ORIGINAL ARTICLE

Upregulation of IL-9 and JAK-STAT Signaling Pathway in Severe Asthma: A Control Study Group of Female Asthmatic Patients

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ABSTRACT

T helper 9 (Th9) cells and interleukin (IL)-9 are involved in the pathogenesis of autoimmune and inflammatory diseases. The exact role of IL-9 cytokine in patients with severe asthma have not yet been studied adequately. The aim of the present study was to investigate the role of IL-9 and the JAK-STAT signaling pathway in 50 women with severe asthma compared to 39 sex□and age□matched health control (HC). IL-9 levels were assessed in induced sputum (IS) and serum by ELISA. IL-9, JAK-1, and STAT5 mRNA and protein levels were assessed by qRT-PCR analysis and western blot respectively. IL-9 levels in severe asthmatic patients were higher than those in HC. Allergic asthmatic patients expressed more IL-9 protein than non-allergic patients. The mRNA levels and protein expression for IL-9, JAK1, pJAK1, STAT5, and pSTAT5 were also significantly elevated in severe asthma relative to HC. These results suggested that IL-9 cytokine and JAK-STAT activation signaling have an essential role the inflammatory process of severe asthmatics.

Key words: Severe asthma, IL-9, $CD4^+$ $IL\text{-}9^+$, JAK/STAT signaling pathway

INTRODUCTION

Asthma is associated with mucosal inflammation driven by innate and adaptive immune responses and airway hyperresponsiveness resulting in bronchoconstriction caused by proinflammatory mediators.[1] Genetic studies in humans have linked asthma and bronchial hyper-responsiveness to human chromosome 5q31-q33, which contains the gene for IL-9, and allelic association.[2] Th9 cells are a more specialized IL-9-producing cell than Th2, Th17 and Treg. [3] Genome-wide differential gene expression in response to dust mite allergen identified IL-9 as potential inducer of severe asthma exacerbations.[4] IL-9 stimulation of human airway epithelial cells resulted in goblet cell hyperplasia and mucin production.[5] The IL-9 activities are mediated by a specific hemopoietin receptor that activates a JAK/STAT pathway. [6] IL-9 expression is increased in lungs of asthmatic patients and IL-9R expression is found in the lungs of asthmatic individuals but not healthy controls.[7] IL-9 activates various types of immune and non-

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immune cells carrying the membrane bound IL-9R. IL-9R signaling has been shown to result in activation of JAK1, JAK3 and STAT transcription factors (STAT1, STAT3, and STAT5).[8] The exacerbation of severe asthma is the result of an imbalance between the Th cells favoring a strong induction of inflammatory mediators into the airways. In this sense, it would be interesting to further study the expression of Th9 / IL-9 in PBMCs and in IS as an inflammatory focus. IL-9 along with JAK-STAT signaling have not been explored in severe asthmatic patients. Therefore, we hypothesized that modulation of IL-9 cytokines which signal via JAK-STAT pathway may account for the immune alterations seen in severe asthmatic patients. We performed this study in a group of severe asthmatic females, attended in a specialized severe asthma unit. In addition, we performed associations among IL-9 levels and JAK-STAT signaling pathway.

MATERIALS AND METHODS

Study Populations

Fifty severe asthmatic patients and 39 non-asthmatic controls were recruited for this study (Department of Paediatric and Respiratory Diseases; A. Mami Hospital of lung diseases, Ariana, Tunisia) and investigated for their induced sputum. The diagnosis of severe asthma was established according to the ATS/ERS criteria. [9] Due to the specificity of our department, all patients were female as we recently reported. The control group (all females, aged 45–58 years) comprised 39 healthy volunteers with no history of obstructive lung disease. The study protocols were reviewed and approved by the ethics committees of our hospital (A. Mami Hospital, Ariana, Tunisia), and informed written consent was obtained from all participating subjects.

