

ORIGINAL PAPER

Type III IFN- λ mRNA expression in sputum of adult and school-aged asthmaticsDominique M. A. Bullens^{*,†}, A. Decraene^{*}, E. Dilissen^{*}, I. Meyts[†], K. De Boeck[†], L. J. Dupont[‡] and J. L. Ceuppens^{*}^{*}Clinical Immunology, Department of Experimental Medicine, [†]Pediatrics, Department of Woman and Child and [‡]Pneumology, Department of Internal Medicine, Katholieke Universiteit Leuven (KULeuven), Leuven, BelgiumClinical and
Experimental
Allergy

Summary

Background The increased susceptibility of asthmatics to rhinovirus infection has recently been related to deficient IFN- λ 1 (IL-29) and IFN- λ 2/3 (IL-28) production by bronchial epithelial cells and macrophages.**Objectives** Here, we studied IFN- λ mRNA expression in the airways of stable asthmatics in comparison with healthy subjects and in relation to asthma symptoms, non-invasive parameters of airway inflammation and lung function parameters.**Methods** Airway cells were obtained by sputum induction, in 14 healthy and 35 asthmatic adults and 12 asthmatic school-aged children. IFN- λ was studied at the mRNA level by quantitative RT-PCR.**Results** Asthmatic adults have increased sputum IL-28 mRNA but similar IL-29 mRNA expression in comparison with healthy subjects. In asthmatics, both sputum IL-28 and IL-29 mRNA expression correlate with the sputum CD3 γ mRNA expression (reflecting infiltrated T cells). IL-28 (but not IL-29) mRNA levels correlate with the relative and absolute number of eosinophils present in the sputum sample. Sputum IL-29 mRNA (but not IL-28) correlates negatively with asthma symptoms in steroid-naïve patients and is significantly higher in steroid-treated than in steroid-naïve patients. Finally, both IL-28 and IL-29 mRNA levels are higher in asthmatic children than in asthmatic adults.**Conclusion** Our results show that asthmatic subjects have substantial type III IFN- λ mRNA levels in the airways. Our data furthermore suggest that IL-29 could have an immunoprotective role in the lower airways.**Keywords** airway inflammation, asthma, type III IFN*Submitted 12 November 2007; revised 14 April 2008; accepted 23 April 2008*

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Introduction

Asthma is histologically characterized by inflammatory cell infiltration of the bronchial mucosa, epithelial cell desquamation, goblet cell hyperplasia and thickening of the submucosa [1]. The role of T cells [2] and neutrophils [3] in this asthmatic inflammatory process is now well defined; furthermore, eosinophils, mast cells and basophils, as well as damaged epithelial cells, release inflammatory mediators and recruit additional inflammatory cells [1].

The majority of asthma-exacerbations, both in allergic and non-allergic asthma, are associated with viral infections [4]. The recently discovered type III IFNs, IFN- λ 1 (IL-29) and IFN- λ 2/3 (IL-28), can be induced by viral

infection in several cell lines and dendritic cells and show antiviral activity by a mechanism similar to but independent of type I interferons [5, 6]. Type III IFN- λ s are most likely necessary for antiviral immunity in the lung [7]. Indeed, a defect in the *in vitro* virus-induced production of type III IFNs by bronchoalveolar lavage cells of allergic asthmatic individuals has been reported recently [7]. Moreover, experimental *in vivo* rhinovirus infection revealed that this defect in type III IFN- λ production was related to the severity of the viral illness and the degree of lung function decline during asthma exacerbation [7]. IFN- λ s in the lungs are most probably produced by epithelial cells, monocytes and/or macrophages [7].

The role of viral infections in the pathogenesis of persistent asthma is becoming the subject of intensive research. The production of the type III IFN, IL-29, is induced by viral agents and has been shown to down-regulate *in vitro* IL-13 production [8], an important mediator of airway hyper-reactivity [9]. We therefore wanted to study the presence of IL-28 and IL-29 in the airways of stable asthmatics with no evidence of viral infection in comparison with healthy controls.

Induced sputum can be used to evaluate the airway inflammatory process by cell and protein analysis. Also, cytokine secretion patterns can be measured by quantitative real-time RT-PCR. When mRNA levels for cytokines are normalized to a house-keeping gene, in order to correct for differences in the cell number and amount of cDNA among the different samples studied, some of the problems associated with protein measurements in sputum samples can be avoided [10, 11]. We used this technique in order to study the presence of IL-28 and IL-29 in the airways of stable adult asthmatics in comparison healthy individuals. Furthermore, we correlated IL-28 and IL-29 mRNA expression with asthma symptoms, lung function parameters and non-invasive parameters of airway inflammation. Finally, we studied sputum IL-28 and IL-29 mRNA expression in stable asthmatic schoolchildren.

