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Original Article

Time Trends of Th1 and Th2 Cytokines in Induced Sputum of Asthmatic Subjects During Acute Upper Respiratory Viral Infections

Ding Zhang,◆ Jingwen Xia,◆ and Xiaodong Chen*

Department of Respiratory Disease, Huashan Hospital, Fudan University, Shanghai, China

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ABSTRACT

Fundamentals: Many asthma exacerbations are caused by respiratory viral infections that induce the interplay between Th1 and Th2 immune responses. However, the time trends for Th1 and Th2 immune responses during these phenomena have not been well studied.
Objective: To identify possible mechanisms underlying the link between respiratory viral infections and asthma exacerbations.
Patients and methods: We recruited 40 adults aged 21-58 years for 4 groups. A. Healthy, B. Healthy with viral infection, C. Mild to moderate asthma and D. Same as C, but with viral infection. Th1 and Th2 cytokines in induced sputum samples during the course of acute upper respiratory viral infections in otherwise healthy and asthmatic individuals were monitored. IL-4, IL-5 and IFN-γ were assayed by ELISA. Viral infection symptoms and asthma severity scores were monitored. Time trends were analyzed using linear mixed models.
Results: IL-4 and IL-5 levels in groups C and D were higher than in groups A and B. IFN-γ levels and viral infection symptoms scores in group B spiked by day 2 and rapidly declined by day 7, while in group D, IFN-γ and symptoms scores for viral infection and asthma peaked much later (days 3-5) and slowly declined. The ratios of IL-4 and IL-5 to IFN-γ in group D were significantly higher than in group C.
Conclusions: Infection-induced asthma exacerbations may be due to impaired anti-viral Th1-immune responses. There appears to be a critical window of 3-5 days for therapeutic intervention.

Tendencias temporales de las concentraciones de citocinas Th1 y Th2 en esputo inducido de pacientes asmáticos durante infecciones víricas agudas de las vías respiratorias superiores

Palabras clave:
Infección vírica de las vías respiratorias superiores
Exacerbación del asma
Interleucina 4
Interleucina 5
Interferón-γ
Equilibrio Th1/Th2
Esputo inducido

RESUMEN

Fundamento: Muchas de las exacerbaciones del asma se deben a infecciones víricas de las vías respiratorias que inducen una interacción de respuestas inmunitarias entre Th1 y Th2. Sin embargo, las tendencias temporales de estas respuestas durante estos fenómenos no se han estudiado con detalle.
Objetivo: Identificar los posibles mecanismos subyacentes de la relación entre las infecciones víricas respiratorias y las exacerbaciones del asma.
Pacientes y métodos: Seleccionamos 40 adultos, de 21-58 años de edad, en 4 grupos: A, sanos; B, sanos con infección vírica; C, con asma leve o moderada, y D, igual que C pero con infección vírica. Durante el curso de una infección vírica aguda de las vías respiratorias superiores se monitorizaron las citocinas Th1 y Th2 en muestras de esputo inducido en individuos por lo demás sanos y en pacientes asmáticos. La interleucina (IL) 4, la IL-5 y el interferón gamma (IFN-γ) se analizaron mediante un método ELISA. Se monitorizaron las puntuaciones de los síntomas de infección vírica y de gravedad del asma. Las tendencias temporales se analizaron mediante la utilización de modelos mixtos lineales.

*Corresponding author.
E-mail address: xdchen8@hotmail.com (X. Chen).
◆Both authors have contributed equally to the investigation.

Resultados: En los grupos C y D los valores de IL-4 e IL-5 fueron mayores que en los grupos A y B. En el grupo B, los valores de IFN-γ y las puntuaciones de síntomas de infección vírica fueron máximos en el día 2 y disminuyeron rápidamente en el día 7, mientras que en el grupo D los valores de IFN-γ y las puntuaciones de síntomas de infección vírica y de asma alcanzaron un máximo mucho más tarde (días 3-5) y disminuyeron lentamente. En el grupo D, los cocientes IL-4 e IL-5:IFN-γ fueron significativamente más altos que en el grupo C.

Conclusiones: Las exacerbaciones del asma inducidas por las infecciones pueden deberse a un deterioro de las respuestas inmunitarias antivíricas Th1. Parece identificarse un intervalo decisivo de 3-5 días para instaurar una intervención terapéutica.

