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Research article

Co-inhibitory receptors in female asthmatic patients: Correlation with IL-17 and IL-26

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Abstract: *Background:* Asthma is an immunological disorder in which T helper 2 (Th2)-type cells and inflammatory cytokines have a prominent role in its pathogenesis. B- and T-lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1) are co-inhibitory receptors that regulate T cell activation. *Objective:* In the present study of asthmatic patients we measured the soluble isoforms of BTLA (sBTLA), CTLA-4 (sCTLA-4) and PD-1 (sPD-1) in induced sputum fluid with the aim to evaluate their utility as responsible for exacerbation. *Methods:* Eighty patients with asthma and 30 healthy controls (HC) were included in the study. Sputum fluid concentrations of sBTLA, sCTLA-4 and sPD-1 were measured with ELISA. Comparisons were made with Mann-Whitney U test and correlations with IL-17, IL-26 levels and FEV1 (%) were assessed with Spearman's Rank correlation test. *Results:* sBTLA levels were significantly higher in the severe and moderate asthmatic patients compared to healthy controls. Significant differences were observed between severe and moderate asthmatics ($p < 0.0001$). No significant differences were found between mild asthmatics and healthy controls ($p = 0.799$). Soluble PD-1 levels were higher in severe and moderate asthmatic patients compared to HC and no significant difference was observed between these two asthmatic groups ($p = 0.124$). Mild asthmatics and control subjects expressed similar sPD-1 levels ($p = 0.856$). Soluble CTLA-4 was exclusively

expressed in certain severe asthmatic patients. IL-17 inflammatory cytokine was significantly correlated with BTLA and sPD-1. IL-17 and IL-26 cytokines were highly expressed in sputum asthmatic groups compared to sputum from HC. Severe asthmatic group was characterized by the highest levels of both IL-17 and IL-26 mediators. Soluble BTLA correlates positively with IL-17 ($r = 0.817$; $p < 0.0001$) and IL-26 ($r = 0.805$; $p < 0.0001$) inflammatory cytokines. IL-17 and IL-26 levels were associated with the asthma clinical severity from severe to mild asthma ($p < 0.0001$). The inflammatory cytokines IL-17 and IL-26 were positively correlated with the percentages of macrophages, PNN and FEV1 (%). *Conclusion:* Here, we provide the first report on the increased expression of sBTLA and sPD-1 in induced sputum of severe asthmatics. IL-26 and IL-17 appeared as a novel proinflammatory axis. Both sBTLA and sPD-1 might be involved in the pathogenesis of asthma and were associated with a poor prognosis.

Keywords: severe asthma; induced sputum; sBTLA; sCTLA4; sPD-1; IL-17; IL-26

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1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized mainly by Th2 lymphocyte-mediated immune responses and associated with bronchial hyper-responsiveness, airflow obstruction, and airway remodelling. Th2-biased inflammation is associated with leukocytes recruitment and type-2 cytokines production. However other inflammatory pathways have been identified in asthmatic patients, depending on the clinical phenotype. Previous studies demonstrated that hyperactive Th17 and Th2 immune responses and their associated cytokines were involved in the development of asthma [1–3].

Antigen-specific activation of naive T lymphocytes requires double signals; T cell receptor (TCR) recognition of antigen bound to MHC on antigen presenting cells (APCs), and a second signal delivered through the interaction between the co-receptor CD28 and its cognate ligands CD80 and CD86 on the same APC [4]. The magnitude of the immune response is regulated by an intricate network of co-signalling cell-surface bound receptors and their corresponding ligands. Programmed Death 1 (PD-1), Cytotoxic T Lymphocyte-associated Antigen 4 (CTLA-4) and B- and T-lymphocyte Attenuator (BTLA) are three well characterized co-inhibitory receptors, through which cell activation and proliferation can be abated. CTLA-4 competes with CD28 for interaction with the CD80 and CD86 ligands on APCs, and by interfering with co-stimulation CTLA-4 can have an attenuating effect on T cell activation [5]. PD-1 is expressed on T-cells upon activation, and has two ligands; PD-L2, which is limited to APCs, and PD-L1, which is expressed on various immune cells [6]. BTLA is broadly expressed: on B and T lymphocytes, macrophages, dendritic cells and NK cells and can be either up- or down-regulated after stimulation depending on the cell type [7]. Its ligand Herpes Virus Entry Mediator (HVEM) is also expressed on both sides of the APC-lymphocyte entity. BTLA-HVEM as well as PD-1-PD-L1/PD-L2 interactions may convey an inhibiting signal [6]. Negative co-stimulatory pathways are important both in the defence against infection and in maintaining peripheral tolerance and immune homeostasis, which is illustrated by the fact that

mice deficient in PD-1, BTLA or CTLA-4 develop autoimmune and lymphoproliferative disease [8]. While increased PD-1 expression on T cells is an established marker of immune paralysis in inflammatory diseases; the roles of CTLA-4 and BTLA remain to be determined [9].

