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Original Article

Th1/Th2 cytokine pattern in Arab children with severe asthma

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Abstract: Background: Bronchial Asthma has recently emerged as one of the most prevalent diseases in Arab countries. Environmental and geographical influences were shown to be the reasons of the variations in the rates of prevalence; no analyses have nevertheless yet been performed on the immunologic background associated with this condition in Arabic children. Objectives: To examine the cytokine production from T cells in children with and without asthma, and to determine the role of the most related cytokine patterns in childhood asthma. Methods: A total of 195 Saudis children (98 asthma pediatric patients and 97 healthy controls) were randomly selected from the Riyadh Cohort Study for inclusion. Asthma was based on established pediatric diagnosis and medications taken. Results: Significant differences were observed between the two groups, thus, GMCSF, INF-γ, IL-5, IL-6, IL-8 and IgG-3 were reduced in patients compared to controls; in these same patients IgE, resistin, IL-4 and IgG-4 were significantly increased. In contrast with these results no differences between patients and controls were seen in CRP, TNF-α, IL-1, IL-2, IL-7, IL-10, IL-13, IgG-1, IgG-2, IgG-A and IgG-M. Result of a principal component analysis suggested that IL4. INF-γ and IgE are major players in the pathogenesis of asthma in Arabic children. Conclusion: These are the first data obtained in asthmatic children in Saudi; data herein confirm that this disease is associated with a profound degree of immune impairment independently of the peculiar genetic of the analyzed individuals, and of the environmental conditions that are present in this part of the world.

Keywords: Children, serum, cytokine, severe asthma

Introduction

Atopic diseases and asthma represent increasing health problems in Arab societies and are the most frequent chronic illnesses in childhood [1-3]. Atopy can be defined as the genetic tendency to develop the classic allergic diseases atopic dermatitis, allergic rhinitis, and asthma. Atopy involves the capacity to produce IgE in response to common environmental proteins.

The development of atopy is favored by a shift towards Th2-like cytokines (interleukin-4 (IL-4), IL-5, IL-13) in humans promoting the production of IgE antibodies [4, 5]. Several potential determinants being responsible for development of atopy and asthma have been proposed such as lack of severe and repeated infections [6, 7], obesity and lack of physical exercise [8], decreased family size [9-11], changing dietary habits [12, 13] and increased indoor allergen exposure [14, 15].

Two types of T helper (Th) clones Th1 and Th2 cells were described by the late 1980s in mice, which were differing in cytokine secretion patterns and other functions [16]. Th1 cells secrete INF-γ and IL-2 which activate macrophages and cytotoxic T cells to kill intracellular organisms, whereas Th2 cells secrete IL-4, IL-9, IL-10 and IL-13 which help B cells to secrete protective antibodies [17-19]. The concept subsequently was applied to human immunity [19-21] and a Th1/Th2 cytokine imbalance was subsequently associated with numerous immunological dis-

eases in humans [22-24], including several chronic conditions such as atopy and asthma. Thus, the concept that inflammatory processes are regulated by a complex network of mutually interacting cytokines emerged; tsi network; determines the prognosis of chronic disorders, [25, 26]. Many studies have been conducted on the activity of T cell cytokines in asthma in adults and in pediatric patients; results confirmed that a severe imbalance in cytokine production is indeed responsible for this disease in both settings [27].

Asthma patterns expressed during childhood might be present up to adulthood [28, 29]. The understanding of asthma during childhood is a task that requires an understanding of the natural history of the disease. Children with significant symptoms in the first years of life, especially those with a family history of asthma, are likely to develop persistent respiratory symptoms later in life [30]. Defining which children are at risk for persistent asthma could allow the definition of mechanisms associated with different phenotypes, better management and, potentially, for reduced morbidity and mortality. No studies have been performed on the immunopathogenesis of pediatric Asthma in Saudis despite the rapid increase in the prevalence of this condition that has been observed in recent years. Differences in the genetic background and in environmental conditions might result in peculiar difference within the Th1/Th2 paradigm in Saudi children and warrant an in-depth analysis of the pathogenesis of asthma in this setting.