Sputum Induction and Processing

The procedure of sputum induction and processing were the same in control and asthma group and was preceded by inhalation of 400 µg of salbutamol and subsequent pulmonary function testing as we recently reported. [10] All patients were pre-treated with two puffs of salbutamol (400 µg) and inhaled a nebulized solution of normal saline followed by increasing concentrations (3%, 4% and 5%) of hypertonic saline using an ultrasonic nebulizer (ULTRA-NEBTM 2000, USA).

Measurement of Serum and Sputum IL-9

Sputum fluid, Serum IL-9 levels were measured using an enzyme-linked immunosorbent assay kit (ELISA) as we recently reported.[11] IL-9 level was measured using specific ELISA kits (R&D Systems, Minneapolis, MN, USA). Each sample was tested in duplicate. The results were expressed as pg/mL and the detection limit of this assay was 0.5 pg/mL.

RNA Extraction and Real-Time PCR Analysis

The isolation of peripheral blood mononuclear cells, sputum cells and the determination of mRNA expression was performed by qRT-PCR analysis as we previously reported. [10] The β-actin mRNA (internal control) was quantified in the same way as the IL-9 mRNA, using the forward and reverse primers: β-actin, 5′-ATGACTTCCAAGCTGGCCGT-3′ and 5′-CCTCTTCAAAAACTT CTCCACACC-3′; IL-9: forward 5′ GGGCATCAGAGACACCAAT-3′, reverse5′-GGACGGAGAGACA CAAGCA-3′; JAK1 forward: 5′-GTCTTTTGCTGCACTGGTGG-3′; reverse: 5′-CCCTGAGGGCTCGTTTCAAT-3′; STAT5 forward 5′-CAGACCAA GTTTGCAG CCAC-3′; reverse: 5′-CACAGCACTTTGTCAGGCAC-3′; and β-actin: forward 5′-GCAGAAGGAGATTAC TGCTCT-3′, reverse 5′-GCTGATCCACATCTG CTGGAA-3′ was used as an endogenous reference. Real-time RT–PCR was performed on a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) and mRNA levels were quantified using SYBR Premix Ex Taq™ II. Data were analysed using the Rotor-Gene real time analysis software version 6.0.

Statistical Analysis

Analysis of data was performed by SPSS version 20 software (SPSSInc). Kolmogorov-Smirnov test was used to check the normal distribution of the parameters. Spearman's correlation test was performed to measure the association between different parameters. P value lessthan 0.05 was considered as significant.

RESULTS

IL-9 Protein Expression in Severe Asthmatic Patients

Serum and sputum IL-9 were highly expressed in severe asthma [serum: 7.75 ± 1.69 pg/mL; sputum: 27.43 ± 6.77 pg/mL; $P < 0.0001$] compared to HC [serum: 5.21± 1.56 pg/mL; sputum: 4.79 ± 1.42 pg/mL; $P < 0.0001$] (Figure 1a). Sputum IL-9 level in asthmatics was five-fold higher than in serum value. Among asthmatic group, 27 patients' present allergies (Mite) and 23 patients were without allergy. IL-9 was found highly expressed in allergic patients (serum: 8.40 ± 1.59 pg/mL; sputum: 30.81 ± 6.64, pg/mL) compared to non-allergic asthma (serum: 6.98 ± 1.49 pg/mL; sputum: 23.45 ± 4.40, pg/mL). Significant correlation was observed between sputum IL-9 and IgE levels ($r = 0.506$; $p = 0.0002$) and between sputum IL-9 and FEV1 (% predicted) ($r= 0.68$; $p = 0.0001$). IL-9 mRNA expression and IL-9 protein were highly expressed in sputum from severe asthmatics as compared to non-asthmatic controls (Figure 1b, 1c).