Material and methods

Subjects

The adult asthmatic study population has been described in detail previously [10]. In brief, patients were recruited between September 2002 and August 2004 (with the exclusion of April, May and June because of the tree-pollen and grass-pollen season). Five additional steroid-naïve patients with mild asthma [based on Global Initiative for Asthma (GINA) criteria] [12] were recruited between September 2006 and February 2007 (Table 1). All patients had 'stable' asthma; they had no exacerbation at the time of or in the 2 months before sputum induction (based on a questionnaire). Only sputum samples with sufficient RNA quantities for RT-PCR analysis could be used for this study. Samples were available from 35 asthmatic subjects (12 women, 23 men), not taking systemic steroids and 14 healthy controls (12 women, 2 men) between 18 and 65 years. Patients attended the outpatient clinic of either the Pneumology or the Allergy Departments of our University Hospital. Asthma diagnosis was based on prior or current proof of reversibility of forced expiratory volume in 1 s (FEV_{1s}) $\geq 12\%$ and 200 mL after inhalation of salbutamol (conforming to ATC criteria). Thirteen patients regularly used inhaled corticosteroids (ICS); 'non-users' either had never used ICS or had not used them since at least 3 months.

Table 1. Asthma patient's characteristics: (a) adult and (b) childhood

	Number of patients
<i>(a)</i>	
Male/female	23/12
Allergic/non-allergic*	18/16
% FEV_{1s} $< 70\%$ of expected value	2 (1 on ICS [‡])
% FEV_{1s} 70–90% of expected value	10 (5 on ICS)
% FEV_{1s} $> 90\%$ of expected value	23 (7 on ICS)
Mild asthma [§]	20 (2 on ICS)
Moderate-to-severe asthma [§]	15 (11 on ICS)
<i>(b)</i>	
Male/female	7/5
Allergic/non-allergic	11/1
% FEV_{1s} 70–90% of expected value	3 (3 on ICS)
% FEV_{1s} $> 90\%$ of expected value	9 (6 on ICS)
Mild asthma [§]	2 (0 on ICS)
Moderate-to-severe asthma [§]	10 (9 on ICS)

*Based on detection of specific IgE antibodies; analysis was not performed in one patient.

[‡] FEV_{1s} expressed as % of the expected value for age, length and weight.

[‡]ICS, inhaled corticosteroids [< 500 µg/day BDP or equivalent ($n = 3$), 500–1000 µg/day BDP or equivalent ($n = 2$) and ≥ 1000 µg/day BDP or equivalent ($n = 7$)] all children: < 500 µg BDP or equivalent.

[§]Disease severity based on revised GINA criteria, mild intermittent and mild persistent asthmatics are grouped as 'mild'; moderate and severe asthmatics are grouped as 'moderate-to-severe'. Fisher's exact test between ICS use and revised GINA classification: ***: $P = 0.0009$

BDP, beclomethasone dipropionate; FEV_{1s} , forced expiratory volume in 1 s; GINA, Global Initiative for Asthma.

Among asthmatic subjects, 18 were classified as allergic according to the presence of serum-specific IgE antibodies for relevant aero-allergens [10]. Healthy non-smoking volunteers were recruited among the University students and co-workers. Healthy controls had normal spirometry, no present clinical symptoms of upper or lower airway disease, asthma symptom score (ASS) of zero and did not use any anti-asthma medication.

Twelve children with stable moderate-to-severe persistent asthma (five females, seven males), aged 8–12 years (mean age 10.0 years old), not taking systemic steroids were recruited between September 2002 and February 2007 at the paediatric outpatient clinic of our University Hospital (Table 1). Patients experienced symptoms during at least 1 year. Asthma diagnosis was based on reversibility of $FEV_{1s} \geq 12\%$ after inhalation of salbutamol. Spirometry was performed according to ATS guidelines [13] and all had $FEV_{1s} > 80\%$ of the expected value (Table 1). Nine patients regularly used ICS [< 750 µg/day beclomethasone dipropionate (BDP) or equivalent]; 'non-users' either had never used ICS or did not use them since at least 3 months. Among asthmatic children, 11 were classified as allergic according to the presence of serum-specific IgE antibodies for house dust mite ($n = 9$) and/or pets ($n = 9$)

and a clinical history suggestive of allergic responses to those allergens.

Disease activity was evaluated using ASS [14], which correlates significantly with the validated (Dutch translated) Juniper score [10, 15]. In subgroups of adult patients, bronchial hyperresponsiveness was quantified as the histamine concentration that led to a 20% decrease in FEV₁ (PC₂₀). Fractional concentration of nitric oxide in exhaled air (FE_{NO}) was measured on-line during single breath exhalation at an expiratory flow rate of 200 mL/s using Ecophysics CLD 700 AL MED (Dürnten, Switzerland) [14].

The study was approved by the local ethical committee of the Faculty of Medicine (Leuven) and informed consent was obtained from patients and/or parents.

Sputum induction

Sputum was induced with an aerosol of inhaled hypertonic saline in increasing concentrations of 3%, 4% and 5% for 7 min in adults and one dose of 5% for 7 min in kids (unless a fall in FEV₁ >10% occurred, in which case the procedure was stopped) generated by a De Vilbiss Nebulizer (Ultra-NebTm 2000 model 200HI, De Vilbiss Health care Inc., Somerset, PA, USA) after pre-treatment with 400 µg of inhaled salbutamol.

Sputum processing, differential cell count and quantitative mRNA

Sputum was processed by a modification of the technique described by Pizzichini et al. [16] and described in detail elsewhere [10]. In 29 adult patients and four children, absolute cell numbers after isolation were available. Differential cell count and RNA isolation were also described previously [10, 11].