Materials and methods

Study subjects

A total of 195 Saudis children ages 17 years old and below, (98 asthma pediatric patients and 97 healthy controls) were randomly selected from the Riyadh Cohort Study for inclusion. The parent or guardian of each child was asked to answer a questionnaire consisting of demographic information that included dietary questions, area of residence (near the factory, high-traffic area, etc.), presence of a smoker at home and other pertinent questions related to asthma. Asthma was based on established pediatric diagnosis. Since most of the subjects were asymptomatic during the course of the study, parents were asked if the child had history of wheezing upon exhalation, frequent episodes of chest tightness and hyper-expansion of thorax with use of accessory muscles.

Anthropometrics

Anthropometry included height (rounded off to the nearest 0.5 cm) and weight (rounded off to the nearest 0.1 kg), which were measured using an appropriate international standard scale (Digital Person Scale; ADAM Equipment, Milford, CT, USA), as well as waist and hip circumference in centimeters, which were measured using a standard tape measure. Mean systolic and diastolic blood pressure readings (in mmHg; average of two readings) were taken using appropriate cuffs.

Biochemical parameters

Fasting blood was collected at primary healthcare centers. Blood was drawn, centrifuged and processed on the same day. Both whole blood and serum were placed in plain polystyrene tubes. Serum was delivered to BRP for storage at -20°C. Fasting serum glucose levels, and complete lipid profile (triglycerides, total cholesterol, LDL- and HDL-cholesterol) were determined using routine laboratory methods (Konelab, Espoo, Finland). This biochemical analyzer was calibrated routinely prior to the analysis of all serum samples using quality control samples provided by the manufacturer (Thermo Fisher Scientific, Espoo, Finland). LINCOplex, human multiplex immunoassay kit based on Luminex 100 system platform (Luminex Corporation, Austin, TX, USA) was used for determination of three different panels by simultaneous detection of a great variety of, cytokines, interleukins and immunoglobulines. The standard protocol for the Process was followed. The concentrations of analyte in each sample were calculated with a five parameter model using Luminex IS software ver. 2.3. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated to evaluate IR status using the formula; glucose (mmol/L) X insulin (μIU/mL)/22.5 [31].

Statistical analysis

Data represented by Mean ± standard deviation. Skewed data was either log or Square root transformed. Non Gaussian Variables were represented by Median and Inter quartile ranges.

Table 1. Characterization of asthmatic children compared to non-asthma control subjects

Parameters	Control	Asthma	*P* value
N	98	97	
Age (Years)	13.5 ± 3.5	13.7 ± 3.0	0.80
BMI (kg/m^2)	21.1 ± 5.5	21.3 ± 6.6	0.81
CRP (μg/ml)	1.4 ± 0.88	1.6 ± 0.84	0.86
Resistin (ng/ml)	17.6 ± 1.0	24.1 ± 1.5	< 0.001
TNF-α (pg/ml)	5.7 ± 1.1	6.5 ± 1.2	0.43
GMCSF (pg/ml)	1.9 ± 0.8	1.2 ± 0.80	0.04
INF-γ (pg/ml)	2.4 ± 0.74	1.6 ± 0.69	0.03
IgE (pg/ml)	71.4 (154.2)	104.3 (184.8)	0.02
IgG-1 (pg/ml)	9.3 ± 0.65	10.2 ± 0.88	0.18
IgG-2 (pg/ml)	5.7 ± 0.80	6.0 ± 0.74	0.74
IgG-3 (pg/ml)	1.2 ± 0.58	1.0 ± 0.60	0.04
IgG-4 (pg/ml)	0.47 ± 0.04	0.70 ± 0.05	0.03
IgG-A (pg/ml)	1.7 ± 0.37	1.8 ± 0.37	0.37
IgG-M (pg/ml)	1.6 ± 0.34	1.6 ± 0.36	0.89
IL-1 (pg/ml)	0.45 ± 0.05	0.37 ± 0.050	0.42
IL-2 (pg/ml)	0.83 ± 0.05	0.74 ± 0.05	0.44
IL-4 (pg/ml)	12.4 ± 2.0	20.6 ± 2.9	0.04
IL-5 (pg/ml)	0.92 ± 0.05	0.60 ± 0.05	0.01
IL-6 (pg/ml)	6.4 ± 1.3	4.2 ± 1.5	0.02
IL-7 (pg/ml)	9.3 ± 1.0	9.3 ± 1.1	0.94
IL-8 (pg/ml)	6.6 (15.2)	6.2 (9.1)	0.04
IL-10 (pg/ml)	12.1 ± 1.3	11.0 ± 1.6	0.43
IL-12 (pg/ml)	1.8 ± 0.11	1.5 ± 0.13	0.50
IL-13 (pg/ml)	4.3 ± 1.0	4.9 ± 1.3	0.56