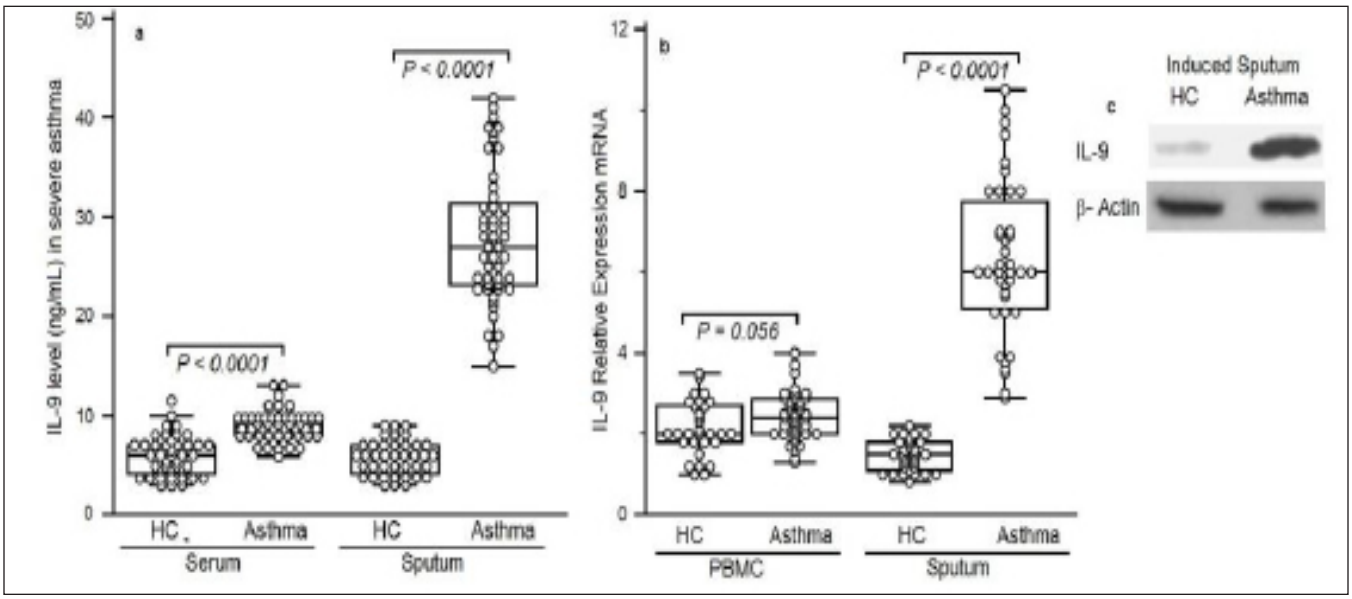
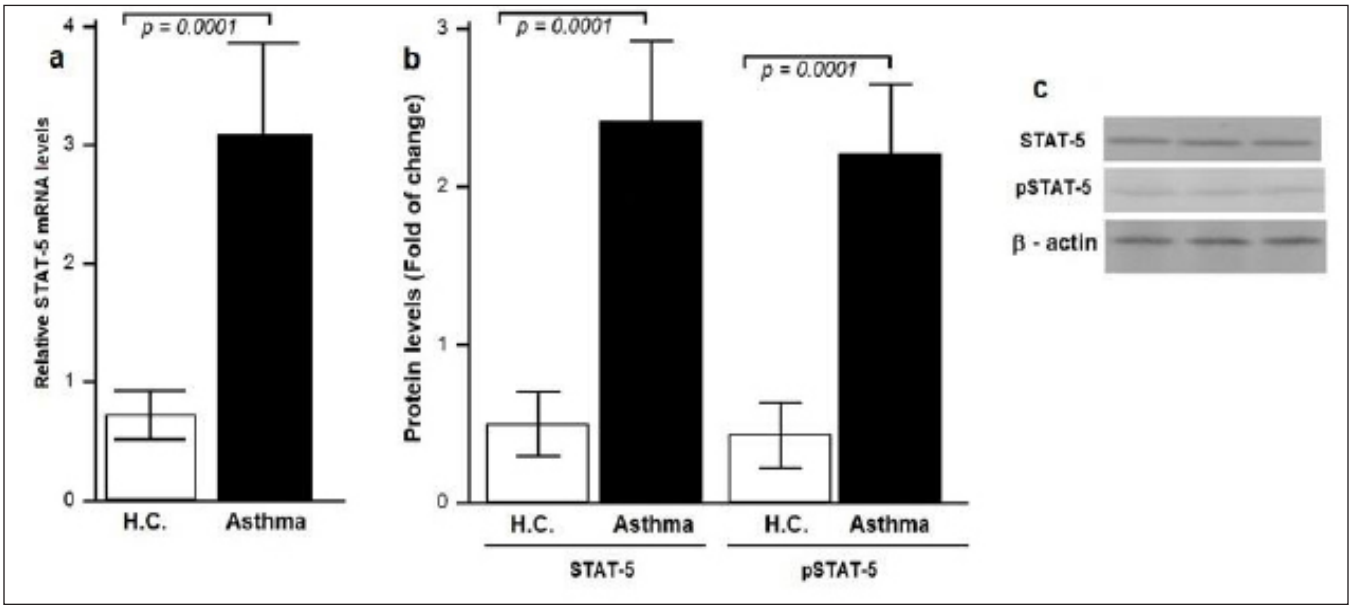


