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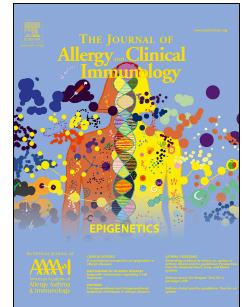
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Enhanced local production of interleukin-26 in uncontrolled compared with controlled adult asthma

Ellen Tufvesson, PhD¹, Prajakta Jogdand, PhD², Karlhans Fru Che, PhD³, Bettina Levänen, PhD³, Jonas S Erjefält, PhD², Leif Björmer, MD, PhD¹, Anders Lindén, MD, PhD^{3,4}

¹) Department of Clinical Sciences Lund, Respiratory Medicine and Allergology, Lund University, Lund, Sweden

²) Airway Inflammation, Department of Experimental Medical Science, Lund University, Lund, Sweden

³) Unit for Lung and Airway Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

⁴) Department of Respiratory Medicine and Allergy, Karolinska University Hospital Solna, Stockholm, Sweden

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*¹) Corresponding author:

Anders Lindén, M.D., Ph.D.

Unit for Lung and Airway Research, Institute of Environmental Medicine,
Karolinska Institutet, PO Box 210, SE-171 77 Stockholm, Sweden

E-mail: anders.linden@ki.se, Phone: +46 (0) 70 090 2286

Conflict of interest

The authors have no competing interests for the contents of this article.

Key words: airway obstruction; AK-155; BAL; cytokine; FENO; ICS; mild; moderate; severe; steroid; Th17

Short title: IL-26 in adult asthma

CAPSULE SUMMARY

The level of local production of IL-26 in the airways bears potential as a biomarker for characterizing adult patients with asthma in terms of poor disease control.

TO THE EDITOR:

Current pharmacotherapy is insufficient for patients with poorly controlled asthma [1,2]. To facilitate the development of novel and effective therapy, we need to improve the mechanistic understanding of this phenotype [3].

The cytokine interleukin (IL)-26 is produced by Th17 cells [4]. It potentiates the chemotactic response of human neutrophils *in vitro* and enhances endotoxin-induced accumulation of neutrophils and macrophages in the airways *in vivo* [4,5]. It also displays direct anti-bacterial properties in experimental models *in vitro* and *in vivo* [6]. Moreover, there is evidence for enhanced IL-26 protein in the airways for poorly controlled compared with controlled asthma, among children with the non-eosinophilic endotype [7]. However, it is not known what is the case in adult patients with asthma; nor in healthy volunteers [7]. Here, we addressed these matters by quantifying airway IL-26 protein in the airway lumen and tissue, as well as its gene expression in local leukocytes. We also related these outcomes to clinical characteristics and measures of inflammation.

We included 32 adult patients treated according to GINA guidelines [1,2], out of which 19 by definition had uncontrolled (asthma control test, ACT $\geq$ 20) and 13 had controlled asthma (ACT<20), plus eight healthy volunteers (**Table S1**). All patients reported weekly respiratory symptoms and displayed a positive metacholine provocation test. All subjects underwent clinical characterization and bronchoscopy. The latter included bronchial and transbronchial biopsies followed by bronchoalveolar lavage (BAL). The Online Supplement contains additional methodological information.

The majority of all patients displayed sensitization against common airborne allergens (**Table S1**). The subgroup with uncontrolled asthma had a lower median FEV₁ (% predicted) and was more responsive to methacholine than the subgroup with controlled asthma (**Table S1**). The subgroup with uncontrolled asthma had treatment with a somewhat higher but still moderate

dose of inhaled corticosteroids (ICS) and more frequently utilized beta₂-adrenoceptor agonists, compared with the subgroup of controlled asthma. However, there was no statistically significant difference in terms of treatment here (**Table S1**).

The median concentration of extracellular IL-26 protein in BAL fluid was lower in the pooled group of all patients with asthma than in healthy volunteers ($p=0.029$; $n=8-32$). In the subgroup of uncontrolled asthma, this IL-26 concentration was higher than in the subgroup with controlled asthma, whereas it still tended to be lower than that in healthy volunteers (**Figure 1A**).

Among all BAL cell subsets, there was a clear trend towards a difference between controlled and uncontrolled asthma for the median neutrophil count only (**Table S2**). In terms of BAL cell counts, the extracellular IL-26 protein displayed a negative correlation with total leukocytes and lymphocytes, in the pooled group of all patients and in the subgroup with uncontrolled asthma (**Figure S1A and B**). Here, there was a corresponding correlation for macrophages in the pooled group of all patients with asthma (**Figure S1C**). For eosinophils there was a corresponding correlation in uncontrolled asthma (**Figure S1D**) but no such correlation for neutrophils (**Figure S1E**).

In the pooled group of all patients with asthma, there was a clear negative correlation between extracellular IL-26 protein and FEV₁ (**Figure S2**) but no corresponding correlation within the subgroups of controlled or uncontrolled asthma. Likewise, we detected no correlations between extracellular IL-26 protein and bronchial responsiveness, F_ENO₅₀, bronchial NO flux, alveolar NO concentration or the dose of ICS among either of the groups with asthma (**Online supplement**).