Two samples independent T-test was done to compare control and asthma. Mann Whitney U test was done to compare control and Asthmatic, wherever variables don't follow Gaussian distribution. *P* values < 0.05 were considered as statistically significant. Pearson's correlation test was performed to examine various correlations. Analyses were performed with the SPSS-PC software, version 16.0 (SPSS Inc, Chicago, IL).

Results and discussion

Subjects characteristics

Characteristics of total of 195 Saudis children < 17 years old (98 asthma pediatric patients and 97 healthy controls) are provided in **Table 1**. Subjects were similar in body mass index (BMI) 21.2 ± 0.1 kg/m^2.

The results showed the presence of a significant decrease in the concentration of GMCSF, INF-γ, IL-5, IL-6, IL-8 and IgG-3 were (1.2 ± 0.80; *P* = 0.04), (1.6 ± 0.69; *P* = 0.03), (0.6 ± 0.05; *P* = 0.01), (4.2 ± 1.5; 0.02), (6.2; *P* = 0.04) and (1.0 ± 0.6; *P* = 0.04) in asthmatic children compared to healthy control (1.9 ± 0.8), (2.4 ± 0.74), (0.92 ± 0.05), (6.4 ± 1.3), (6.6) and (1.2 ± 0.58), respectively. Significantly higher levels of IgE (104.3; *P* = 0.02), Resistin (24.1 ± 1.5 ng/ml; *P* = 0.001), IL-4 (20.6 ± 2.9 pg/ml; *P* = p 0.04) and IgG-4 (0.70 ± 0.05 mg/ml; *P* = 0.03) compared to the healthy control subjects of IgE (71.4), Resistin (17.6 ± 1.0 ng/ml), IL-4 (12.4 ± 2.0 pg/ml) and IgG-4 (0.47 ± 0.04 mg/ml), were seen in the same children compared to controls.

In contrast with these results, no significant differences were detected between asthma patients and healthy control subjects in levels of CRP, TNF-α, IL-1, IL-2, IL-7, IL-10, IL-13, IgG-1, IgG-2, IgG-A and IgG-M. Results showed that there is significant increasing in resistin ($P \leq 0.001$), IgE (*P* = 0.02), IgG-4 (*P* = 0.03) and IL-4 (*P* = 0.04), while significant decreasing were indicated in GMCSF (*P* = 0.04), INF-γ (*P* = 0.03), IgG-3 (*P* = 0.04), IL-5 (*P* = 0.01), IL-6 (*P* = 0.02) and IL-8 (*P* = 0.04).

The key to answering some of the questions related to asthma heterogeneity and to the relationship of genetics to disease expression lies in the careful definition of asthma phenotypes. The Th2 cytokine expression and a concomitant down regulation of secreted Th1 cytokines in allergic diseases was reported in many studies were done in adults. But there are only few data on asthma phenotypes in children, one of the first studies measuring cytokine concentrations in children with allergic disease, revealed a significant increase in the level of IL-4 in serum from atopic asthmatics compared to controls, which correlated with IgE [32]. Although the importance of certain Th2 cytokines, in particular IL-4 and IL-5, in children with atopic asthma have been well documented, recent data in both adults and children has challenged the concept of a Th1/Th2 imbalance, and has put forth some evidence to suggest a Th1 profile in asthma. Other subsequent studies in serum and blood supported the importance of IL-4 in childhood asthma [33, 34]. The differences in IL-4 levels are likely to be dependent on disease severity, since no differences in IL-4 concentrations compared to