To examine the participation of these soluble isoforms in immune regulation, we aimed to measure induced sputum concentrations of the soluble isoforms of PD-1, CTLA-4 and BTLA in patients with asthma and evaluate their usefulness as biomarkers and indicators of severity of disease. We also measured IL-17 and IL-26 as inflammatory mediators and their correlations with these soluble isoforms.

2. Materials and methods

2.1. Patients

Eighty patients with well-defined women asthmatic patients (20 with moderate asthma, 20 with mild asthma and 40 patients with severe asthma) were recruited from the Department of Respiratory Disease, A. Mami Hospital (Ariana, Tunisia), using the criteria set by the Global Initiative for Asthma guidelines [10]. Asthma was diagnosed with exacerbations defined as episodes of a progressive increase in shortness of breath, cough, wheezing, or chest tightness, or a combination of these symptoms, requiring a change in treatment. Detailed definitions of the inclusion and exclusion criteria for the enrolment of asthmatic subjects were reported previously [3,11].

The protocols for the study were reviewed and approved by the ethics committees of our hospital, and informed consent was obtained from all participating subjects. Patients with asthma were treated with regular inhaled glucocorticoids (ICS), but variable daily doses were required to control the symptoms (at the time of evaluation daily ICS dose ranged 200–800 µg/day). Only patients whose asthma was controlled were retained. Table 1 describes the characteristics of the asthmatic patients in the study. Thirty healthy controls (all females) were recruited (aged 45–58 years) with no respiratory nor allergic manifestations. Spirometry was carried out in the patient and the control group. FEV1% was assessed by using a spirolab II.

2.2. Sputum induction and processing

The sputum was induced and processed as previously described [3,11–13]. Cell viability was determined by trypan blue exclusion and cytospins. Cells were stained with May-Grünwald-Giemsa to assess the differential cell count by counting 500 non-squamous cells under a light microscope.

2.3. Enzyme-linked immunosorbent assay

sPD-1 was analyzed with RayBio® Human SP-D ELISA Kit (RayBiotech (Norcross, GA, USA)); All samples were diluted 1:1 in phosphate-buffered saline (PBS) (pH 7.4) and 1% bovine serum antigen (BSA) supplemented with 25 µg/ml heat-inactivated normal goat IgG (DAKO A/S, Rodovre, Denmark) to ensure pre-aggregation of heterophilic antibodies in the samples examined. The minimum detection limit (cut-off) was 0.040 ng/ml, calculated as two standard deviations of the

blanks. Soluble BTLA was analyzed at 1:5 dilution with Human BTLA ELISA Kit, (range detection 0.47–30 ng/ml) (Cusabio Biotech (Wuhan, China)). Sputum sCTLA-4 was analyzed at 1:2 dilution with Human sCTLA-4 Platinum ELISA, (detection range 0.16–10 ng/ml) (CTLA-4 (Soluble) Human ELISA Kit; Bender Medsystems, Milano, Italy).

IL-17 concentrations in sputum supernatants were quantified using an Enzyme-Linked Immunosorbent Assay kit (Abcam, Cambridge, United Kingdom). Concentrations below the standard range of the assay (1.6 pg/ml) were set as zero. IL-26 was quantified in induced sputum using commercially available enzyme-linked immunosorbent assay kits (LS-F4914, LifeSpan) tested for non-specific binding as previously described [14]. Sputum fluid samples were blocked for heterophilic antibodies with bovine, murine, and rabbit immunoglobulin G (Jackson Immuno Research). ELISA-Amplification System (ELAST) (NEP116001EA, PerkinElmer) was applied before adding 3,3',5,5'-tetramethylbenzidine (TMB); otherwise, the assays were performed according to the manufacturers protocol. Samples were analyzed in duplicates, and values below the detection limit were assigned the same value as the detection limit, which in this case was 15.63 pg/ml. Optical density (OD) was measured at 450 nm with a reference value of 570 nm (Thermo Scientific, Multiskan GO). Optical densities were converted to concentrations using a four-parametric logistic regression. All ELISA analyses were performed according to the manufacturers' instructions.