In the pooled group of all patients with asthma, we detected no correlation between extracellular IL-26 protein and ACT score ($p=0.21$, $r=-0.23$, $n=31$). However, in uncontrolled asthma, the median ACT score was clearly lower in the subjects with relatively low ($\leq$ median

in all patients) compared to those with relatively high (>median in all patients) extracellular concentration of IL-26 protein (**Figure S3A**). In contrast, the ACT score was similar regardless of whether extracellular IL-26 protein was high or low in the subgroup of controlled asthma (**Figure S3B**). Moreover, there was a clear positive correlation between extracellular IL-26 and IL-8 protein in the pooled group of all patients with asthma (**Figure S4**).

The median level of IL-26 mRNA in BAL cells was higher in the subgroup of uncontrolled compared with the subgroup of controlled asthma (**Figure 1B**). The referred mRNA did not display any correlation with extracellular IL-26 protein (**Table S3**) but it did display a clear positive correlation with $F_{E}NO_{50}$ in all asthma groups (**Figure S5**) and a negative correlation with all BAL cell counts in the subgroup of uncontrolled asthma (**Table S3**).

We detected IL-26-positive cells in central bronchial mucosa and peripheral alveolar lung parenchyma (including luminal cells) from all subjects (**Figure 2A**). The pooled group of patients with asthma displayed lower median immunoreactivity for IL-26 protein (% area; $p=0.045$; $n=8-23$) in the parenchymal tissue, compared with healthy volunteers but there was no corresponding difference in bronchial tissue. In the subgroup of uncontrolled asthma, the referred immunoreactivity in parenchymal tissue tended to be higher than that in the subgroup of controlled asthma, although lower than that in healthy volunteers (**Figure 2B**). There was no corresponding difference in the bronchial mucosa (**Figure 2C**) or in the alveolar lumen (**Figure 2D**).

The current study indicates that extracellular IL-26 protein in the peripheral airways is enhanced in adult patients with uncontrolled compared with controlled asthma. The results are also compatible with both subgroups of asthma having lower extracellular IL-26 protein than do healthy volunteers. Similarly, the data sets on IL-26 gene expression in airway leukocytes, as well as protein in peripheral parenchymal tissue, support the idea that there is enhanced

production of IL-26 in uncontrolled compared with controlled asthma, and that there is less IL-26 protein in the peripheral airways of adult patients with asthma than in healthy volunteers.

Although the ACT score represents a continuous variable rather than a threshold one, this score can be a clinically useful indicator of disease control with a borderline value of 20 as the established “divider” between uncontrolled and controlled asthma [1-3]. Given this fact, our findings in the group defined as having uncontrolled asthma, including poorly controlled and uncontrolled disease, are intriguing. Here, we obtained evidence that, in uncontrolled asthma, low extracellular IL-26 protein signifies patients with an even poorer disease control. Possibly, these patients are unable to respond with enhanced local IL-26 protein; a response that may thus be both reactive and protective. The finding of a negative correlation between extracellular IL-26 protein and FEV₁ (% predicted) in the pooled group of all patients with asthma is also compatible with a reactive and protective cytokine response. Along these lines, the negative correlation between extracellular IL-26 and total leukocytes, lymphocytes and eosinophils in the lumen of patients with uncontrolled asthma is suggestive of a reactive mechanism that serves to protect from excessive mobilization of key leukocytes as well. The clear and positive correlation between gene expression for IL-26 in airway leukocytes and F_ENO₅₀ in all groups of patients with asthma is also suggestive of a reactive and protective role for IL-26.

Even though both the gene expression for IL-26 in airway leukocytes and extracellular IL-26 protein did display a higher median level in uncontrolled than in controlled asthma, there was no positive correlation between these two outcomes in patients or in healthy volunteers. We think that this can be explained as follows: Extracellular IL-26 protein in BAL samples reflects the production and release from several cellular sources, including structural cells such as bronchial epithelial cells and lung fibroblasts, as well as leukocytes [4,9,9]. In

contrast, the IL-26 mRNA that we quantified originates exclusively from luminal leukocytes in the airways.

Similar the case for extracellular IL-26 protein, we obtained evidence for a negative correlation between gene expression for IL-26 in airway leukocytes and counts for total leukocytes and lymphocytes in uncontrolled asthma. In the same sub-group, there was also a negative correlation for this gene expression and counts for neutrophils and macrophages. For neutrophils, this may seem paradoxical, since extracellular IL-26 and IL-8 protein do correlate in a positive manner in the pooled group of all patients with asthma, with indicated trends in the sub-groups. We suspect that this finding relates to the fact that IL-26 can exert both pro- and anti-inflammatory actions in terms of release of the very same neutrophil-mobilizing cytokines, depending upon the cellular target [4].

With the exception of three patients with controlled asthma, all patients had treatment with a narrow range of low to moderate doses of ICS. This fact makes it impossible to determine the role of ICS with respect to the difference in local IL-26 production between uncontrolled and controlled asthma. However, we recently demonstrated that treatment with a glucocorticoid effectively inhibits the release of IL-26 protein in human lung fibroblasts *in vitro* [9]. Thus, from a hypothetical point-of-view, ICS may account for the average decrease in extracellular IL-26 protein that we observed in the pooled group of all patients with asthma.