Real-time quantitative RT-PCR was performed for IL-28, IL-29, CD3 γ and 18S rRNA in the ABI prism 7700 Sequence Detector System (Applied Biosystems, Foster City, CA, USA) as described [10, 11]. The primer and probe sequences for CD3 γ were published previously [11]. The primer and probe sequences for IL-28 and IL-29 were:

IL28A- forward primer (fw) ctg cca cat agc cca gtt ca
 IL28A- reverse primer (rv) aga agc gac tct tct aag gca tct t
 IL28A- Taqman probe (tp) tct cca cag gag ctg cag gcc tt t a
 5'Fam 3'Tamra
 IL29-fw gga cgc ctt gga aga gtc act
 IL29-rv aga agc ctc agg tcc caa ttc
 hIL29-tp agt tgc agc tct cct gtc ttc ccc g 5'Fam 3'Tamra

cDNA plasmid standards, consisting of purified plasmid DNA specific for each individual target, were used to quantify the target gene in the unknown samples, as described [17]. All results were normalized to 18S rRNA to compensate for differences in the amount of cDNA. The

lower limit of detection for both IL-28 and IL-29 mRNA levels was 10 copies, respectively, found at 37 and 35 threshold cycles (CT). All but one of the samples had IL-28 and IL-29 mRNA levels above the detection limit. Owing to limitation in the amount of cDNA available, IL-29 mRNA could not be measured in four of the sputum samples.

Statistics

Statistical analyses were performed with the help of GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) using the two-tailed Mann-Whitney *U*-test. Correlations were studied by the Spearman's non-parametric test. Contingency tables were analysed by Fishers' exact test. A difference was considered to be significant when $P \leq 0.05$.

Results

Sputum interferon- λ levels in healthy and asthmatic subjects

As shown in Figs 1a and b, sputum cells of both healthy ($n = 14$) and asthmatic ($n = 35$) individuals express IL-28 and IL-29 mRNA. Sputum IL-28 mRNA (Fig. 1a) but not IL-29 mRNA (Fig. 1b) levels are significantly higher in asthmatic than in healthy individuals, both in the steroid-naïve and in the steroid-treated patient group ($P = 0.02$ and 0.01 , respectively). IL-28 mRNA levels are comparable between steroid-treated and steroid-naïve patients (compare the open and closed symbols in Fig. 1a, $P = 0.8$). Sputum IL-28 and IL-29 mRNA levels correlated significantly with each other in control individuals as well as in asthmatic individuals, whether patients were steroid naïve or steroid treated ($r = 0.9$, $P < 0.0001$ in controls; $r = 0.7$, $P = 0.0007$ and $r = 0.9$, $P = 0.0005$ steroid naïve and steroid treated, respectively, data not shown).

Sputum IL-29 mRNA levels were significantly higher in steroid-treated than in steroid-naïve asthmatic patients (median IL-29 mRNA levels 0.4 and 0.1, respectively, $P = 0.04$) [compare open ($n = 10$) and closed ($n = 21$) symbols in Fig. 1b]. Sputum IL-29 mRNA levels were also significantly higher in the steroid-treated patient group than in healthy controls ($P = 0.04$). No statistically significant difference in IL-28 or in IL-29 mRNA levels could be observed when patients treated with low-dose ICS (< 1000 µg BDP or equivalent) were compared with patients treated with high-dose ICS (≥ 1000 µg BDP or equivalent) ($P = 1$ and $P = 0.9$, respectively).

Allergic asthmatics tended to have higher IL-28 mRNA levels than non-allergic asthmatics (median 0.02 and 0.004, respectively, $P = 0.09$), whereas sputum IL-29 mRNA levels were comparable in allergic and non-allergic asthmatics (median 0.06 and 0.2, respectively, $P = 0.7$, data not shown).