2.4. Statistical analysis

Data are shown as dot plots. The one way ANOVA, independent sample *t*-test, Bonferroni correction, Mann-Whitney test, Wilcoxon test and Kruskal-Wallis H test were applied using the SPSS17.0 software (SPSS Inc., Chicago, Illinois, USA). A p-value less than 0.05 were considered statistically significant.

3. Results

3.1. Demographic and functional patient characteristics

The demographic and functional characteristics of the asthmatic patients and healthy subjects are summarized in Table 1. Asthmatic patients were no smoker. Overall there was no difference in BMI between asthmatics and healthy controls.

3.2. Sputum cell counts

Results are given in Table 1. The percentage of lymphocytes, eosinophils and neutrophils were higher in asthmatic groups ($p = 0.001$) compared to healthy subjects. The percentage of sputum macrophages tended to be higher in healthy controls compared to all asthmatic groups ($p < 0.005$). The percentages of macrophages were not significantly different between the 3 asthmatic groups. Severe asthmatic patients expressed low lymphocytes percentage compared to mild and moderate asthmatics ($p = 0.002$). Eosinophils were increased in severe asthma compared to mild and moderate

asthma ($p = 0.0001$). Low significant differences was observed between mid and moderate asthmatics in the eosinophil percentages ($p = 0.0042$).

Table 1. Demographic, functional and inflammatory characteristics of the validation cohort population.

Screening cohort	Healthy subjects	Mild asthma	Moderate asthma	Severe asthma
Number	30	20	20	40
Demographic characteristics				
Age (years), median (Range)	54.7 (27–74)	58 (51–66)	55.7 (55–60)	55.87 (26–75)
Functional characteristics				
FEV1 (% pred)	109 ± 15.3	97.6 ± 12.8[†]	92.56 ± 11.90[†]	56.72 ± 14.72[†]
FEV1/FVC ratio	80.7 ± 6.7	74.7 ± 6.3[†]	65.4 ± 7.8[†]	61.1 ± 69.8[†]
Sputum characteristics				
Lymphocytes	8.2 ± 4.95	20.47 ± 10.5[†]	20.5 ± 4.9[†]	13.5 ± 7.6[†]
Macrophages (%)	58.3 ± 16.9	41.47 ± 19.87[†]	39.7 ± 5.7[†]	44.2 ± 6.7[†]
Neutrophils (%)	21.8 ± 18.9	31.86 ± 14.80[†]	38.2 ± 10.7[†]	32.2 ± 12.4[†]
Eosinophils (%)	0.0 ± 0.00	3.96 ± 2.3[†]	2.75 ± 1.4[†]	9.2 ± 2.4[†]

Data are expressed as distributions (yes/no), means ± SD, or medians with range. Comparisons were done with Pearson X_2 tests or the Kruskal-Wallis test, followed by the Mann-Whitney U test with the Bonferroni correction. [†]: $p < 0.005$ versus healthy subjects. FEV1: forced expiratory volume in 1s; FVC: forced vital capacity. FEV1 (% pred) and FEV1/FVC ratio were significantly decreased in severe asthmatic patients compared to mild ($p < 0.0001$) and moderate ($p < 0.0005$) asthmatics. The percentage of lymphocytes was decreased in severe asthmatic compared to mild and moderate ($p < 0.0001$) asthma patients.

3.3. Comparison of sBTLA and sPD-1 in asthmatic patients and healthy controls

Soluble BTLA sputum concentrations were highest in the asthmatic patients (42.48 ± 26.74 ng/ml; range: 10–105 ng/ml) compared to HC (16.79 ± 4.048 ng/ml; range: 9.0–24.9 ng/ml). Significant higher sBTLA were present in severe asthmatics (64.42 ± 20.63 ng/ml; range: 30–105 ng/ml) compared to mild (16.43 ± 3.60 ng/ml; range: 10–23) and moderate (24.35 ± 3.29 ng/ml; range: 18–29.3 ng/ml) asthmatic patients. No significant differences were observed between mild asthmatics and HC ($p = 0.799$) (Figure1A).