Introduction

Asthma is a serious public health concern. The Global Initiative for Asthma (GINA) has reported that, worldwide, 300 million individuals suffer from asthma.[1] This load is aggravated by the fact that many asthmatic patients can suffer exacerbations of their symptoms due to external causes not related to their underlying disease. Prospective studies have shown that 85% of these exacerbations in children and about half in adults are due to viral infections.[2,3] According to our clinical observations, frequently asthma exacerbations take place 3-5 days after an individual contracts a viral infection of the upper airways.[4] Currently, studies that aim to determine the possible mechanism of the causal relationship between viral infections and asthma exacerbations have been focused on the interaction between immune responses Th1 and Th2.

These 2 groups of helper T lymphocytes are differentiated by the cytokines they release: the Th1 cells predominantly produce gamma interferon (IFN-γ), while the Th2 cells produce interleukin (IL) 4 and IL-5. In healthy individuals homeostasis between Th1 and Th2 is very important. A change in the Th population with Th cells replaced by Th1 cells tends to decrease the production of Th2 cells, whereas an increase of Th2 cells suppresses the production of the Th1 cells. During a viral airway infection (predominance of Th1 type) the immune responses can modulate the deviation of the immune system towards a Th2 response, present in asthma, and, in fact, the immune status established in asthma may alter the course of antiviral responses. It was considered that inhibition of production of IL-4 and IL-5 should be effective for asthma treatment. Furthermore, although using this strategy eosinophilia was suppressed, it has not been shown to be useful from the clinical point of view.[5-7] The lack of efficacy of IL-12 or IFN-γ administration, which suppresses Th2 activity, has also been reported.[8]

One of the problems to determine optimum treatment modalities is that time trends of Th1 and Th2 immune responses have not been studied in detail during acute viral infections. Among the various non-invasive methods to assess airway inflammation, induced sputum examination is a relatively simple strategy. Freezing an induced sputum sample immediately after collection for its subsequent processing at the convenience of the laboratory technicians prevents problems caused by enzyme contents in this body fluid that digest fluid and cell components if the sample is not frozen. Different research teams have tested this technique over the last years.[9-12]

In this study, we use this method to assess time dependent changes in IL-4 and IL-5 (Th2 cytokines) and IFN-γ (Th1 cytokines) in induced sputum samples from asthmatic patients and healthy control individuals with or without airway viral infections. The aims of this study were to establish the antiviral airway immune response patterns in absence or presence of asthmatic immune responses and to identify the possible mechanisms where the relationship between viral airway infections and asthma exacerbations is based.

Individuals and Methods

Individuals

The research committee of the Huashan Hospital affiliated to the Fudan University approved the study and all the individuals provided their written informed consent to take part on this study. We selected a total of 40 adults, from 21 to 58 years old (22 men and 18 women). They were distributed in 4 groups (10 per group) based on their airway health status.

Group A included healthy individuals, group B included individuals with a viral infection of the upper airways, but otherwise healthy; group C included individuals with controlled, mild to moderate asthma; group D included individuals with controlled, mild to moderate asthma, and viral infection of the upper airways.

For all groups, the exclusion criteria were the following: history of severe cardiopathy, hypertension, chronic bronchitis, glaucoma, prostate hypertrophy, thyroid conditions, diabetes, anaemia or liver and renal failure. Pregnant or lactating women were also excluded. Other inclusion/exclusion criteria for each of the 4 groups were the following: the group A and B individuals did not have a history of allergic or immunological diseases, had not received a viral vaccine during the last 12 months and had not taken part in any clinical drug trial during the last 3 months; in group B, individuals with a diagnosis of an acute viral infection of the upper airways, based on signs, symptoms, systematic laboratory tests and exams for viral detection were included; in group B, individuals with suspected bacterial infections based on signs, symptoms and laboratory tests were excluded. For example, WBC counts were, as a minimum, 10.0?10^9/l, or the percentage of neutrophils was as a minimum 80% (greater than the reference value) or chest X-ray indicated pneumonia, including viral pneumonia. All the individuals in groups A and B were personnel of the Huashan Hospital affiliated to Fudan University, in Shanghai, China. Groups C and D were asthmatic ambulatory patients, monitored in Huashan Hospital affiliated to Fudan University. The patients were diagnosed with asthma based on criteria developed by the Chinese Society of Respiratory Diseases, and classified as mild or moderate. The patients received regular treatment according to GINA guidelines; they were treated with inhaled budesonide (Turbuhaler®) (100-400 μg) as maintenance treatment and they were in remission. Patients in groups C and D had not received a viral vaccine during the last 12 months and had not taken part in any clinical drug trial during the previous 3 months. In the patients of group B a diagnosis of acute viral infection of the upper airways was made based on signs, symptoms, systematic laboratory tests and virus detection. Patients in group D were excluded if a bacterial infection was suspected based on signs, symptoms and laboratory tests. The patients in this group were also excluded if the symptoms became deteriorated after upper airway infection and sputum induction could not be achieved.