Ellen Tufvesson, Ph.D., Department of Clinical Sciences Lund, Respiratory Medicine and Allergology, Lund University, Lund, Sweden

Prajakta Jogdand, Ph.D., Airway Inflammation, Department of Experimental Medical Science, Lund University, Lund, Sweden

Karlhans Fru Che, Ph.D., Unit for Lung and Airway Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Bettina Levänen, Ph.D., Unit for Lung and Airway Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Jonas S Erjefält, Ph.D., Airway Inflammation, Department of Experimental Medical Science, Lund University, Lund, Sweden

Leif Bjermer, M.D., Ph.D., Department of Clinical Sciences Lund, Respiratory Medicine and Allergology, Lund University, Lund, Sweden

Anders Lindén, M.D., Ph.D., Unit for Lung and Airway Research, Institute of Environmental Medicine, Karolinska Institutet, and Department of Respiratory Medicine and Allergy, Karolinska University Hospital Solna, Stockholm, Sweden

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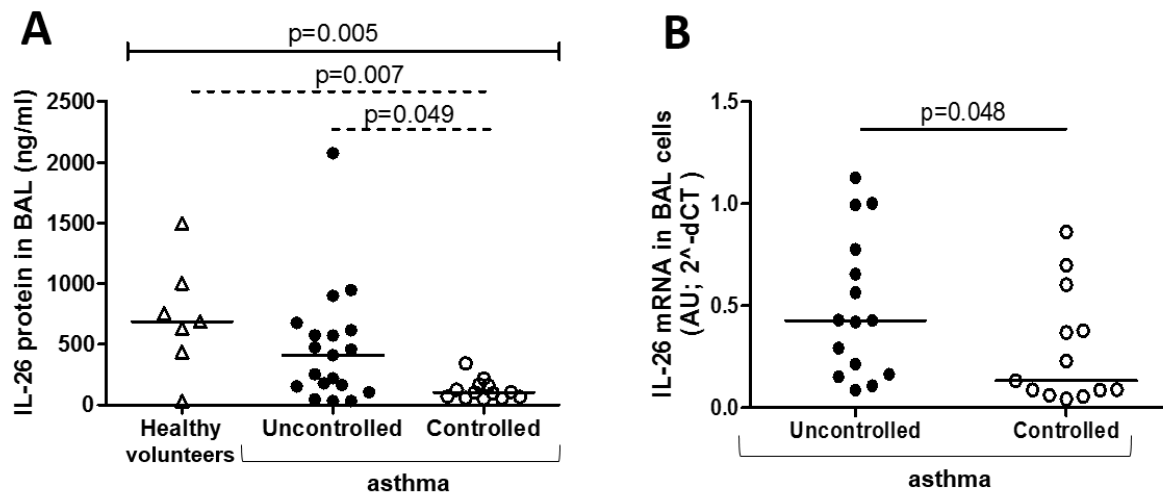
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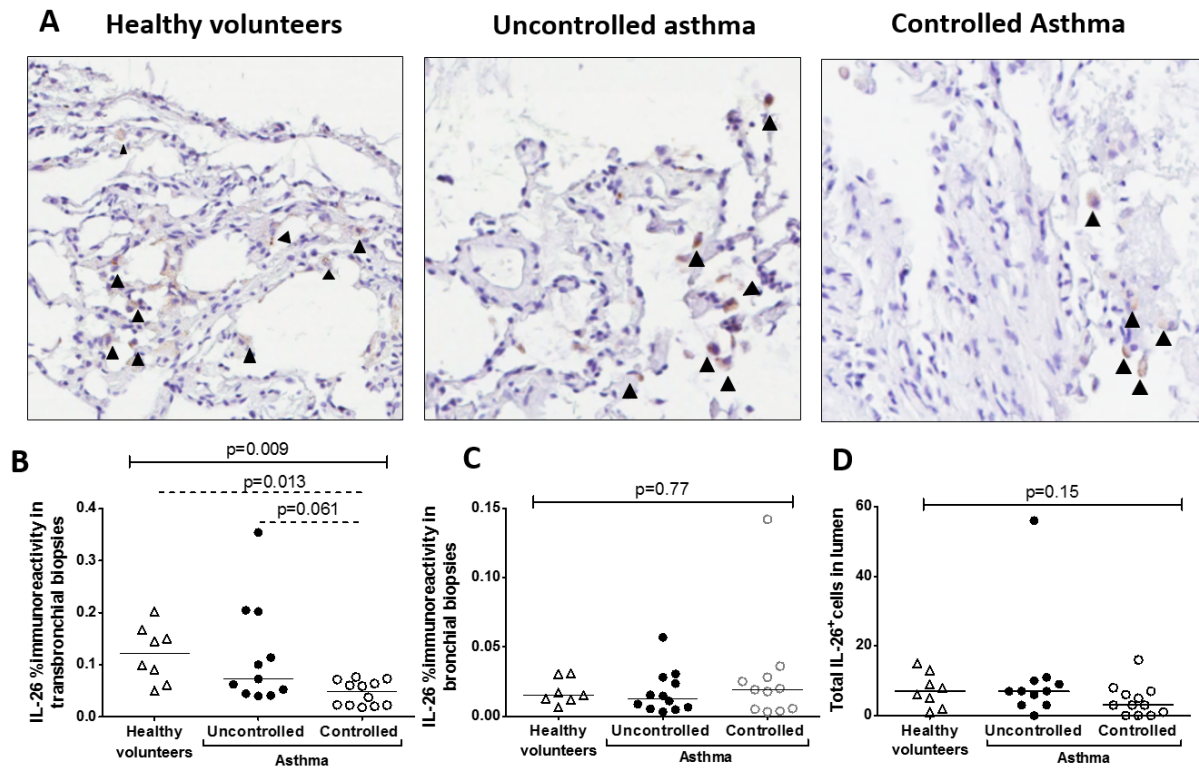
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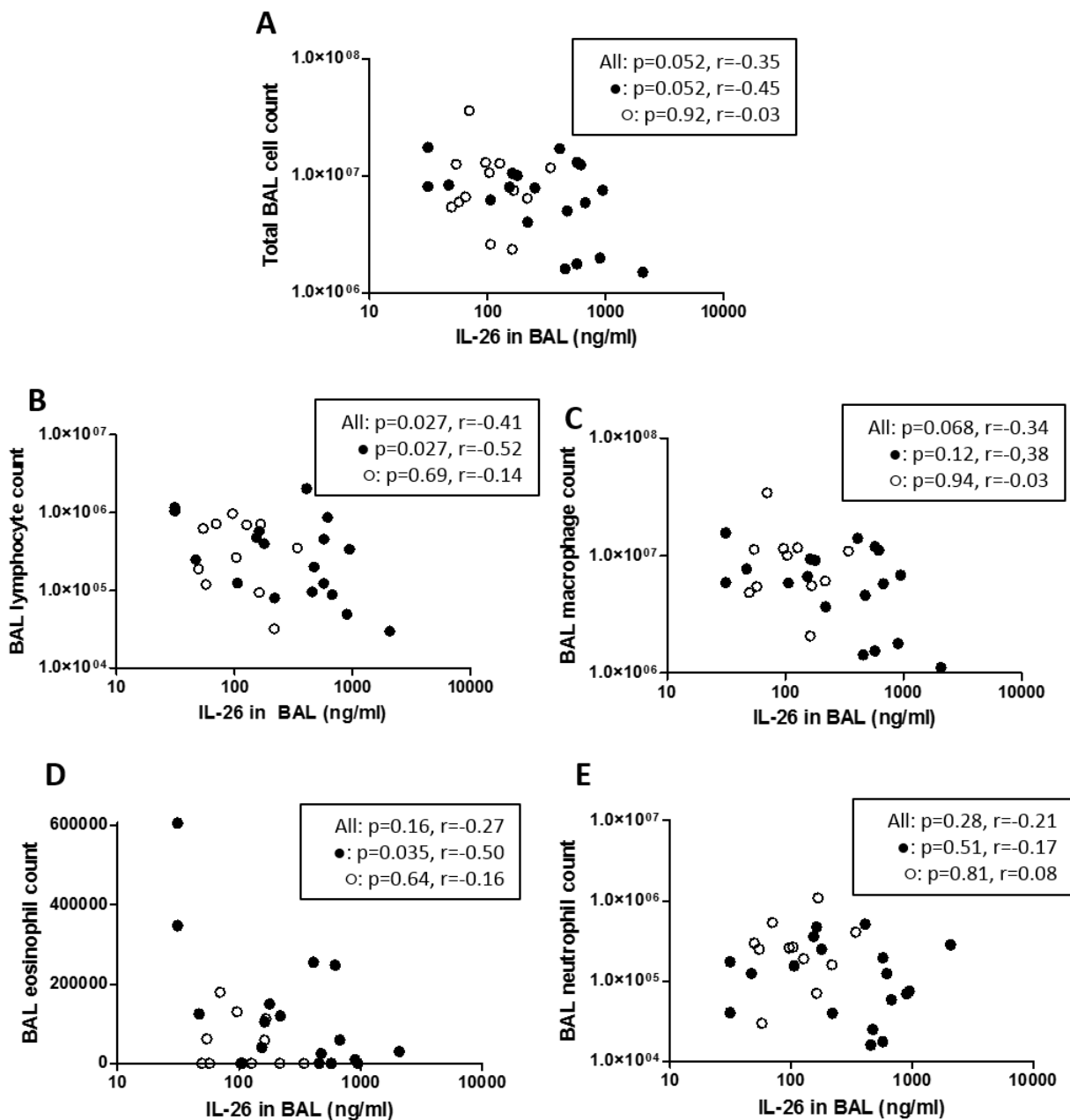
FIGURE LEGENDS

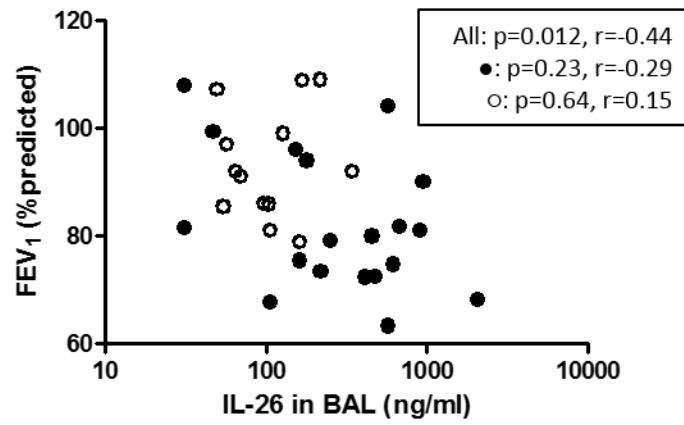
Figure 1. Extracellular IL-26 protein in BAL fluid (A) and IL-26 mRNA in BAL cells (B) from patients with uncontrolled and controlled asthma compared to healthy volunteers. Data is presented as individual values and median. Solid line=comparison between several groups, dotted lines=pairwise comparisons.