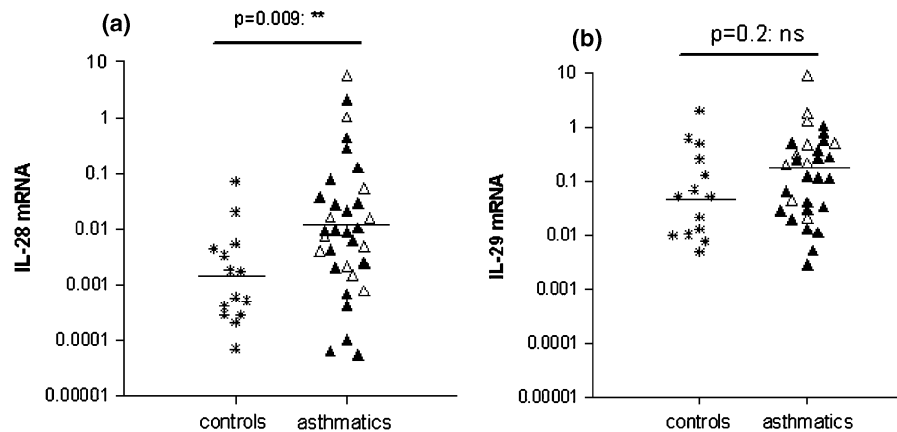


Fig. 1. IL-28A and IL-29 mRNA levels in induced sputum: comparison between healthy controls and asthmatics. Cells were isolated from induced sputum of healthy controls ($n = 14$) and asthmatic patients ($n = 35$). RNA was extracted and $1 \mu\text{g}$ RNA was transcribed to cDNA, which was used in duplicate to perform real-time RT-PCR using AB-universal Mastermix, with either 18S rRNA (a, b) or IL-28A- (a) and IL-29- (b) specific primers and VIC- (18S rRNA) or FAM- (cytokines) labelled specific probes. Results were quantified by the use of a cDNA plasmid-standard and expressed as the ratio of cDNA copy numbers for IL-28A (a) or IL-29 (b) divided by the cDNA copy numbers for 18S rRNA multiplied by 10^4 [17]. Open symbols represent patients treated with inhaled corticosteroids (ICS), closed symbols represent patients without ICS treatment. Comparisons were performed using two-tailed Mann-Whitney U -test. The median is indicated with a horizontal line. ** $P < 0.01$ and NS, not significant.

Table 2. *Differential cell counts in sputum of control subjects and asthmatic adults and children

	Macrophages	Neutrophils	Eosinophils	Lymphocytes	Epithelial cells
Controls (A) ($n = 11$)	61.7 ± 4.3	36.4 ± 4.3	0.4 ± 0.2	1.6 ± 0.4	15.7 ± 4.8
Mild asthmatics (B) ($n = 13$)	63.5 ± 4.3	29.7 ± 3.8	2.7 ± 0.9	4.1 ± 1.1 (1)	8.3 ± 2.6
Moderate-severe no ICS (C) ($n = 4$)	32.7 ± 6.6 (2)	57.3 ± 8.9 (3)	6.0 ± 2.2 (4)	3.9 ± 1.5	3.6 ± 1.8
Moderate-severe+ICS (D) ($n = 11$)	38.6 ± 6.7 (2)	52.1 ± 6.5	5.7 ± 2.8	3.4 ± 0.9	4.7 ± 1.3
Kruskal-Wallis test comparing A, B, C and D	$P = 0.002$	$P = 0.02$	$P = 0.04$	$P = 0.06$	$P = 0.2$
Asthmatic children+ ICS, (E) ($n = 5$)	34.9 ± 3.9	49.8 ± 8.1	4.8 ± 3.6	10.6 ± 5.16	22.12 ± 5.2
MWU test comparing D and E	$P = 1$	$P = 0.9$	$P = 0.5$	$P = 0.2$	$P = 0.1$

*Cytospins were prepared, stained with May Grünwald Giemsa and the percentage of macrophages, neutrophils, eosinophils and lymphocytes among the leucocytes as well as the percentage of epithelial cells on the cytopins was counted. As all parameters passed normality test, values represent mean and standard error of the mean cell number in percentage. (1) controls vs. mild asthmatics, $P = 0.01$; (2) controls vs. moderate-to-severe asthmatics without ICS, $P = 0.01$; mild vs. moderate-to-severe asthmatics both without and with ICS, $P = 0.001$; (3) mild asthmatics vs. moderate-to-severe asthmatics with ICS, $P = 0.01$; (4) controls vs. moderate-to-severe asthmatics without ICS, $P = 0.01$.

ICS, inhaled corticosteroids; MWU, Mann-Whitney U -test.

Sputum interferon- λ levels in relation to sputum differential inflammatory cell count

Sputum IL-28 and IL-29 mRNA levels were compared with CD3 γ mRNA levels, reflecting lymphocytic inflammation [11] ($n = 35$), and with the percentage of eosinophils and neutrophils among the leucocytes and the percentage of epithelial cells among all cells present in cytopins ($n = 29$, Table 2). The absolute amount of cells present in the sputum sample after processing ($n = 29$) was similar among all groups (data not shown, Kruskal-Wallis $P = 0.3$).

We found a significantly positive correlation between sputum IL-28 (but not IL-29) mRNA levels and the percentage of eosinophils among leucocytes (Fig. 2a) as well as the absolute amount of eosinophils in the sputum sample in steroid-naïve patients (Fig. 2c). As shown in Figs 3a and b, sputum IL-28 and IL-29 mRNA levels

correlated significantly with the CD3 γ mRNA levels in asthmatics. However, no correlation between the IL-28 and IL-29 mRNA levels and the absolute amount of lymphocytes present in the sputum sample could be found (data not shown). Furthermore, and to our surprise, a significantly negative correlation between the percentage of macrophages [but not the absolute amount of macrophages (data not shown)] present on the cytospin and both IL-28 and IL-29 mRNA levels was found (Figs 3c and d). We also found a significantly positive correlation between the percentage of neutrophils on cytospin and sputum IL-29 mRNA levels ($r = 0.6$, $P = 0.001$, Fig. 3f) mainly in steroid-treated patients. However, IL-28 and IL-29 mRNA levels did not correlate with the absolute amount of neutrophils present in the sputum sample in asthmatic patients ($r = 0.2$, $P = 0.3$ and $r = 0.1$, $P = 0.3$, respectively, data not shown). We also observed a significant positive