In severe asthmatic patients, sputum levels of sPD-1 were increased (6.077 ± 1.59 ng/ml; range: 3.5–9.2) compared with mild asthmatics (3.27 ± 0.98 ng/ml; range: 2–6) and healthy controls (3.23 ± 0.75 ng/ml; range: 1.5–5) (Figure 1B). No significant differences were observed between severe and moderate asthmatic patients (5.44 ± 1.2 ng/ml; range: 3.0–7.0; $p = 0.124$). Patients with mild asthma expressed similar levels than HC ($p = 0.856$). Soluble PD-1 was associated to FEV1 (% pred) in asthmatic patients ($r = 0.574$; $p = 0.0007$) (Figure 1C). Soluble CTLA-4 was detectable at low level in only 10 over 30 severe asthmatics (0.7 ± 0.2 ng/ml).

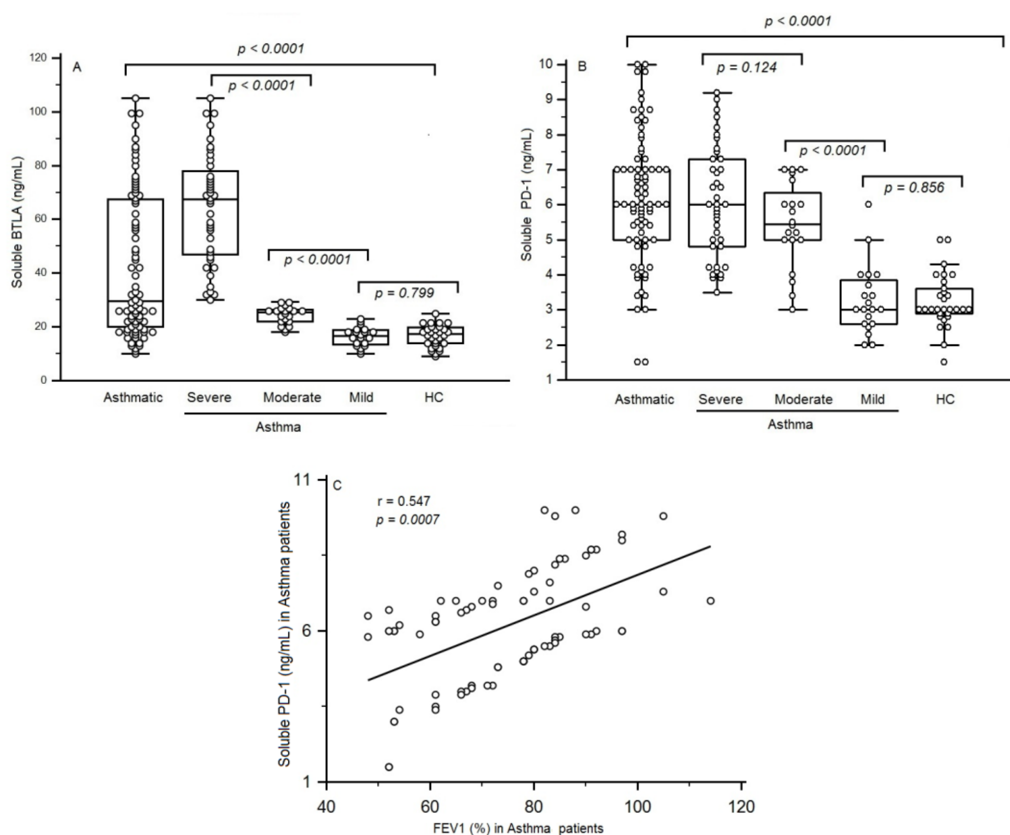

Figure 1. Induced sputum sBTLA and sPD-1 concentrations in asthmatic patients. [A]: sBTLA concentrations in the asthmatic patients. [B]: sPD-1 concentrations in the induced sputum from asthmatic patients. sBTLA and sPD-1 were quantified by ELISA. The lines inside the boxes indicate the median; the outer borders of the boxes indicate 25th and 75th percentiles; the bars extending from the boxes indicate the 10th and 90th percentiles. The mean values were compared and the p values are indicated at the figures. [C]: Association of sputum sPD-1 concentrations with FEV1 (%) in asthmatic patients. Pearson's correlation coefficient was shown in the figure. The "r" value indicates the calculated regression coefficient.

3.4. IL-17 levels in induced sputum

Levels of sputum IL-17 were highest in asthmatic patients: severe (31.50 ± 7.78 pg/ml), moderate (22.77 ± 2.90 pg/ml) and mild (18.72 ± 2.4 pg/ml) compared to healthy controls (3.22 ± 1.92 pg/ml; p < 0.0001) (Figure 2A). Significant differences were found between the 3 asthmatic stages (p < 0.0001). Significant correlation was observed between IL-17 and the sBTLA co-inhibitors (Figure 2B). Positive correlation was found between IL-17 cytokine and sPD-1 in asthmatic patients (r = 0.627; p = 0.0004).