Individuals were told to go to hospital after 24 hours, as soon as they experience signs of upper airway viral infection. All of them

Table 1
Score for symptoms of upper airways viral infection

	Score		
Symptoms	Mild (1)	Moderate (2)	Serious (3)
Temperature (°C)	37.5 ≤ T < 38	38 ≤ T < 38.5	T ≥ 38.5
T	Intermittent, < 5 times/day, with no alterations of daily life or work patterns	From mild to severe	Frequent, > 10 times/day, alteration of daily life and sleep patterns
Rhinitis, nasal congestion or sneezing	Slight, mild, < 5 times/day, with no alterations of sleep or breathing patterns	From mild to severe	Aqueous, abundant, severe, > 10 times/day, alteration of sleep or breathing patterns
Headaches, pharyngitis, myalgias	Mild, with no alteration of work or sleep patterns	From mild to severe	Alterations of daily life and sleep patterns

agreed to undergo systematic laboratory tests and a chest X-ray. All individuals were followed during 7 days; sputum was induced daily and the score for upper airway viral infection symptoms and asthma were registered daily.

Diagnostic Criteria for Upper Airway Infections

1. Clinical features: patients caught a cold and had fever, chills, sweating, discomfort, pharyngitis, rhinitis, nasal congestion, sneezing, headaches, malaise and joint pains. Physical examination indicated erythema and pharyngeal inflammation and lung auscultation did not detect dry or humid crackles.
2. Laboratory tests: systematic haemogram, WBC count ≤ 10.0?10^9/l or neutrophils ≤ to 80% (or not higher than baseline).
3. Imaging: chest X-rays did not show any lung condensation or signs of infection. Old lesions were excluded.

Symptom Score for Viral Airway Infections and Asthma

The symptoms of viral airway infection were scored in the following way: 1, mild; 2, moderate, and 3, severe. Table 1 includes the criteria used to assign these scores. Diagnosis, degree and treatment of asthma were carried out according to GINA[13] criteria. Table 2 shows the criteria used to assign symptoms scores for asthma.

Sputum Induction and Sample Processing

Sputum was induced between 8-10 am daily during 7 days, according to what has been previously described.[14] To summarise, after basal spirometry and pre-treatment with salbutamol, individuals inhaled 3, 4 and 5% hypertonic saline solution during 7 minute periods. After each period, they tried to expectorate sputum into plastic containers.

The sputum samples were poured into Petri plates immediately after induction. Mucus plugs from lower airways were chosen, put in Eppendorf® tubes and 100 mL of dimethyl sulfoxide were added (Merck, Germany). The tubes were hermetically closed, stirred during 30 s, coded and stored in a freezer at –70 °C.

A day-shift laboratory assistant processed the frozen sputum samples using a previously described protocol,[15] with a few slight modifications. The Eppendorf tubes were thawed to room temperature and their contents were weighed. A volume (μL) of the dithiothreitol solution (Sputolysin, Calbiochem Corp, La Jolla, California, USA) in a 1:10 dilution with distilled water equal to 4 times the weight of the sputum (in mg). It was homogenised using the mixer, and then, the tube was placed in a rocking platform shaker. A volume of buffered Dulbecco saline with phosphates was added equivalent to the dithiothreitol solution used, and the suspension was filtered using a gauze. Cell viability was assessed by exclusion with trypan blue. The cell suspension was centrifuged and the supernatant was frozen at –70 °C for subsequent detection of IL-4, IL-5 and IFN-γ.