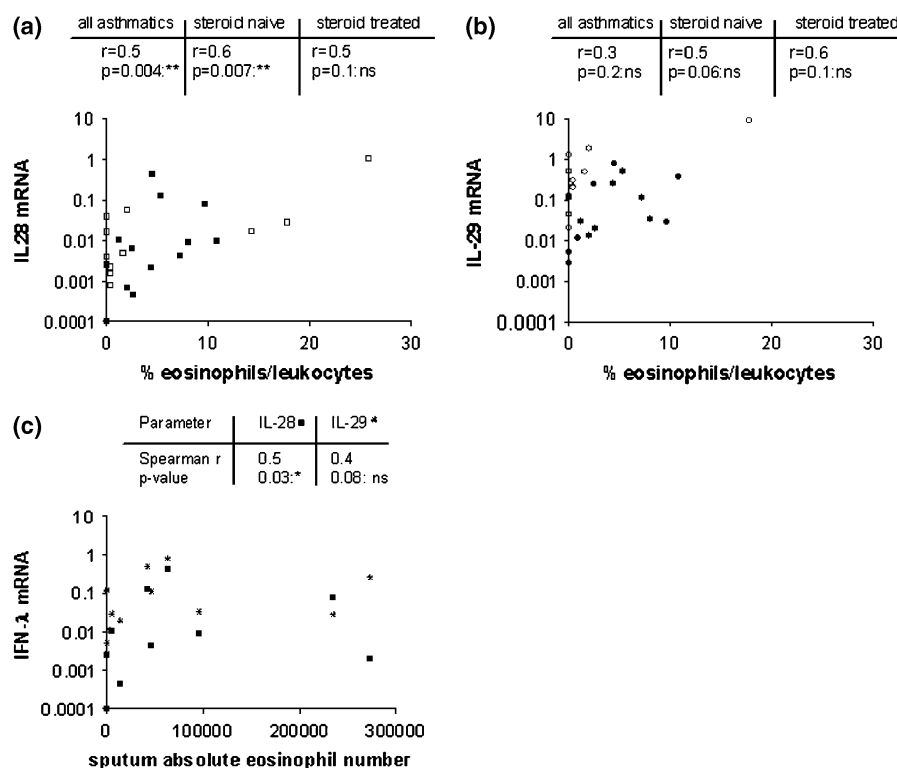


Fig. 2. Correlation of sputum IL-28A and IL-29 mRNA levels with eosinophils. Cells were isolated from induced sputum of asthmatic patients and counted after processing. IL-28A and IL-29 mRNA levels were determined as explained in Fig. 1. Cytospins were prepared, stained with May Grünwald Giemsa and the percentage of eosinophils among the leucocytes was counted (a, b) as well as the absolute amount of eosinophils present in the sample after processing in steroid-naïve patients (c). Open symbols represent patients treated with inhaled corticosteroids (ICS) and closed symbols represent patients without ICS treatment. Spearman's non-parametric correlation studies were performed. * $P < 0.05$, ** $P < 0.01$ and NS, not significant.

correlation between sputum IL-29 mRNA (but not IL-28 mRNA) levels and the percentage of epithelial cells (representing epithelial shedding [18]) in asthmatics (Figs 3g and h).

Sputum interferon- λ levels in relation to asthma symptoms, non-invasive parameters of airway inflammation and lung function parameters

As indicated in Fig. 4, IL-29 mRNA levels but not IL-28 mRNA levels (see Table 3) correlated negatively with the ASS in steroid-naïve asthma patients. No significant correlation could be observed between sputum IL-28 and IL-29 mRNA levels on the one hand and either FE_{NO} , histamine PC_{20} or $FEV_1\%$ levels on the other (data not shown).

Sputum interferon- λ levels in asthmatic schoolchildren

Sputum IFN- λ levels were studied in 12 moderate-to-severe persistent asthmatic schoolchildren, aged between 6 and 12 years, the majority treated with ICS. As sputum induction in an age-matched healthy control group failed to recover enough sputum RNA for cytokine mRNA quantification, we compared the IL-28 and IL-29 sputum mRNA levels with the amounts present in sputum from

asthmatic adults. Asthmatic children have significantly higher sputum IL-28 (median 2.4) and IL-29 (median 26.84) mRNA levels than asthmatic adults ($P < 0.0001$ for both cytokines). A significant difference persisted if only moderate-to-severe adult asthmatics (Fig. 5) were compared with the school-aged asthmatic kids. No correlation could be found between the ASS and either sputum IL-28 or IL-29 mRNA levels in the school-aged patient group. As almost all kids were steroid treated, no separate analysis of the sputum cytokine expression was performed in the steroid-naïve group. Although there was a tendency towards increased $FEV_1\%$ and $FEF_{50\%}$ levels in children with higher sputum IL-28 and IL-29 mRNA levels, these correlations were not significant ($r = 0.5$, $P = 0.06$ and $r = 0.4$, $P = 0.1$ between $FEV_1\%$ and IL-28 and IL-29, respectively; and $r = 0.4$, $P = 0.09$ and $r = 0.3$, $P = 0.2$ between $FEF_{50\%}$ and IL-28 and IL-29, respectively).