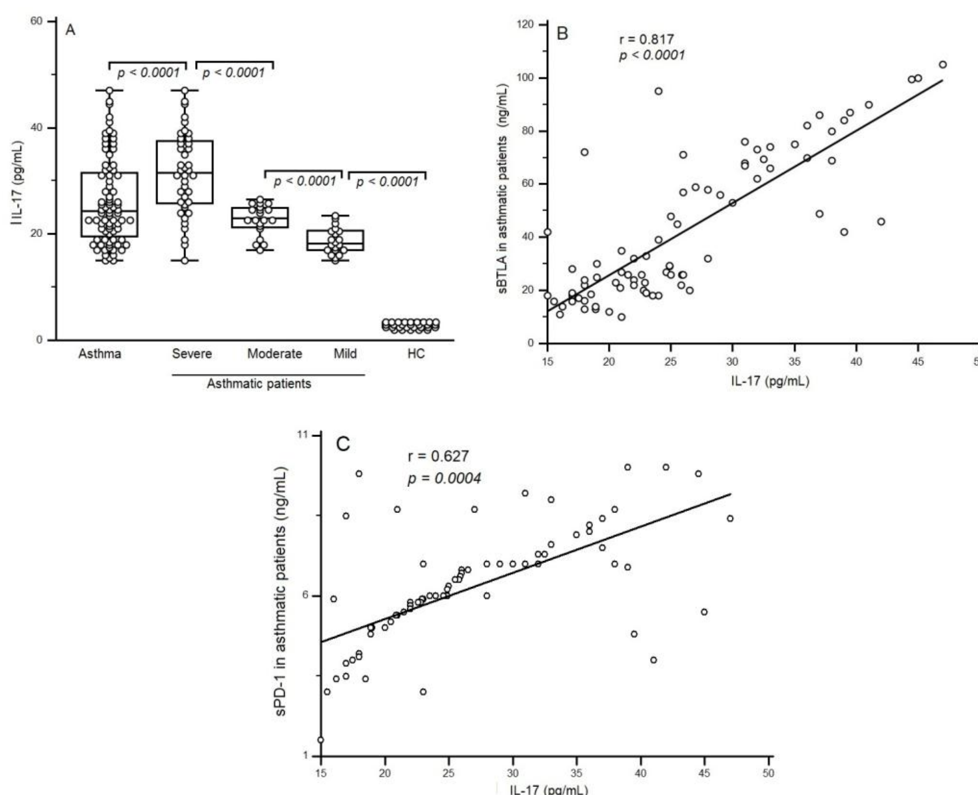

Figure 2. IL-17 expression in induced sputum from asthmatic patients. [A]: IL-17 levels in asthmatic patients according to their clinical severity. The lines inside the boxes indicate the median; the outer borders of the boxes indicate 25th and 75th percentiles; the bars extending from the boxes indicate the 10th and 90th percentiles. The mean values were compared and the p values are indicated at the figures. [B]: Association of sputum IL-17 concentrations with sputum sBTLA in asthmatic patients. [C]: Association of sputum IL-17 concentrations with sputum sPD-1 in asthmatic patients. Pearson's correlation coefficient was shown in the figure. The "r" value indicates the calculated regression coefficient.

3.5. Levels of sputum IL-26

We quantified IL-26 by ELISA in the sputum of asthmatic patients and controls. Values are expressed as mean ± standard deviation [SD] (Figure 3A). IL-26 concentrations were higher in asthmatic patients (3.66 ± 1.77 ng/ml; $p < 0.0001$) than in healthy controls (1.526 ± 0.52 ng/ml) (Figure 3A). In severe asthma, IL-26 levels were overexpressed (4.94 ± 1.6 ng/ml; $p < 0.0001$) as compared to moderate (2.57 ± 0.34 ng/ml) and mild asthma (2.21 ± 0.69 ng/ml). The concentration of IL-26 levels was significantly associated with the asthma clinical severity from severe to mild stages (Figure 3A). Low significant difference was observed between mild and healthy controls ($p = 0.0456$). No correlations were observed between IL-26, and sPD-1 or sCTLA-4. However, significant correlation was observed between sBTLA and IL-26 in asthma ($r = 0.885$; $p < 0.0001$) (Figure 3B).