Table 2
Score of asthma symptoms

Signs	Score	Degree
Cough	0	No
	1	Intermittent, ≤ 5 times/day, with no alterations of daily life or work patterns
	2	5 times/day ∼10 times/day, moderate
	3	Frequent day and night, ≥ 10 times/day, alteration of daily life and sleep patterns
Panting	0	No
	1	Intermittent, with no alterations of daily life or work patterns
	2	Moderate alteration of daily movements and sleep patterns
	3	Evident, makes it impossible to remain in a lying position, alteration of daily movements and sleep patterns
Wheezing	0	No
	1	At times, deep and rapid breathing
	2	Sporadic bilateral, pulmonary sibilances
	3	Diffuse bilateral, pulmonary sibilances

Laboratory Tests

On the first day of the first viral infection symptoms, ELISA equipment (Simcere, Nanjing, China) was used to detect airway viruses according to manufacturer's instructions. The viral spectrum identified with this equipment was rhinovirus, syncytial airway virus, flu virus, parainfluenza, adenovirus and coronavirus viruses. The concentrations of IL-4, IL-5 and IFN-γ in the supernatant were determined using ELISA (Rapid BioLab, Calabasas, CA, USA) equipment. The results were expressed in pg/mL.

Statistical Analysis

Due to the reduced size of the sample, a non-parametric Kruskal-Wallis test was used to compare the 4 groups. Means and standard deviations were determined for age and haematological test indexes. Distributions by gender (number, percentage) were compared using the Fisher exact test. Cytokines were measured on repeated tests during 7 days; a linear mixed model was used to test the time trends and compare groups. Means and standard deviations were calculated for cytokines and IL-4/IFN-γ and IL-5/IFN-γ ratios. If a time trend or a group effect was statistically significant (post-hoc analysis), a Tukey test was performed. Statistical significance was established with a value of $p < 0.05$. For statistical analysis the statistical program SPSS (version 15.0, SPSS Inc., Chicago, USA) was used.

Results

Baseline Characteristics of Individuals

We selected a total of 40 adults, from 21 to 58 years old: 22 men (55%) and 18 women (45%). As described in Methods, they were

distributed in 4 groups (A, B, C or D) based on their airway health status (10 by group). Table 3 includes a summary of baseline characteristics. When comparing the 4 groups, no significant differences were found in terms of age, gender, basophile percentage, platelet count, RBC count or haemoglobin values.

Furthermore, significant differences were found in the 4 groups regarding WBC counts and WBC formula. In patients with upper airway infections (groups B and D) higher WBC counts, an increase in the percentage of eosinophils, lymphocytes and monocytes and a lower percentage of neutrophils than in healthy individuals (group A) or patients that only had asthma (group C) were detected.

Virus Detection in the Induced Sputum Samples

To detect virus in induced sputum samples ELISA equipment was used. As expected, in group A and C samples no virus was detected. In group B, 5 individuals had a rhinovirus infection, 3 individuals had a flu virus infection and 2 individuals had a syncytial airway virus infection. In group D, 6 individuals had a rhinovirus infection, 3 individuals had flu virus infection and 1 individual had a syncytial airway virus infection.

Time Trends of Th1 and Th2 Cytokines in Induced Sputum

Figure 1 shows variations over time of the concentrations of IL-4, IL-5 and IFN-γ in the induced sputum samples. Regarding IL-4 (fig. 1A), patients with asthma alone (group C) or both with asthma and upper airway infection (group D) had higher values throughout the observation period compared to healthy individuals (group A). In patients that only had infection of the upper airways (group B), IL-4 values were similar to those reported by healthy individuals (group A). Regarding time trends, IL-4 values increased on day 4 and decreased after day 5 (for time trends both $p < 0.001$) in groups B and D. For group C, IL-4 values remained stable up to day 4, increased significantly by day 5 and decreased slightly up to day 7 ($p = 0.004$). In group A no significant changes were detected ($p = 0.759$).

Regarding IL-5 (fig. 1B), both group C and group D reported higher values than those observed in group A before day 3, although the difference between group A and C disappeared after day 4. IL-5 values in groups A and C did not change significantly over time (both $p > 0.05$). Whereas, IL-5 values in groups B and D increased significantly after 5 days, did not change up to day 6 [sic] and decreased on day 7 ($p = 0.06$ for group B and $p < 0.001$ for group D).

For IFN-γ (fig. 1C), group C had lower values than group A on days 1 and 2, but similar values after day 3. Initially, lower values were found in group D than in group A on day 1; however, in the group D, IFN-γ values were higher than in Group A after day 2, although the differences on days 2 and 7 were not statistically significant. Once again, in groups A and C, IFN-γ values did not vary significantly during the observation periods (both $p > 0.05$).