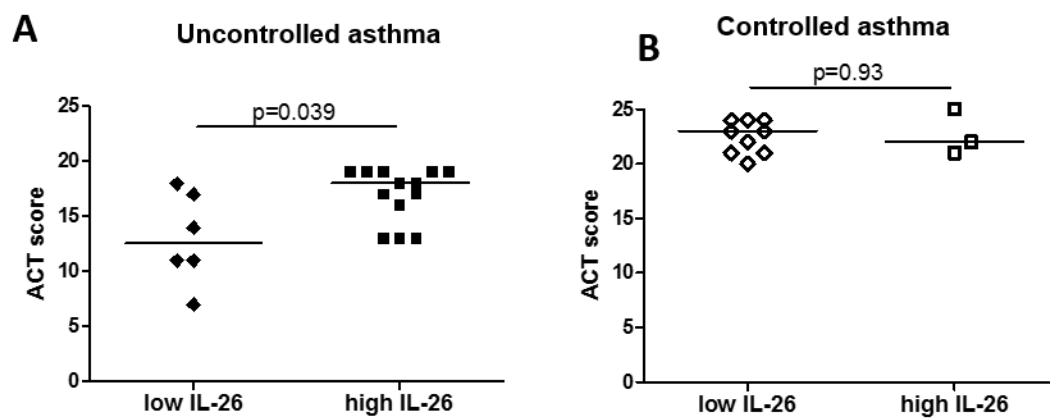
Figure 2. Immunoreactivity for IL-26 protein in lung tissue from patients with uncontrolled and controlled asthma compared to healthy volunteers. Photographs (A) show immunoreactivity in alveolar parenchyma. Arrows depict IL-26 positive cells. Graphs show % area of immunoreactivity in alveolar parenchyma (B) and bronchial tissue (C), as well as number of IL-26-positive cells in the alveolar lumen (D). Solid line=comparison between several groups, dotted lines=pairwise comparisons.

