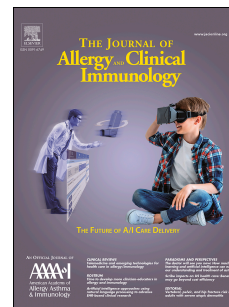


Journal Pre-proof

Clinical and histopathological predictors of therapeutic response to bronchial thermoplasty in severe refractory asthma

Maha Zohra Ladjemi, PhD, Leonarda Di Candia, PhD, Nicolas Heddebaut, MS, Camille Techoueyres, MD, Eloise Airaud, MS, David Soussan, Marie-Christine Dombret, MD, Fatima Hamidi, MS, Noëlline Guillou, MS, Pierre Mordant, MD, PhD, Yves Castier, MD, PhD, Séverine Létuvé, PhD, Camille Taillé, MD, PhD, Michel Aubier, MD, Marina Pretolani, PharmD, PhD



PII: S0091-6749(21)00005-1

DOI: <https://doi.org/10.1016/j.jaci.2020.12.642>

Reference: YMAI 14923

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 21 October 2019

Revised Date: 8 December 2020

Accepted Date: 17 December 2020

Please cite this article as: Ladjemi MZ, Di Candia L, Heddebaut N, Techoueyres C, Airaud E, Soussan D, Dombret M-C, Hamidi F, Guillou N, Mordant P, Castier Y, Létuvé S, Taillé C, Aubier M, Pretolani M, Clinical and histopathological predictors of therapeutic response to bronchial thermoplasty in severe refractory asthma *Journal of Allergy and Clinical Immunology* (2021), doi: <https://doi.org/10.1016/j.jaci.2020.12.642>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.

Clinical and histopathological predictors of therapeutic response to bronchial thermoplasty in severe refractory asthma

Maha Zohra Ladjemi, PhD^{a,b,*†}, Leonarda Di Candia, PhD^{a,b,*‡}, Nicolas Heddebaut, MS^{a,b},
Camille Techoueyres, MD^{a,b}, Eloise Airaud, MS^{a,b}, David Soussan^{a,b},
Marie-Christine Dombret, MD^{a,b,c,d}, Fatima Hamidi, MS^{a,b}, Noëlline Guillou, MS^{a,b,§},
Pierre Mordant, MD, PhD^{a,b,d,e}, Yves Castier, MD, PhD^{a,b,d,e}, Séverine Létuvé, PhD^{a,b},
Camille Taillé, MD, PhD^{a,b,c,d}, Michel Aubier, MD^{a,b}, Marina Pretolani, PharmD, PhD^{a,b}

^a Inserm UMR1152, Physiopathologie et Epidémiologie des Maladies Respiratoires, Université de Paris, Faculté de Médecine, F-75018, Paris, France; ^b Laboratory of Excellence, INFLAMEX, Université Sorbonne Paris Cité and DHU FIRE, Paris, France; ^c Département de Pneumologie A, hôpital Bichat-Claude Bernard, F-75018, Paris, France; ^d Assistance Publique des Hôpitaux de Paris, Paris, France; ^e Département de Chirurgie Thoracique, hôpital Bichat-Claude Bernard, F-75018, Paris, France.

* these authors equally contributed to this work

† present address: Institut Cochin, U1016 Inserm/CNRS UMR8104, Université de Paris, F-75014, Paris, France

‡ present address: Scinopsis, F-83600, Fréjus, France

§ present address: Centre d'Immunologie et des Maladies Infectieuses (CIMI), Inserm UMR1135/CNRS ERL8255, Université Pierre et Marie Curie, F-75013, Paris, France

Corresponding author: Marina Pretolani, PharmD, PhD, Inserm UMR1152, Université de Paris, Faculté de Médecine, 16 rue Henri Huchard, F-75018, Paris, France

e-mail: marina.pretolani@inserm.fr; phone: (+33) 1 57 27 75 86; fax: (+33) 1 57 27 75 51

Word count in the body of the manuscript: 3542

This article has an Online Repository at www.jacionline.org

Sources of support: This study was supported by Boston Scientific, Marlborough, MA, USA, by AstraZeneca, Gaithersburg, MD, USA and by Legs Poix, Chancellerie des Universités, Paris, France

Disclosure of potential conflict of interest: M. Pretolani's institution has received funding from AstraZeneca; S. Létuvé received funding from AstraZeneca and GSK; C. Taillé reports grants and personal fees from Sanofi, GSK, AstraZeneca, Novartis, outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Abstract

Background. Phenotypes and endotypes predicting optimal response to bronchial thermoplasty (BT) in severe asthmatics remain elusive.