It is interesting to highlight that IFN-γ values in group B reached a maximum on day 3, and, afterwards, decreased rapidly. On day 7 the IFN-γ values were similar to those of day 1. However, maximum IFN-γ values from group D were identified on day 5 and decreased on day 7, when the concentration was 477.0 pg/mL, which were similar to the values observed on day 3.

We also calculated the ratios of Th2/Th1: IL-4/IFN-γ and IL-5/IFN-γ cytokines. The daily results of these ratios figure on table 4. For group A, these were logically significantly lower than those from groups C and D (all $p < 0.05$). Regarding time trends, significant variations were detected in these ratios in groups B and D ($p < 0.001$ for both groups), although the time trends for IL-4/IFN-γ and IL-5/IFN-γ were different.

In group B, the IL-4/IFN-γ ratio on day 4 or later was significantly greater than the values detected on day 3 or previously ($p < 0.001$); the IL-5/IFN-γ ratio decreased slightly from day 1 to 3; afterwards, it increased significantly form day 4 to 6, whereas on days 6 and 7 it was similar to day 1 ($p = 0.001$). In group D, the IL-4/IFN-γ ratio increased significantly after day 3 ($p < 0.001$); on days 5 and 6, the IL-5/IFN-γ ratio was significantly higher than on day 1, but on day 7 it was not significantly different to the value seen on day 1 ($p = 0.124$).

Time Trends of Scores for Viral Infections and Asthma

The symptom scores for airway viral infection (table 1) and asthma symptoms (table 2) were registered daily for the 30 individuals from groups B, C and D, accordingly. Table 5 includes the daily results of these scores. For asthma scores, group D scores on day 1 were similar to those of group C. However, asthma scores increased in group D after day 2, and reached their maximum on day 4 ($p < 0.001$). Compared to group C, group D reported higher scores for asthma symptoms on days 2-7 (all $p < 0.05$). For group D, asthma symptom scores on days 3, 6 and 7 were similar.

For upper airway symptom scores, similar scores from groups B and D were identified before day 4; afterwards, the scores for group B were significantly lower than those of group D. The maximum scores of group B were registered on day 2, after that, they decreased rapidly up to day 7 ($p < 0.001$). The scores for group D increased rapidly from day 1 to 4 and decreased up to day 7 ($p < 0.001$). The scores registered on days 6 and 7 were similar to those of day 2.

Discussion

In this study, in order to better understand the complex interactions between viral infections and asthma, we continuously

Table 3
Baseline characteristics

	Group A (n = 10)	Group B (n = 10)	Group C (n = 10)	Group D (n = 10)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Value of p
Age (years)	41.4 ± 10.0	41.1 ± 11.6	39.7 ± 7.2	40.7 ± 13.1	0.987
Men (n [%])	7 (70.0)	4 (40.0)	5 (50.0)	6 (60.0)	0.715
Haematology					
Basophils (%)	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.3	0.5 ± 0.2	0.866
Eosinophils (%)	2.1 ± 0.5	1.7 ± 0.4	2.8 ± 0.6	5.1 ± 1.2	< 0.001*
Lymphocytes (%)	28.0 ± 2.3	37.1 ± 3.1	28.6 ± 2.3	35.0 ± 2.0	< 0.001*
Neutrophils (%)	63.4 ± 4.1	54.0 ± 2.7	63.8 ± 2.7	52.7 ± 1.9	< 0.001*
Monocytes (%)	5.0 ± 1.3	6.7 ± 0.5	4.5 ± 1.0	6.5 ± 0.4	< 0.001*
Platelets (10^9/l)	267.2 ± 54.4	300.1 ± 48.9	297.5 ± 57.6	313.3 ± 65.6	0.322
RBC (10^{12}/l)	4.7 ± 0.5	4.7 ± 0.5	4.9 ± 0.5	4.9 ± 0.6	0.731
Haemoglobin (g/l)	141.2 ± 13.5	140.7 ± 10.9	142.6 ± 10.6	140.6 ± 8.2	0.951
WBC count (10^9/l)	5.7 ± 0.6	6.8 ± 1.0	5.6 ± 0.8	6.8 ± 0.8	0.003*

Group A, control, healthy; group B, infection of upper airways; group C, asthma; group D, asthma+infection of upper airways.
SD indicates standard deviation.