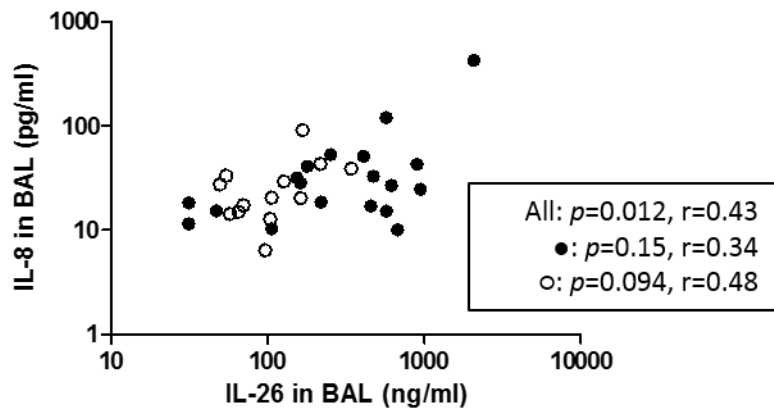












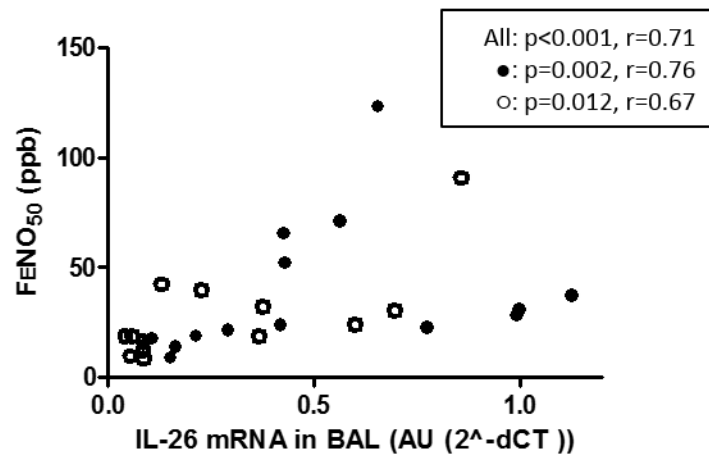


Table S1. Clinical characteristics

	Healthy volunteers [†]	All asthma	Uncontrolled asthma	Controlled asthma	<i>p</i>
Number (n)	8	32	19	13	
Female/Male, n	5/3	14/18	9/10	5/8	0.73
Age (years)	23 (22-28)	34 (25-49)	42 (25-50)	29 (25-35)	0.11
Sensitization, n (%)	0	26 (81) [‡]	16 (84) [†]	10 (77) [†]	0.73
Former smokers, n (%)	0	6 (19)	4 (21)	2 (15)	0.69
ACT, score	n.a.	20 (16-22)	17 (13-19)	23 (21-24)	n.a.
FEV ₁ (L)	3.7 (3.3-5.1)	3.5 (3.8-4.2)	3.2 (2.4-4.0)	4.1 (3.3-4.8)	0.009
FEV ₁ (% pred)	98 (91-113)	86 (76-97)	80 (73-94)	92 (86-103)	0.013
PD20 (µg)	>2,000	398 (127-1,143)*	265 (84-510) [#]	1,100 (296-2,000) [§]	0.035
Positive methacholine (n)	0	29*	18 [#]	11 [§]	n.a.
FeNO, ppb	15 (9-17)	24 (16-39)	28 (18-59)	19 (14-36)	0.34
Bronchial NO flux (nL/s)	0.7 (0.5-1.0)	1.2 (0.7-1.6)	1.3 (0.7-2.5)	1.0 (0.7-1.6)	0.54
Alveolar NO (ppb)	2.1 (2.0-2.4)	2.7 (1.7-3.4)	2.7 (1.8-3.5)	2.7 (1.7-3.4)	0.98
ICS, n (%)	0	29 (91)	19 (100)	10 (77)	0.55
ICS (µg daily dose)	0	800 (400-800)	800 (400-800)	400 (200-800)	0.32
LABA, n (%)	0	16 (50)	11 (58)	5 (38)	0.49
SABA, n (%)	0	22 (69)	14 (74)	8 (62)	0.72
LTRA, n (%)	0	8 (25)	3 (16)	5 (38)	0.22

Data is presented as median (IQR) or n (%). ACT=Asthma control test; Sensitization is defined as a positive skin prick test to one or more airborne allergens (birch, timothy grass, mugwort, cat, dog, horse, moulds and/or house dust mite); [‡], [†] and [†]=11, 8 and 3 patients, respectively, were sensitized to house-dust mite); FEV₁=forced expiratory volume in 1 second; PD20=provocative dose of methacholine giving rise to a fall in FEV₁ of >20%; ICS=inhaled corticosteroids (equivalent dose to budesonide); LABA=long-acting beta₂-adrenoceptor agonist daily; SABA=short-acting beta₂-adrenoceptor agonist when needed; LTRA=leukotriene receptor antagonist daily; *, [#] and [§]=3, 1 and 2 patients, respectively, did not perform the methacholine provocation test for reasons of clinical safety (FEV₁ <65 % predicted); *p*=significance value for comparison between uncontrolled and controlled asthma; n.a.=not applicable; [†]=for technical reasons, BAL could be obtained from 7 of the healthy volunteers only.

Table S2. BAL cell number per total BAL sample.

	Uncontrolled asthma n=18	Controlled asthma n=11	<i>p</i>
Total BAL cell count ($\times 10^{-6}$)	7.8 (3.5-11.0)	10.6 (6.0-12.7)	0.47
BAL lymphocyte count ($\times 10^{-6}$)	0.29 (0.09-0.65)	0.35 (0.12-0.71)	0.74
BAL lymphocyte proportion (%)	5 (3-6)	4 (2-6)	0.47
BAL macrophage count ($\times 10^{-6}$)	6.3 (3.2-9.8)	10.0 (5.5-11.5)	0.32
BAL macrophage proportion (%)	90 (87-92)	92 (89-95)	0.34
BAL eosinophil count ($\times 10^{-6}$)	0.05 (0.00-0.18)	0.00 (0.00-0.11)	0.24
BAL eosinophil proportion (%)	1 (0-2)	0 (0-1)	0.16
BAL neutrophil count ($\times 10^{-6}$)	0.12 (0.04-0.26)	0.26 (0.16-0.41)	0.055
BAL neutrophil proportion (%)	2 (1-3)	3 (2-4)	0.16

Data are presented as median (interquartile range) with *p*-values from the Mann-Whitney non-parametric test. Data is presented as number of cells in total BAL or as relative cell counts as % of all BAL cells. For technical reasons, BAL cell numbers could be obtained from a limited number of patients with uncontrolled (n=18) and controlled (n=11) asthma only..

Table S3. Correlations according to the Spearman rank correlation test for IL-26 mRNA in BAL cells and IL-26 protein concentration or cell counts in BAL samples.

	IL-26 mRNA in BAL cells		
	All asthma (n=24-27)	Uncontrolled asthma (n=13-14)	Controlled asthma (n=11-13)
BAL IL-26 protein	<i>p</i> =0.38, <i>r</i> =0.18	<i>p</i> =0.82, <i>r</i> =0.068	<i>p</i> =0.68, <i>r</i> =-0.13
Total BAL cell count	<i>p</i><0.001, <i>r</i>=-0.68	<i>p</i><0.001, <i>r</i>=-0.82	<i>p</i> =0.071, <i>r</i> =-0.52
BAL lymphocyte count	<i>p</i>=0.025, <i>r</i>=-0.46	<i>p</i>=0.003, <i>r</i>=-0.75	<i>p</i> =0.81, <i>r</i> =-0.82
BAL macrophage count	<i>p</i><0.001, <i>r</i>=-0.73	<i>p</i><0.001, <i>r</i>=-0.87	<i>p</i> =0.10, <i>r</i> =-0.52
BAL eosinophil count	<i>p</i> =0.50, <i>r</i> =-0.14	<i>p</i>=0.012, <i>r</i>=-0.67	<i>p</i> =0.37, <i>r</i> =0.30
BAL neutrophil count	<i>p</i> =0.06, <i>r</i> =-0.39	<i>p</i>=0.014, <i>r</i>=-0.67	<i>p</i> =0.75, <i>r</i> =0.11

ONLINE SUPPLEMENT

Ethics

The study protocol was approved by the Regional Board for Ethical Review in Lund (# LU-412/03) and all participating subjects gave oral and written informed consent, in accordance with the recommendations of the code of ethics of the World Medical Association (Declaration of Helsinki).