Discussion

This manuscript evaluates for the first time the presence of the type III IFN- λ s, IL-28 and IL-29, in the airways in stable asthma. We demonstrate that sputum IL-28 mRNA levels are significantly increased in asthmatics in

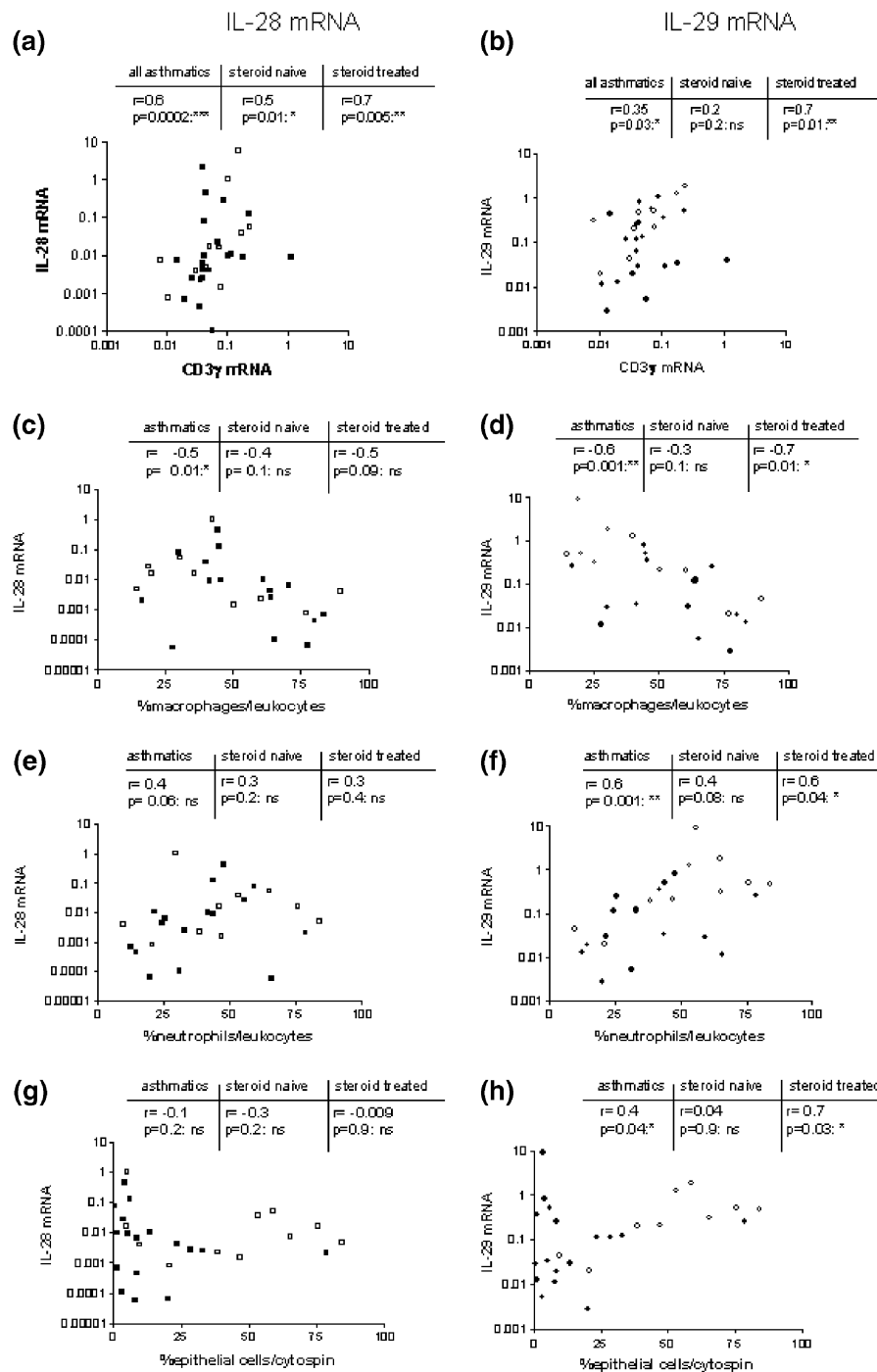


Fig. 3. Correlation of sputum IL-28A and IL-29 mRNA levels with the CD3 γ mRNA levels, the percentage of macrophages and neutrophils among leucocytes and the percentage of epithelial cells on the cytospin. Cells were isolated from induced sputum of asthmatic. IL-28A, IL-29 and CD3 γ mRNA levels were determined as explained in Fig. 1. Cytospins were prepared as explained in Fig. 2 and the percentages of macrophages and neutrophils among the leucocytes as well as the percentage of epithelial cells on the cytospins were determined. Open symbols represent patients treated with inhaled corticosteroids (ICS) and closed symbols represent patients without ICS treatment. Spearman's non-parametric correlation studies were performed. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and NS, not significant.

comparison with healthy individuals, whereas sputum IL-29 mRNA levels are similarly present in asthmatic and healthy individuals. Type III IFN- λ s are most likely necessary for antiviral immunity in the lung [7]. In the

cited manuscript, *in vitro* stimulation by rhinoviruses induced significantly higher type III IFN- λ production by bronchoalveolar cells from healthy than from atopic asthmatic individuals. As our study subjects, asthmatic as