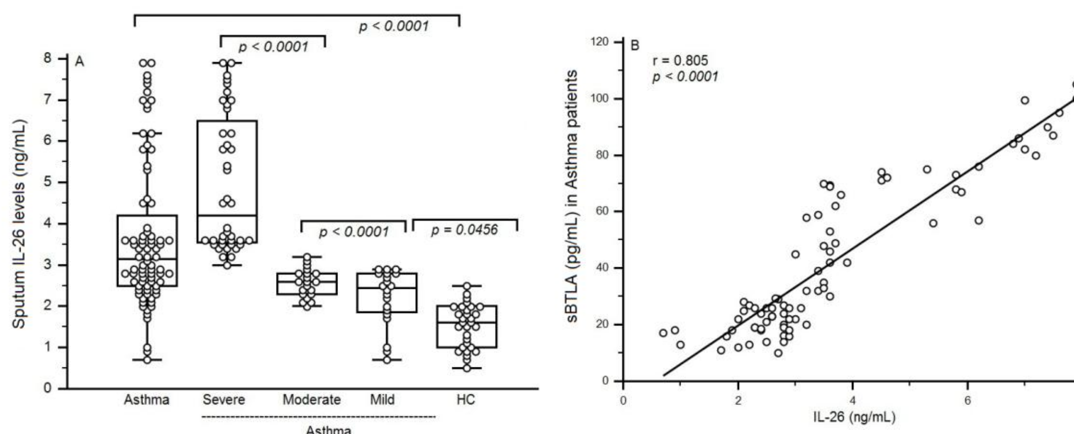

Figure 3. Interleukin 26 in sputum fluid from severe asthmatic patients. [A]: Induced sputum IL-26 levels in asthmatic. [A]: IL-26 concentrations in the asthmatic patients was quantified by ELISA. The lines inside the boxes indicate the median; the outer borders of the boxes indicate 25th and 75th percentiles; the bars extending from the boxes indicate the 10th and 90th percentiles. The mean values were compared and the p values are indicated at the figures. [B]: Correlation of sBTLA and IL-26 level in asthmatic patients. Pearson's correlation coefficient was shown in the figure.

3.6. IL-26 in severe asthmatic patients and its correlation with IL-17, FEV1 (%), percentages of macrophages and neutrophils.

Significant correlations were found between IL-26 and IL-17 levels, between IL-26 and the percentage of macrophages (Table 2), between IL-26 and FEV1% and between IL-26 and PNN%. Figure 4 represents correlations observed in severe asthmatic patients.

Table 2. Correlation between IL26 and IL-17, IL-26 and the percentage of macrophages, IL-26 and forced expiratory volume [FEV (%)] and between IL-26 and PNN cells in severe, moderate and mild asthmatic patients. Correlation was tested by Spearman's rho (r) and the level of significance is indicated. In all tests the level of significance was a two-sided, p-value of less than 0.05.

	IL-26/IL-17	IL-26/% of macrophages	IL-26/FEV1%	IL-26/% PNN
Severe asthma	r = 0.569 (p = 0.0001)	r = 0.656 (p = 0.0001)	r = 0.872 (p = 0.0001)	r = 0.552 (p = 0.0002)
Moderate asthma	r = 0.736 (p = 0.0002)	r = 0.587 (p = 0.0003)	r = 0.734 (p = 0.0001)	r = 0.547 (p = 0.0002)
Mild asthma	r = 0.512 (p = 0.0021)	r = 0.572 (p = 0. 0004)	r = 0. 537 (p = 0.0006)	r = 0.526 (p = 0.0003)