Table 4
Variations of ratios between IL-4/IFN-γ and IL-5/IFN-γ during one week

	Group A (n = 10)	Group B (n = 10)	Group C (n = 10)	Group D (n = 10)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Value of p[a]
IL-4/IFN-γ ($10^{?1}$)					
Day 1	0.9 ± 0.4[b]	0.7 ± 0.2[b]	4.8 ± 2.0[c]	5.2 ± 1.6[c]	< 0.001 *
Day 2	0.9 ± 0.4[b]	0.5 ± 0.2[b]	5.2 ± 1.5[c]	3.5 ± 0.7[c]	< 0.001 *
Day 3	0.8 ± 0.4[b]	0.5 ± 0.2[b]	4.5 ± 1.3[c]	8.6 ± 2.8[d]	< 0.001 *
Day 4	0.8 ± 0.3[b]	0.8 ± 0.4[b]	4.1 ± 1.5[c]	8.4 ± 1.8[d]	< 0.001 *
Day 5	1.0 ± 0.5[b]	1.2 ± 0.5[b]	6.3 ± 1.6[c]	9.9 ± 2.5[d]	< 0.001 *
Day 6	1.0 ± 0.3[b]	1.3 ± 0.6[b]	5.1 ± 1.4[c]	9.5 ± 2.2[d]	< 0.001 *
Day 7	1.0 ± 0.5[b]	1.3 ± 0.6[b]	5.8 ± 1.9[c]	9.7 ± 3.0[d]	< 0.001 *
IL-5/IFN-γ ($10^{?1}$)					
Day 1	0.3 ± 0.2[b]	0.4 ± 0.2[b]	1.4 ± 0.7[c]	1.8 ± 0.8[c]	< 0.001 *
Day 2	0.3 ± 0.1[b]	0.2 ± 0.1[b]	1.4 ± 0.5[c]	1.9 ± 0.5[c]	< 0.001 *
Day 3	0.2 ± 0.1[b]	0.2 ± 0.1[b]	1.1 ± 0.5[c]	2.7 ± 1.0[d]	< 0.001 *
Day 4	0.4 ± 0.2[b]	0.3 ± 0.1[b]	1.1 ± 0.4[c]	2.6 ± 0.7[d]	< 0.001 *
Day 5	0.3 ± 0.1[b]	0.4 ± 0.2[b]	1.0 ± 0.5[c]	2.8 ± 0.8[d]	< 0.001 *
Day 6	0.3 ± 0.1[b]	0.5 ± 0.3[b]	1.2 ± 0.4[c]	3.4 ± 1.0[d]	< 0.001 *
Day 7	0.3 ± 0.1[b]	0.5 ± 0.3[b]	1.2 ± 0.5[c]	2.8 ± 0.7[d]	< 0.001 *

Group A, control, healthy; group B, infection of upper airways; group C, asthma; group D, asthma+infection of upper airways.
IFN indicates interferon; IL, interleukin; SD, standard deviation.
* Indicates significant difference, p < 0.05.
[a] A mixed linear model was used.
[b,c,d] Different letters indicate significant differences between both groups, p < 0.017.

Table 5
Variations of scores for upper airway viral infections and asthma during one week[1]

	Group B (n = 10)	Group C (n = 10)	Group D (n = 10)	
	Mean ± SD	Mean ± SD	Mean ± SD	Value of p[a]
Score of asthma symptoms				
Day 1	–	0.3 ± 0.5	0.2 ± 0.4	0.714
Day 2	–	0.2 ± 0.4	1.2 ± 0.4	0.015 *
Day 3	–	0.3 ± 0.5	2.3 ± 0.5	0.001 *
Day 4	–	0.4 ± 0.5	3.2 ± 0.4	< 0.001 *
Day 5	–	0.3 ± 0.5	3.1 ± 0.6	< 0.001 *
Day 6	–	0.3 ± 0.5	2.3 ± 0.7	0.002 *
Day 7	–	0.2 ± 0.4	1.8 ± 0.4	0.002*
Symptom score for airway infections				
Day 1	1.7 ± 0.7	–	1.6 ± 0.5	0.624
Day 2	3.9 ± 0.9	–	2.9 ± 0.7	0.141
Day 3	3.7 ± 1.2	–	4.0 ± 0.7	0.141
Day 4	2.9 ± 0.7	–	4.6 ± 0.5	0.141
Day 5	1.9 ± 0.6	–	3.3 ± 0.5	0.018 *
Day 6	0.9 ± 0.6	–	2.5 ± 0.5	0.002 *
Day 7	0.2 ± 0.4	–	2.3 ± 0.5	< 0.001 *