Figure 1. Serum and Sputum IL-9 protein levels in severe asthmatic patients. (a): IL-9 was quantified by ELISA in serum and in sputum fluid of severe asthmatic patients and non-asthmatic control (HC). (b): Expression of IL-9 mRNA. (c): Western blot expression of IL-9 in induced sputum.

Upregulation of JAK-STAT Signaling is Associated with Severe Asthma

We evaluate the role of JAK1, pJAK1, STAT5 and pSTAT5 transcription factors signalling pathways in severe asthmatics using RT-PCR and western blot analysis. STAT-5 mRNA and JAK-1 mRNA were highly expressed in severe asthmatics compared to HC (Figure 2a, 2d). In the same way, protein expression of STAT-5, pSTAT-5, JAK-1 and pJAK-1 were increased in asthmatics (Figure 2b, c, e, f). Our data suggest that JAK1 signaling may play a significant role in severe asthmatic patients, and may be a clinically useful disease marker. These results provide evidence that STAT5 and JAK-1 signalling may also be a key indicator of immune alterations in severe asthmatic patients.



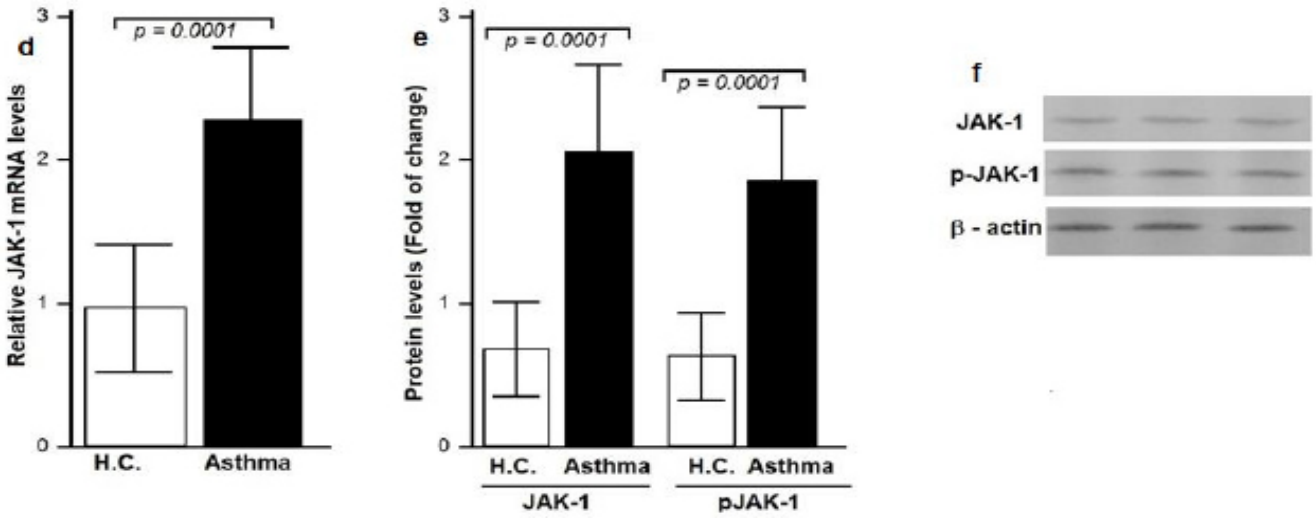


Figure 2. Relative expression of STAT5 and JAK-1 in severe asthmatic patients by QRT-PCR (a, d). Protein levels of STAT5, pSTAT-5, JAK-1 and pJAK-1 and JAK1 analysed by western blot (b, c, e, f). β-actin served as the standard. Data are expressed as means ± SD.

DISCUSSION

In this study we show, for the first time, that the IL-9 is up-regulated in the IS of severe asthmatic patients. We have also shown that IL-9 is correlated with FEV1, in asthmatic smokers and especially in patients with allergy. Our data reported increased significant concentration of IL-9 in severe asthmatics and especially in patients with allergic asthma. Finally, we reported that mRNA and protein expression for IL-9, JAK1, pJAK1, STAT5, and pSTAT5 were also significantly elevated in severe asthmatics women relative to non-asthmatic controls. These results suggested that IL-9 and JAK-STAT activation signalling have an essential role in immune dysfunction of our severe asthmatic group.