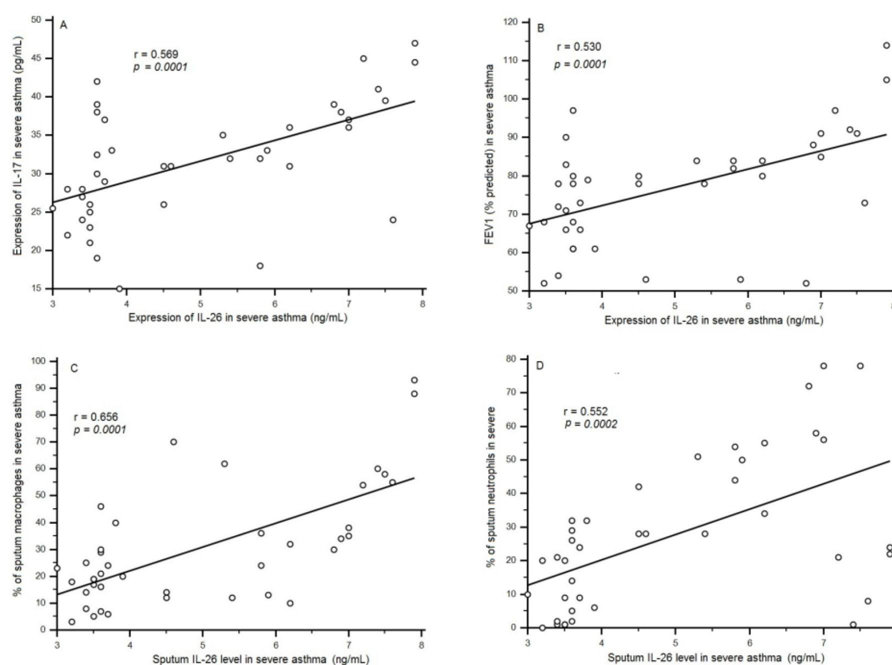

Figure 4. Expression of IL-26 and its correlations with IL-17, FEV1 (%), sputum macrophages and sputum neutrophils in severe asthmatic patients.

4. Discussion

In the present study we found that sputum concentrations of the soluble isoform of the co-inhibitory receptor B- and T-lymphocyte Attenuator (sBTLA) were low in healthy individuals and elevated in asthmatic patients, particularly in severe asthmatics. Evaluation of sBTLA as a dynamic marker confirmed an association to disease severity. In the same way, soluble Programmed Death 1 (sPD-1) concentrations were higher in patients with severe asthma than in healthy subjects. Level of sPD-1 was associated with the asthma clinical severity. Soluble Cytotoxic T Lymphocyte-associated Antigen 4 (sCTLA-4) concentrations were low or undetectable. Previous studies on soluble CTLA-4 show increased plasma concentrations in several autoimmune disorders, but low or undetectable levels in healthy individuals. There might be certain pathogenic mechanisms present in autoimmune disease, but not in asthma. Perhaps due to the limited sensitivity of commercially available ELISA kits, sCTLA-4 is difficult to study. IL-17 and IL-26 were highly expressed in asthma sputum and correlate with sBTLA. IL-17 and IL-26 inflammatory axis was correlated to sputum cells: macrophages and neutrophils.

This is to our knowledge the first study of soluble co-inhibitory molecules and IL-26 in induced sputum from asthmatics. Soluble BTLA was highly expressed in severe asthmatic patients. The existence of sBTLA from healthy individuals has been demonstrated by Wang et al. [15]. The BTLA-HVEM pathway is complicated by the fact that HVEM has multiple partner molecules through which cross-linking results in either a co-stimulatory (LIGHT and LTα) or a co-inhibitory (BTLA and CD160) signal [16]. Therefore BTLA might be a more fine-tuned immune regulator than CTLA-4. The origin of sBTLA remains to be elucidated. Perhaps it is generated through alternative

splicing of mRNA as has been demonstrated for sPD-1 and sCTLA-4 [17–19]. Another potential source of the measured BTLA is proteolytic cleavage of the outer part of the membrane-bound receptor, possibly representing a means of controlling T cell inhibition. In this study, sputum sBTLA concentrations were highest in the severe stage of asthmatics suggesting that sBTLA is a marker for an activated pathway in the immune system, triggered by inflammation. sBTLA concentrations and disease severity in the asthmatic cohort were associated; sBTLA and IL-17 were correlated and IL-17 concentration had higher levels than those with mild or moderate asthmatics. IL-26 was associated with the asthma clinical severity from mild to severe and correlates significantly with the macrophages and neutrophils percentages. The most important data found was the correlation of IL-26 with FEV1 (%). By using Spearman rank test and multiple regression analysis, our research showed that sputum IL-26 in severe asthmatics was closely related to sputum macrophage and neutrophil percentages. Sputum IL-17 and IL-26 should be of clinical relevance in the pathogenesis of asthma.