Group A, control, healthy; group B, infection of upper airways; group C, asthma; group D, asthma+infection of upper airways. A dash indicates a non-determined value.
SD indicates standard deviation.
* Indicates significant difference, p < 0.05.
[a] A mixed linear model was used.

monitored the concentrations of cytokines (IFN-γ, IL-4 and IL-5) in induced sputum samples of asthmatic patients and healthy individuals in the control group, with or without viral airway infections, and registered the scores for asthma and upper airways viral infection symptoms during a 7-day period. We observed that individuals presented with low values for IL-4, IL-5 and IFN-γ in induced sputum, with barely any fluctuations during the 7 days and that their level of IFN-γ in the sputum increased rapidly (on day 2) up to high values when these patients contracted viral infection of the upper airways. We also found that viral infection symptoms showed at an early stage and remitted very fast. This shows that healthy individuals have effective antiviral immunity. In induced sputum, IFN-γ values had a negative correlation with symptoms, as observed on previous studies.[16,17]

We found that individuals with asthma, even in the remission stage, presented with higher values of IL-4 e IL-5 and lower values of IFN-γ than healthy individuals and higher ratios of IL-4/IFN-and IL-5/IFN-γ ($p < 0.01$). These results confirm that asthmatic individuals have a history of immune system deviation towards a Th2 response, which is consistent with data from research in animals and in clinical settings.[18-21] Furthermore, during acute exacerbations of asthma, a predominant Th 2deviation is evident.[22] Recent tests indicate that this abnormal deviation towards Th2 activity in asthmatic patients could be related to a decrease in the function of other types of Th cells, i.e., regulator T cells. These cells seem to reduce Th2 activity in healthy individuals.[23] The population of natural aggressor T cells could also play a role in asthma pathogenesis.[24]

IFN-γ values in induced sputum of patients with asthma, even during remission, increased to a lower degree and more slowly than in non-asthmatic individuals. Asthmatic individuals also had a higher viral infection symptom score than healthy individuals. It has been argued that asthmatic patients are not more vulnerable to viral airway infections than patients who do not have asthma, even though they may suffer more serious consequences.[25] This indicates the possibility that they may suffer deterioration of their antiviral responses and, in consequence, develop more prolonged infections that can cause severe airway obstruction and wheezing. It is interesting to highlight that the production of IFN-β, IFN-γ and IFN-λ in response to airway viruses could also be impaired.[26-28]

There is evidence that proves that an insufficient production of IFN-β in the airways of asthmatics patients causes a viral infection of epithelial cells. Once infected, these cells can show an inefficient apoptotic response. Consequently, virus can rapidly multiply in these cells and enter the airways, so that the viral load is much higher than that of healthy patients.[28,29] Furthermore, virus induced IFN-γ secretion is positively related to lung function in asthmatic individuals.[30] Globally, these findings indicate that deterioration of IFN response could lead to more serious clinical features in viral airway infections in patients suffering from asthma. We also found that symptom scores in these asthmatic patients were much higher than in asthmatic patients without upper airway viral infection. These findings are consistent with a "likely to exacerbations" phenotype.[31,32]

However, we did not find any significant differences in IL-4/IFN-γ and IL-5/IFN-γ ratios between healthy individuals with or without