Study population

The inclusion criteria for the patients with asthma were mild to moderate disease according to GINA guidelines [1], a report of weekly respiratory symptoms and a positive metacholine test to confirm bronchial hyperresponsiveness. All study subjects underwent skin prick test and completed the Asthma Control Test (ACT). All subjects were required to be non-smokers at the time of investigations, with a smoking cessation date $\geq$ one year prior to the inclusion in the current study. A historic tobacco load up to 10 pack-years was accepted for inclusion. For the control group, we included healthy volunteers, for which any respiratory symptom, sensitization to airborne allergens or confirmed bronchial hyperresponsiveness constituted exclusion criteria. We required that all subjects had to be free of clinical signs of respiratory tract infection during at least three weeks prior to investigation, to avoid confounding influence by microbes on the cytokine signalling or other aspects of the pathophysiology of asthma. All investigations were performed out of season for inhaled pollens. Bronchial responsiveness was assessed through a methacholine provocation test (i.e. $PD_{20}\text{-FEV}_1 \leq 2,000$ μg) performed as previously described [2].

Certain data sets from the study population that we utilized have been published elsewhere, in studies addressing specific aims other than those addressed in the current study [3-7].

Exhaled nitric oxide

A NIOX Flex instrument (Aerocrine AB, Stockholm, Sweden) was used to measure exhaled nitric oxide (NO) at flow rates of 50 (providing $F_{E}NO_{50}$), 100, 200 and 300 mL/s, 3-4 times at each flow. Estimations of alveolar NO concentration and bronchial NO flux were made in a two-compartment linear model [8,9] where flow rates of 100-300 mL/s were utilized.

Bronchoscopy

Bronchoscopy was performed with local anaesthetics in accordance with clinical standard routine, as previously described in detail [6]. A flexible bronchoscope (Olympus IT60, Tokyo, Japan) with a working channel of 2.6 mm, was utilized together with a biopsy forceps (Olympus FB211D).

Bronchial and transbronchial biopsies

The detailed clinical procedures for tissue sampling have been described elsewhere [6]. Briefly, central biopsies of the bronchial mucosa were taken in segmental and sub-segmental carina of the right upper lobe. Transbronchial biopsies of the alveolar lung parenchyma were performed by means of biopsy forceps guided by fluoroscopy in the right lower lobe. Tissues for the transbronchial biopsies were taken from the periphery at a minimum of 2 cm from the chest wall, to minimize the risk for pneumothorax.

Bronchoalveolar lavage

After biopsy sampling, bronchoalveolar lavage (BAL) was performed (3 x 50 mL) using body-tempered and sterile phosphate buffered saline (PBS) in the right middle lobe. The median (IQR) volume of the recovered BAL fluid was 86 (74-91) mL. The harvested BAL sample was filtered (100 μ m pore size) and centrifuged (200 x g during 5 minutes at +4° C) to get separate cells and debris from the fluid component. The sample supernatant was the harvested and the obtained cell-free BAL fluid was frozen (-80° C) until further analysis. The

yielded pellet of BAL cells was thereafter re-suspended in PBS and a portion was utilized for Cytopsin slides for differential counting after staining with May-Grunewald/Giemsa, as previously described [6]. The quantity of BAL leukocytes was assessed using total and cell differential counts for the total BAL volume obtained. An additional fraction of the BAL cells were lysed for RNA preparation of total unsorted BAL cell mRNA (limited by BAL cell availability to n=15 for uncontrolled asthma).

Quantification of extracellular IL-26 protein in BAL samples

We analyzed cell-free BAL fluid samples for the quantifications of extracellular concentrations of IL-26 protein, utilizing a commercial ELISA (Cusabio Biotech®) as previously described [10].

Quantification of mRNA levels in unsorted BAL cells

The preparation of RNA was performed followed by cDNA synthesis and semi-quantitative real-time PCR, using Fast SYBR Green® Master Mix (Applied Biosystems™). Beta-actin was used as an endogenous control, and results were expressed as $\Delta\Delta C_t$ (relative values). Specific information on primer sequences has been published elsewhere [10].

Tissue processing

The tissue biopsies were handled as previously described [4]. Briefly, these biopsies were placed in a buffered formaldehyde (4%) solution, dehydrated, and embedded in paraffin for storage. Sections (3 μ m) were then made using a microtome and stained with Mayer's haematoxylin. Thereafter the tissues were examined histologically at low power magnification to select biopsies that had a well preserved overall morphology. The sections were coded for blinded handling and, based on the morphological criteria only, two bronchial and two transbronchial biopsies were chosen from each subject.