Our study suggested a key role for IL-17 and IL-26 during an asthma episode by showing a gradual rise in sputum with increasing asthma severity. Furthermore, we found a significant correlation between sputum IL-26 and neutrophils. To our knowledge, the role of IL-17 and IL-26 in neutrophilic asthmatic airway inflammation is supported, mainly from mouse models of asthma and the important pathologic effects of IL-17 in neutrophilic inflammation in other diseases, such as psoriasis [20], while much less from asthma patients [21]. For the first time, we reported a significant association between sputum IL-26 and the severity of asthma, leading to the assumption that IL-17/IL-26 axis is essential in the induction of an exacerbation, of the airway inflammation. It is intriguing that we observed an association for sputum IL-26 and % FEV1. In fact, the role of IL-17/IL-26 in asthma is complex and may have different functions. IL-26 favours the generation of inflammatory cells mainly through the induction of IL-17 production. Beside its inflammatory role, IL-26 also binds to self DNA and promotes the secretion of IFN-α by pDCs, which acts as an "alarmin" to activate the innate immune system and activate a response to tissue or cell damage. These new findings suggest an intriguing mechanism of action for IL-26 in asthma pathology.

The precise functions of the soluble form of PD-1 are not well described, and sPD-1 has been reported to exhibit both functional antagonism [22,23] and agonism [24]. Although sPD-1 is known to be bioactive, and an association with disease has been proposed [18]. The correlation of sPD-1 with FEV1% in asthmatic patients could indicate an ongoing attempt to dampen the immune activity in the severe stage of disease, where protective mechanisms are overruled by the high degree of inflammation. Significant positive correlation between sPD-1 and FEV1% ($r = 0.743$; $p < 0.0001$) was observed in severe asthmatic patients. Induced sputum was depicted to have high levels of inflammatory mediators IL-17, TNF-α, IL-6 [25–28].

Levels of IL-17 were highly expressed in asthma patients [3,27–30]. Recently Ricciardolo et al. [31] investigated the role of Th17 cytokines (IL-17A and IL-17) expression in nasal/bronchial biopsies obtained from atopic/non atopic mild-to-severe asthmatics. They observed a significant increased expression of IL-17F in severe asthma which was correlated to neutrophils, airway obstruction and disease exacerbation in severe asthma. The overexpressed IL-17F was also able to recognize frequent exacerbation phenotype potentially at risk of asthma death [31].

The results of our present study indicate that the sputum concentration of IL-26 was significantly increased in asthma patients compared with controls and the elevation correlated positively with disease severity. IL-17 was significantly correlated to the new IL-26 inflammatory cytokine. IL-26 is constitutively expressed in sputum of asthmatic patients which probably indicate its intrinsic production involving inflammation and matrix inflammation. The increased IL-26 level in sputum indicates its substantial and inducible release in the lung. Griffith et al. [32] indicated that IL-26 acted on different cell types in the lungs and it would be interesting to investigate its role during inflammatory airway conditions. Ohnuma et al. [33] reported that IL-26^{+}CD26^{+}CD4^{+} T cell infiltration appears to play a significant role in the lung of obliterative bronchiolitis [33]. They also demonstrated that human IL-26 induced collagen deposition in obliterative bronchiolitis of murine allogeneic transplantation model and that IL-17 was shown to be involved in the pathogenesis of obliterative bronchiolitis associated with chronic rejection [34]. We confirmed the implication of IL-26 in the processes of exacerbation in the lung of asthmatic disease. IL-26 is involved and plays a critical role in antibacterial host defence of human lungs. Che et al. [35] reported that IL-26 is abundantly produced and released by alveolar macrophages and possibly by local helper and cytotoxic T cells [35]. We reported significant positive correlation between IL-26 and the percentage of macrophages in asthma. IL-26 produced by alveolar macrophages can recruit the neutrophils via induction of chemotaxis factors such IL-8 and leukotaxin n-formyl-methionyl-leucylphenylalanine (fMLP) [36]. Both IL-8 gene and protein expression may play a key role in asthma pathogenesis [37].

5. Conclusion

Taken together, our results suggest that, although soluble co-inhibitory receptors do not appear to be good markers to diagnose asthma, sBTLA or sPD-1 may be of interest as a prognostic indicator, perhaps in combination with classical biomarkers, and that further studies on the immune pathways involving sBTLA may be of value in clarifying the pathogenesis in human asthma. Finally, this is an observational study and no conclusions can be drawn in terms of the underlying immunological mechanisms. The interesting finding should be the correlations observed in severe asthma with IL-17 and IL-26. Future mechanistic studies are required to examine the biological activity of sBTLA, sPD-1 and why high levels are associated with disease severity. It would be interesting to more investigate the role of IL-26 during inflammatory airway conditions such as asthma, where damage to epithelial cells is more prevalent. Our data provides a valuable contribution to our knowledge of the control of pathogens in the lungs, in particular the role that IL-26 plays in antibacterial host defensiveness of asthma lungs.

Conflict of interest

The authors declare no conflicts of interest in this paper.

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