Objective. To compare clinical characteristics and hallmarks of airway inflammation and remodelling before and after BT in responders and partial responders severe asthmatics refractory to oral steroids and to omalizumab.

Methods. Twenty-three severe refractory asthmatics were divided into BT-responders (n=15) and BT-partial responders (n=8), according to the decrease in asthma exacerbations at 12 months after BT. Clinical parameters were compared at baseline and 12 months after BT and hallmarks of airway inflammation and remodelling were analysed by immunohistochemistry in bronchial biopsies before and 3 months after BT.

Results. At baseline, BT-responders were around 8 years younger ($P=0.02$), had greater incidence of atopy, higher numbers of blood eosinophils (both $P=0.03$) and IgE levels, higher epithelial interferon (IFN)- α expression, higher mucosal eosinophils and IL-33-positive cells ($P\leq 0.05$) than BT-partial responders. A reduction in blood eosinophils, serum IgE, type 2 (T2) airway inflammation, mucosal IL-33-positive cells and mast cells, associated with an augmented epithelial MUC5AC and IFN- α/β immunostaining were noted after BT in responders, whereas mucosal IL-33-positive cells were augmented in BT-partial responders. Most of these changes correlated with clinical parameters. Sub-epithelial membrane thickening and airway smooth muscle area were similar in the two patient groups at baseline and after BT.

Conclusion. By reducing allergic T2 inflammation and increasing epithelial MUC5AC and anti-viral IFN- α/β expression, BT may enhance host immune responses and thus attenuate exacerbations and symptoms in responders. Instead, targeting IL-33 may provide a clinical benefit in partial responders.

Key words: Bronchial epithelium, T2-type inflammation, MUC5AC, IL-33, mast cells, IFN- α/β , exacerbations, asthma control, bronchial thermoplasty

KEY MESSAGES

- Bronchial thermoplasty (BT) may provide various degrees of clinical benefit for severe refractory asthmatics, depending on their phenotype and endotype. At baseline, BT-responsive severe asthmatics were younger, had higher incidence of atopy, circulating eosinophils, and IgE levels, and greater expression of mucosal T2 markers and IL-33 than partial responders.

- Treatment with BT reduced systemic and mucosal allergic/T2 inflammation, while augmented epithelial MUC5AC and IFN- $\alpha\beta$ expression in BT-responders, but not in -partial responders. In contrast, BT-partial responders had greater numbers of mucosal IL-33-expressing cells after BT than BT-responders. Most of these changes correlated with main clinical parameters of asthma control and symptoms.

CAPSULE SUMMARY

Atopic severe asthmatics with T2-type inflammation are more susceptible to benefit from BT treatment, which results in decreased systemic and mucosal inflammation and increased epithelial MUC5AC and anti-viral interferons' expression.

ABBREVIATIONS

ACT, asthma control test; AQLQ, asthma quality of life questionnaire; ASM, airway smooth muscle; BT, bronchial thermoplasty; ICS, inhaled corticosteroids; IFN, interferon; IL, interleukin; IL-13R α 2, IL-13 receptor α 2; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity, IQR, interquartile range; OCS, oral corticosteroids; T2, Type 2; SBM, sub-epithelial basement membrane.