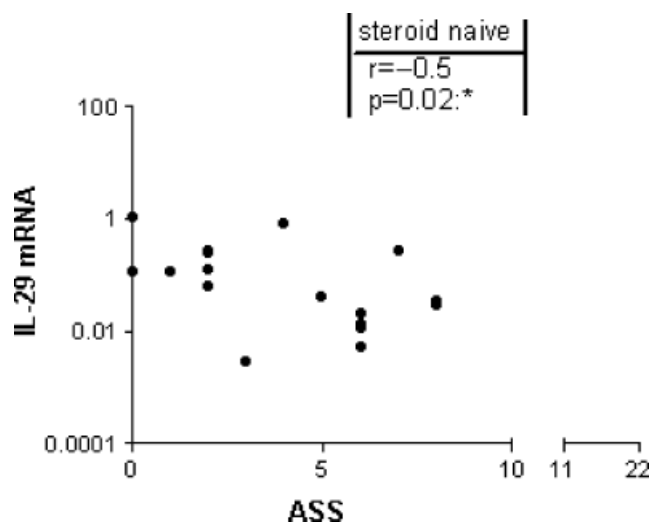


Fig. 4. IL-29 mRNA levels in correlation to asthma symptom score (ASS) in steroid-naïve patients. IL-29 mRNA levels were determined as explained in Fig. 1. Steroid-naïve patients scored their symptoms by using the ASS. A Spearman's non-parametric correlation study was performed.

Table 3. Relation between sputum IL-28 and IL-29 mRNA levels and Asthma Symptom Score (ASS) in steroid-naïve and steroid-treated asthmatic adults

	ASS/22	IL-28 [†]	IL-29
Steroid-naïve patients	Median: 4.5 P_{25} : 2 P_{75} : 6.5	$r = -0.1$ $P = 0.3$ ($n = 18$)	$r = -0.5$ $P = 0.02^*$ ($n = 17$)
Steroid-treated patients	Median: 8 P_{25} : 4.5 P_{75} : 12.5	$r = -0.2$ $P = 0.3$ ($n = 11$)	$r = 0$ $P = 0.5$ ($n = 9$)

[†]IL-28 and IL-29 mRNA levels were determined as explained in Fig. 1. Patients scored their symptoms by using the ASS. Median values of ASS as well as the 25th and 75th percentile of ASS values are shown. Spearman's non-parametric correlation study was performed.

* $P < 0.05$.

well as healthy individuals, did not report recent viral infection, we hypothesize that the presence of IL-28 and/or IL-29 mRNA in the airways of asthmatics might be constitutively expressed and its expression might be further triggered by chronic inflammation. Our data indicate that IL-28 and IL-29 mRNA expression in stable asthmatics without current viral infection is not defective.

In the steroid-naïve group, IL-29 mRNA levels correlated negatively with asthma symptoms as evaluated by ASS. This could mean that in the absence of anti-inflammatory treatment, the induction of IL-29 is related to milder asthma symptoms and IL-29 could therefore play an immunoprotective role. However, no correlation could be found between sputum IL-29 mRNA levels and other parameters used to evaluate airway inflammation or asthma severity (e.g. exhaled NO and FEV₁%). This might

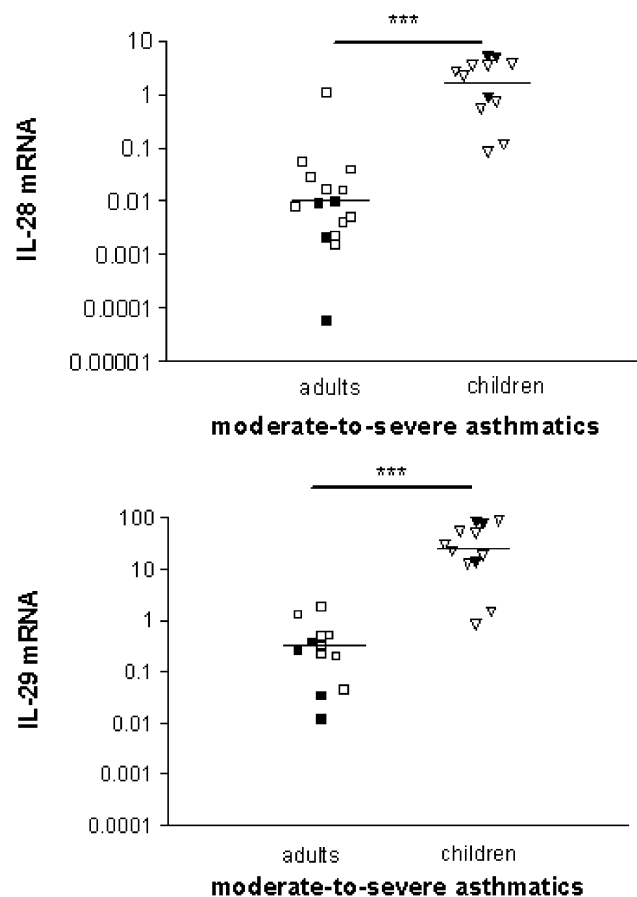


Fig. 5. IL-28A and IL-29 mRNA levels in induced sputum: comparison between moderate-to-severe asthmatic adults and children. Cells were isolated from induced sputum of moderate-to-severe asthmatic adults ($n = 15$) and children ($n = 12$). IL-28 and IL-29 mRNA levels were determined as explained in Fig. 1. Open symbols represent patients treated with inhaled corticosteroids (ICS) and closed symbols represent patients without ICS treatment. Comparisons were performed using two-tailed Mann-Whitney U -test. The median is indicated with a horizontal line. *** $P < 0.001$.