IL-9 has recently been implicated in determining mucosal immunity and susceptibility to atopic asthma.[12] IL-9 is described as a pro-inflammatory cytokine because of its capacity to support proliferation of B and T cells. In combination with IL-17, IL-9 increases the accumulation of neutrophils and perpetuates inflammation as observed in inflammatory /auto-immune diseases. Th9 cells in asthma have been studied extensively in experimental models in mice but little studied in asthma in human. [13] The major interest in characterizing these helper T cells is none other than identifying treatments. Human studies corroborate observed data in animal.[13] Thus, IL-9 seems to be important in regulating the known risk factors for asthma, and represents an important target identified through genetic means for therapeutic intervention in this disorder. The increased IL-9 levels contribute to general allergic phenotype, characterized by high levels of IgE antibodies to aeroallergens derived from house dust mites, as we reported. The increased IL-9 protein in serum and sputum in severe asthmatics and particularly in our allergic cohort were confirmed in this short paper

T helper cells-derived cytokines are critically associated with asthma pathogenesis and JAK-STAT transduction and activation of transcription signaling is found to be involved in asthma.[14] IL-9 induction depends on the activation of the JAK-STAT pathway.[15] All the IL-9 activities studied so far are mediated by a specific hemopoietin receptor that activates a JAK-STAT pathway.[14] JAK-STAT signalling pathway is a promising route for the effective control of the disease. Early evidence indicates that the JAK-STAT pathway is involved in asthma severity.[16] Our study showed an increased induction of JAK-STAT signaling in severe asthma. The JAK-STAT pathway plays critical roles in survival, cell growth, and differentiation of many types of cells, and is particularly important in controlling T cell differentiation. Together, these results suggest that several potential mechanisms may underlie asthma exacerbation. Our findings suggest that improvement of Th9 cytokine and JAK-STAT signaling might ameliorate severe asthma.

CONCLUSION

Our results suggest IL-9 may play an important role in the

pathogenesis of patients with severe asthma by modulating adaptive and innate immune responses. Our study also showed an increased induction of JAK / STAT. Together, these results suggest that several potential mechanisms may underlie asthma dysregulation. Our results suggest that improving cytokine signaling and JAK / STAT may improve the management of severe asthma.

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