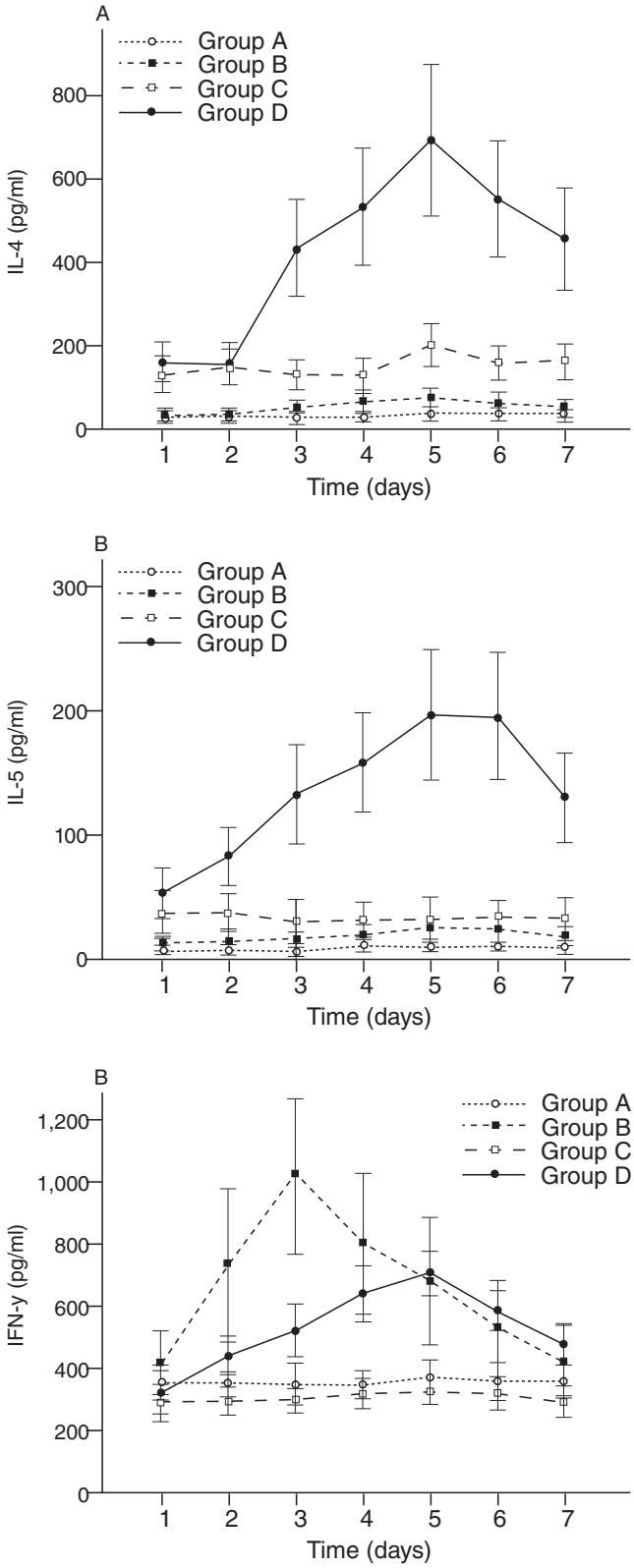

Figure 1. Time trends of cytokines in sputum samples induced during the 7 day observation period. A) Interleukin (IL) 4. B) IL-5. C) Interferon gamma. Group A, healthy individuals; group B, with airway viral infection, but otherwise healthy; group C, with mild or moderate asthma; group D, same as group C, but with airway viral infection (n = 10 per group). The results are means ± standard deviations. A linear mixed method was used to analyse time trends. When a significant time trend was found, a Tukey test was used for post-hoc analysis. Legend: 1A) 1, IL-4 (pg/mL); 2, time (days); 3, group A; 4, group B, 5, group C, and 6, group D; 1B) 1, IL-5 (pg/mL); 2, time (days); 3, group A; 4, group B; 5, group C, and 6, group D; 1C) 1, interferon gamma (pg/mL); 2, time (days); 3, group A; 4, group B; 5 group C, and 6, group D.

upper airway viral infection, which indicates that there were no significant changes of Th1/Th2 homeostasis. Furthermore, we did observe significant differences in these ratios between asthmatic individuals with and without upper airway viral infections. This indicates that there are probably other mechanisms that regulate this homeostasis. Recently, in the course of clinical studies, much attention has been given to regulating T lymphocytes and it has been found that they regulate Th1/Th2 homeostasis through a number of mechanisms.[33]

In this study, we determine that the time trends of changes in IL-4 and IL-5 in induced sputum of patients with asthma and airway viral infections were consistent with fluctuations in asthma symptoms, since they were more serious 3-5 days after contracting the infection. As a result, we concluded that 3-5 days after contracting a viral infection of the upper airways is the timeframe where infection symptoms worsen in patients with asthma. It is possible to recommend administering treatment as soon as possible during this timeframe.

The balance of Th1/Th2 immune responses is inclined towards Th2 cells in patients with mild or moderate asthma, even during remission. Antiviral immunity of the airways is less resistant in these patients and is weaker compared to antiviral immunity in healthy individuals. In asthmatic patients, the viral infection induced Th1 cell response of the upper airways exacerbates the Th1/Th2 imbalance. This indicates the existence of other mechanisms to control this balance. We found that the timeframe during which infection symptoms begin to worsen in patients with asthma is 3-5 days after contracting the infection.

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