Table 2. Correlations between asthma-related traits in a population of 198 children, 7-17 years of age

	Asthma	IgE	INF-γ	IL4	IL8	IL10		Principal Component 1	Principal Component 2
Asthma	1						Asthma	0.75	-0.13
IgE	0.86	1					IgE	0.99	-0.08
INF-γ	0.17	0.09	1				INF-γ	0.50	-0.18
IL4	0.14	0.21	-0.27	1			IL4	0.66	-0.14
IL8	-0.26	-0.19	-0.24	0.07	1		IL8	-0.07	0.99
IL10	-0.39	-0.13	-0.18	0.20	0.49	1	IL10	-0.11	0.30
							% of variance explained by each component	37.8	19.5
							Used Varimax-Rotated factor solution		

Correlations between asthma-related traits in a population of 198 children, 7-17 years of age.

normal controls were seen in children with mild/moderate asthma [35]. Tang et al. were the first to report that an increase in IL-4 and a decrease in INF-γ expression associates with atopy, rather than with full blown asthma [5]. Several studies confirmed the allergen-specific regulation of cytokine production in PBMCs. Thus, house dust mite (HDM) appears to cause up-regulation of Th2 cytokines (IL-4, IL-5, IL-9, IL-10 and IL-13) in atopic children including atopic asthmatics, and conflicting results have been reported on INF-γ -g expression [36, 37]. Little data are available on the role of GMCSF in childhood asthma, possibly due to its expression by both Th1 and Th2 subsets, whereas the only study that has investigated T cell cytokines in sputum from children found that IL-5 and GMCSF concentrations are similar in children with asthma compared to controls [38].

Results of the principal component analysis of all atopic related traits (**Table 2**) showed that two components were retained based on: (a) Eigen values 1 or greater, or (b) components above the break in the screen plot. Varimax orthogonal rotation was used to obtain a set of independent, uncorrelated and best interpretable components. Each of these components is an independent linear combination of the original variables. The components were interpreted based on the loadings that relate the biomarkers to components. The first component explained 37.8% variance compared with 19.5% for second giving a cumulative percentage of 57.3%. Asthma was strongly loaded with the first (r = 0.75) compared to the second component (r = -0.13). For the first component IgE was the strongly loaded (r = 0.99), followed by IL4 (r = 0.66) and INF-γ (r = 0.50).

Our results clearly showed that an imbalance in T cell cytokines is associated with atopic asthma in children. Moreover the results support the importance of GMCSF and INF-γ in childhood asthma and the correlation with IL-4 and IL-5 as Th2 cytokines. These are the first data obtained in asthmatic children in Saudi; data herein indicate confirm that this disease is associated with a profound degree of immune impairment independently of the peculiar genetic of the analyzed individuals, and of the environmental conditions that are present in this part of the world.

In conclusion, the chronicity of asthma has been associated with cytokine-mediated inflammation, in particular from T helper 1 (Th1) and T helper 2 (Th2) cells. Over the past 10 years, a number of studies have unraveled the role of T cell cytokines in childhood asthma even if this research in the pediatric setting has been hampered by ethical and practical difficulties. A large body of evidence suggests that Th2 cytokines are up-regulated in pediatric asthma. However, a number of more recent publications propose that Th1 cytokines may also have inflammatory effects in childhood asthma. In particular, INF-γ role in childhood asthma has been clearly documented. Such reports have questioned the concept of the Th1/Th2 imbalance in such childhood asthma.

This study suggested that, IL-4, INF-γ, IL-5 and GMCSF can used as a biomarkers in understanding severe asthma. We recommended that more further studies to determine the environmental pollution impact in childhood asthma.

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Disclosure of conflict of interest

None.

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