferon gamma elevations in serum of the status asthmaticus of the non-atopic patients with bronchial asthma, indicate strong evidence of T-cell activation even in non-atopic bronchial asthma (27,28).

In conclusion, this study emphasize the role of T cells in asthma and suggest that regulation of their function may be important in the treatment of acute and stable asthmatic children as evident by elevated serum levels of sIL-2R. In addition, serum levels of sIL-2R can't be used as indication of acute asthmatic disease severity or allergic state. This study suggests that the therapeutic strategy for asthma should be targeted at inflammatory phenomena rather than at symptoms alone.

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References

1. Warrier MR, Hershey GK. Asthma genetics: personalizing medicine. J Asthma. 2008; 45:257-64
2. Hanania NA. Targeting airway inflammation in asthma: current and future therapies. Chest. 2008; 133:989-98.
3. Akdis CA, Blaser K, Akdis M. Apoptosis in tissue inflammation and allergic disease. Curr Opin Immunol .,2004; 16:717–23.
4. Lee NA, Gelfand EW, Lee JJ. Pulmonary T cells and eosinophils: coconspirators or independent triggers of allergic respiratory pathology? J Allergy Clin Immunol., 2001; 107:945–57.
5. Rubin LA, Kurman CC, Fritz ME. *et al.* Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J Immunol., 1985; 135: 3172-7
6. Lai KN, Leung JCK, Chow CC, Cockram CS. T lymphocyte activation in euthyroid Graves' ophthalmopathy: soluble interleukin 2 receptor release, cellular interleukin 2 receptor expression and interleukin 2 production. Acta Endocrinol (Copenh), 1989; 120:602-9.
7. .Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. JAMA. 1999; 282: 1440-6.
8. .Avendano LF, Palomino MA, Larrañaga C. Surveillance for respiratory syncytial virus in infants hospitalized for acute lower respiratory infection in Chile (1989 to 2000). J Clin Microbiol., 2003; 41:4879-82.
9. .Palomino MA, Larranaga C, Villagra E, Camacho J, Avendano LF. Adenovirus and respiratory syncytial virus adenovirus mixed acute lower respiratory infections in Chilean infants. Pediatr Infect Dis J., 2004; 23:337-41.
10. Williams SG, Schmidt DK, Redd SC, Storms W; National Asthma Education and Prevention Program. Key clinical activities for quality asthma care. Recommendations of the National Asthma Education and Prevention Program. MMWR Recomm Rep., 2003 ; 52:1-8.
11. Stelmach I, Jerzynska J and Kuna P. A randomized, double-blind trial of the effect of treatment with montelukast on bronchial hyperresponsiveness and serum eosinophilic cationic protein (ECP), soluble interleukin 2 receptor (sIL-2R), IL-4, and soluble intercellular adhesion molecule 1 (sICAM-1) in children with asthma. J Allergy Clin Immunol., 2002; 109:257-63.
12. 12.Hite SA, Huang YT. Microwave-accelerated direct immunofluorescent staining for respiratory syncytial virus and influenza A virus. J Clin Microbiol., 1996; 34:1819-20.
13. Limas CJ, Goldenberg IF, Limas C. Soluble Interleukin-2 Receptor Levels in Patients with Dilated Cardiomyopathy: Correlation with Disease Severity and Cardiac Autoantibodies. Circulation, 1995; 91:631-4..
14. Bousquet J, Corrigan CJ, Venge P. Peripheral blood markers: evaluation and inflammation in asthma. Eur Respir J., 1998; 26:42S-8S
15. Cabalka AK. Physiologic risk factors for respiratory viral infections and immunoprophylaxis for respiratory syncytial virus in young children with congenital heart disease. Pediatr Infect Dis J. 2004; 23:S41-5.
16. Oh JW. Respiratory viral infections and early asthma in childhood. Allergol Int. 2006; 55(4):369-72.
17. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of nasopharyngeal

carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. BMC Infect Dis., 2010; 23; 10:304

18. Noyola DE, Rodríguez-Moreno G, Sánchez-Alvarado J, Martínez-Wagner R, Ochoa-Zavala JR. Viral aetiology of lower respiratory tract infections in hospitalized children in Mexico. Pediatr Infect Dis J., 2004; 23:118-23.
19. Malek-shahi SS, Azad TM, Yavarian J, Shahmahmoodi S, Naseri M, Rezaei F. Molecular detection of respiratory viruses in clinical specimens from children with acute respiratory disease in Iran. Pediatr Infect Dis J., 2010; 29:931-3
20. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet, 2010; 376:826-34.
21. Aebi T, Weisser M, Bucher E, Hirsch HH, Marsch S, Siegemund M. Co-infection of Influenza B and Streptococci causing severe pneumonia and septic shock in healthy women. BMC Infect Dis., 2010; 27:308.
22. Hare KM, Grimwood K, Leach AJ, Smith-Vaughan H, Torzillo PJ, Morris PS et.al. Respiratory bacterial pathogens in the nasopharynx and lower airways of Australian indigenous children with bronchiectasis. J Pediatr., 2010; 157:1001-5.
23. Tang RB, Chen SJ. Soluble interleukin-2 receptor and interleukin-4 in sera of asthmatic children before and after a prednisolone course. Ann Allergy Asthma Immunol. 2001; 86(3):314-7.
24. Lai CK, Chan CH, Leung JC, Lai KN. Serum concentration of soluble interleukin 2 receptors in asthma. Correlation with disease activity. Chest, 1993; 103:782-6.
25. Park CS, Lee SM, Chung SW, Uh S, Kim HT, Kim YH. lnterleukin-2 and Soluble Interleukin-2 Receptor in Bronchoalveolar Lavage Fluid From Patients With Bronchial Asthma. Chest, 1994; 106:400-6.
26. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol., 2000; 105:399–408.
27. .Heath H, Qin S, Rao P, et.al. Chemokine receptor usage by human eosinophils. The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. J Clin Invest. 1997; 99:178-84.
28. Bettiol J, Bartsch P, Louis R, *et al.* Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: relationship with blood and sputum eosinophilia and serum IgE levels. Allergy, 2000; 55:1134-41.

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