After antigen retrieval, immunohistochemistry for IL-26 protein in tissue was performed with the Dako REAL™ EnVision™ Detection System using a mouse monoclonal anti-human IL-26 (MAB 1375, Clone #197505, R&D Systems, Abingdon, UK). Negative controls were established by omitting the IL-26 antibody, as well as by adding an isotype control antibody.

Quantification of protein expression for IL-26 in tissue

The stained tissue sections were scanned using an automated slide scanner with a 20x microscope lens (ScanScope CS™, Aperio, Vista, CA) and high-resolution and digital images were produced. The images were analysed via the image analysing program VIS (Visiopharm Integrator System, Hoersholm, Denmark) and settings were made to detect DAB staining for IL-26 protein as well as the total tissue area (excluding air) in μm^2 . Large vessels, glands and blood were excluded from the analysis. The expression of DAB per tissue area (μm^2) was calculated in two bronchial and two transbronchial biopsies and a respective average for each patient was calculated. This DAB per tissue area is referred to as immunoreactivity for IL-26 protein below.

Statistical analysis

The IBM SPSS statistics 25 software was used for non-parametric data analyses. Unless otherwise stated, the results are presented with median (interquartile range) values. The Kruskal-Wallis test was utilized for comparison between several groups, with subsequent adjustment with Bonferroni correction for multiple test in specific pairwise comparisons. The Mann-Whitney rank sum test was utilized for comparison between two separate groups and Spearman's correlation test was used for correlation analyses. A p -value < 0.05 was regarded as a statistically significant result. n equals the number of independent observations in all data sets.

RESULTS

Endotype of study population

The median BAL neutrophil count displayed a trend towards being somewhat higher in controlled than in uncontrolled asthma, whereas the other cell populations were similar between the groups (**Table S2**). Thus, with reference to BAL leukocyte counts, there was no group-related difference that proved statistically significant.

Extracellular IL-26 protein in BAL samples

Group differences of extracellular IL-26 protein in BAL samples. The exclusion of the six former smokers that had been recruited did not markedly change the group-related differences in median concentrations of extracellular IL-26 protein in BAL samples and the principal statistical outcomes remained unaltered (data not shown).

Associations with leukocytes in BAL samples. For the pooled group of all patients with asthma, the median concentration of extracellular IL-26 protein in BAL fluid did not differ markedly for patients with low ($\leq$ median in the pooled group of all patients) or high ($>$ median in the pooled group of all patients) count for eosinophils or neutrophils, respectively (data not shown).

Associations with sensitization. All but six included patients with asthma were sensitized against inhaled allergens (**Table S1**), making it impossible to address the association between atopy concentrations of extracellular IL-26 protein in BAL samples in a statistically conclusive manner ($p=0.87$ for patients with asthma with compared with without sensitization; $n=6-26$).

Association with clinical data. We did not detect any correlation between the concentrations of extracellular IL-26 protein in BAL fluid and bronchial reactivity ($=PD_{20}$; $p=0.99$; $r=-0.003$; $n=29$) in the pooled group of all patients with asthma. The same was true for $F_{ENO_{50}}$

($p=0.70$, $r=-0.071$, $n=32$), bronchial NO flux ($p=0.86$, $r=-0.032$), alveolar NO concentration ($p=0.17$, $r=-0.25$) and the daily dose of ICS ($p=0.99$, $r=0.001$), respectively.

IL-26 protein in tissue

When the immunoreactivity for IL-26 was expressed as absolute cell counts per area, the outcome mimicked the principal finding of decreased immunoreactivity (% per area) for IL-26 protein in peripheral parenchymal tissue from the pooled group of all patients with asthma compared with the group of healthy volunteers. Thus, this optional way of analyzing immunoreactivity for the pooled group of patients with asthma (18 (12-23) cells/mm²) compared with the group of healthy volunteers (25 (23-30) cells/mm²) proved statistically significant as well ($p=0.023$; $n=11$ for uncontrolled and 12 for controlled asthma).

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LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1. Correlations between extracellular IL-26 protein and leukocyte counts in BAL samples from patients with asthma. (A) Total leukocytes, (B) lymphocytes, (C) macrophages, (D) eosinophils and (E) neutrophils are presented. Data is presented as individual dots (●=uncontrolled and ○=controlled asthma).

Figure S2. Correlation between extracellular IL-26 protein in BAL samples and FEV₁ (% of predicted) in patients with asthma. The data sets are presented as individual dots (●=uncontrolled and ○=controlled asthma).

Figure S3. Asthma control test (ACT) score for patients with high or low extracellular IL-26 protein in BAL samples among patients with uncontrolled (A) and controlled (B) asthma. The data sets represent patients with low ($\leq$ the median of all patients [165 ng/ ml]) compared to high ($>$ median of all patients) IL-26 protein concentrations in BAL. Here, n=12 for uncontrolled asthma since ACT score is missing in one patient.

Figure S4. Correlation between extracellular IL-26 and IL-8 protein in BAL samples from patients with asthma. The data is presented as individual dots (●=uncontrolled and ○=controlled asthma).

Figure S5. Correlation between messenger RNA for IL-26 in BAL cells and FENO₅₀ for patients with asthma. The data is presented as individual dots (●=uncontrolled and ○=controlled asthma).