reflect the discrepancy between asthma control (which could be reflected by the ASS) and airway inflammation (e.g. exhaled NO levels). A similar hint towards a potential protective role is our finding that IL-29 mRNA expression is higher in steroid-treated than in steroid-naïve asthma patients. In other words, steroids might induce IL-29 mRNA. However, steroid-treated patients, with some exceptions, are mainly more severe asthmatics, thus leaving open the possibility that the higher values observed in steroid-treated patients are related to more severe inflammation. In steroid-treated patients, IL-29 mRNA expression correlated significantly with the epithelial cell percentage present in cytopins. This would suggest that epithelial cell shedding might, at least partly, be responsible for the detected IL-29 mRNA. An ongoing prospective study observing the changes in IL-29 mRNA expression after initiation of steroid treatment in

steroid-naïve patients will resolve this question. Finally, the trend in school-aged asthmatics towards a positive correlation between sputum IL-28 and IL-29 mRNA levels and FEV₁% also suggests a protective role of IL-29. In that respect, the significantly higher sputum IL-28 and IL-29 mRNA levels observed in this young age group when compared with adult asthmatics should not necessarily be considered as a sign of instability.

There are other data in the literature also pointing to an immunoprotective role of IFN- λ in asthma. First, it has been demonstrated recently that IL-29 can down-regulate T-helper type 2 cytokine production, especially IL-13 production [8], known to be an important mediator of airway hyper-reactivity [9]. Second, human monocyte-derived dendritic cells treated with IFN- λ acquire a tolerogenic phenotype and induce expansion of CD25⁺ T regulatory cells *in vitro* [19]. Thus, IFN- λ production might be a protective mechanism against allergic sensitization towards inhaled allergens.

Not all our data uniformly point towards a protective role of IFN- λ . In asthmatic patients, sputum IL-28 mRNA levels correlate with the percentage of sputum eosinophils as well as the absolute amount of eosinophils present in the sputum sample in steroid-naïve patients, and with the levels of CD3 γ mRNA, reflecting infiltrating T cells in all patients. Although T cells only represent a minority of cells in the asthmatic airways, the production of T cell cytokines in the airways has a major impact on the physiopathology of childhood as well as adult asthma [2, 20]. T cells and/or eosinophils are probably not the source of the IFN- λ production, but both cytokines might (most likely indirectly) contribute to the recruitment of T cells to the airways [21]. It has indeed recently been described that IL-29 is able to induce CXC chemokine mRNA expression in human peripheral blood mononuclear cells, especially of IFN- γ -inducible T cell α -chemoattractant (CXCL11), IFN- γ -inducible protein 10 (IP-10/CXCL10) and CXCL9. Upon CXCL9, CXCL10 and CXCL11 interaction with their chemokine receptor CXCR3, T cells (in a murine model, even preferentially Foxp3⁺ regulatory T cells) are recruited [22]. It is therefore possible that IL-29- (and perhaps also IL-28-) induced CXC chemokines contribute to T cell recruitment to the airways [21]. Whether the presence of IFN- λ mRNA in the airways causes inflammation and is hence harmful for the asthmatic subject or is caused by a compensatory mechanism in an attempt to reduce the ongoing inflammation is subject of further study.

It is striking that IL-29 mRNA negatively correlates with ASS and positively correlates with the relative numbers of neutrophils and epithelial cells, whereas IL-28 mRNA, on the other hand, correlates with the absolute and relative numbers of eosinophils in the samples. These findings would suggest that IL-29 and IL-28 could either be differentially induced or they could exert different functions in the airways but this has to be studied.

A weakness of our study is that our conclusions are based on cytokine measurements at the gene level only. Protein expression of IFN- λ s in the lower airways of stable asthmatics and healthy individuals is therefore not guaranteed. Although previous work has demonstrated a good correlation between sputum cytokine expression at the protein and at the mRNA level [23], additional invasive techniques (e.g. immunostaining on biopsy material) are necessary to finally prove the expression of IFN- λ in stable asthmatics.

Moreover, the study has been performed in Belgium outside the pollen season, and thus in a time span overlapping the period of prevalent viral infections. Viral cultures or PCR to detect viral colonization were not performed. Although asthmatics and healthy volunteers reported no symptoms related to a current or recent upper or lower respiratory tract infection, we cannot rule out current or recent viral contact or colonization. Viral colonization could especially be present at a younger age and might also explain the differences found between asthmatic children and adult asthmatics [24].

In conclusion, we demonstrate the presence of IL-28 and IL-29 mRNA in the airways in stable asthma. The inverse correlation of IL-29 levels with ASS is compatible with a protective role of this cytokine against asthma symptoms. The relation between IFN- λ and immunoprotective mechanisms, such as the potential recruitment of regulatory T cells to the lower airways, remains to be elucidated.

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