Introduction

Bronchial thermoplasty (BT) is a non-pharmacological treatment option for patients with severe asthma who remain symptomatic despite maximal medication. The initial purpose of this treatment was to reduce airway smooth muscle (ASM) mass in proximal airways, through the delivery of thermal energy *via* a dedicated catheter.^{1,2} Previous investigations by our and other groups have shown that BT markedly decreased ASM mass, but also other structural abnormalities involved in airway narrowing and bronchial (hyper)reactivity, such as the number of neuroendocrine epithelial cells and nerve endings.⁷⁻⁹ These effects correlated with a reduction in the number of severe exacerbations, hospitalisations for asthma and of emergency department visits, and with improved quality of life.¹⁻⁷

Other studies showed that BT decreased the epithelial expression of MUC5AC and of its driver, interleukin (IL)-13,^{10,11} reduced tissue fibrosis, *i.e.* sub-epithelial basement membrane (SBM) thickening, collagen type I deposition,^{7,9,12} and alveolar levels of transforming-growth factor- β ,¹³ and downregulated the production of heat-shock protein 60 by the bronchial epithelium, which promotes fibroblast-mediated remodelling.¹⁴ BT may also target airway inflammation, as shown by a decrease in the proportion of alveolar eosinophils and of the levels of the leukocyte-chemotactic chemokine, CCL5.¹³

In the above-mentioned studies, however, correlations between histopathological/biological alterations and clinical parameters were not investigated.

Importantly, severe asthmatics do not respond homogeneously to BT and some of them show partial or no clinical improvement.^{7,9,15} Therefore, the major current challenge in the clinical practice is to define eligibility criteria for BT, which implies the identification of specific biomarkers for responders.^{9,15}

To answer these questions, we compared demographic and clinical parameters to the nature of airway inflammation and remodelling in 23 severe refractory asthmatics. These patients have been defined as “BT-responders” or “BT-partial responders”, according to changes in the rate of exacerbations 12 months after the procedure, as primary efficacy endpoint.

We compared the epithelial expression of MUC5AC and MUC5B and their ratio, which has been associated with an epithelial Type 2 (T2)-high signature and airway eosinophilia.¹⁶⁻¹⁸

110 We also determined the epithelial levels of the IL-13R α 2 receptor, which binds the T2
111 cytokine, IL-13,¹⁹ of the alarmin, IL-33, which propagates T2 signals through the activation of
112 innate immune cells,^{20,21} and of type I (α/β) interferons (IFN).²² Moreover, we investigated
113 hallmarks of airway inflammation including mucosal eosinophils, neutrophils, IL-13- and IL-
114 17A-positive cells,^{10,23,24} and mucosal and intra-muscular mast cells.^{23,25} Finally, we
115 quantified ASM area and SBM thickening, as validated targets of BT.^{7,8,12}

Methods

Patients and BT procedure

Twenty-three adults with severe uncontrolled asthma were recruited in the Respiratory Diseases department of the Bichat Hospital (Paris, France) (Table E1 in the Online Repository at <http://www.jacionline.org>).^{7,8} Spirometry, number of exacerbations, and scores on asthma control test (ACT) were taken before, 3 and 12 months after BT.^{7,8} BT was performed using the ALAIR® System (Boston Scientific, Marlborough, MA, USA) for a total of 3 sessions, separated by one-month intervals.^{7,8}

A bronchoscopy was performed 2 weeks before the first BT session and 3 months after the last one^{7,8} to collect 4 bronchial biopsies from the lower right lung lobe for immuno-histochemical studies. Control biopsies were obtained from surgically resected bronchial specimens of 10 non-smoker donors of lung transplantation.²⁶

The 23 severe asthmatics were classified into BT-responders (n=15) and BT-partial responders (n=8), using a cut-off of <3 exacerbations for responders and ≥3 exacerbations for partial responders during the 12 months following BT. Indeed, this cut-off value was one of the eligibility criteria for including severe refractory asthmatics in this study and, therefore, all patients had at least 3 severe exacerbations requiring oral corticosteroid (OCS) bursts the year prior to inclusion. The 15 responders had sub-optimal controlled asthma (mean ACT score of 16.6), whereas the 8 partial responders remained uncontrolled (mean ACT score of 8.6) (Table 1).^{27,28}

This protocol was approved by the CPP Ile-de-France I Ethics Committee (n° 2012-sept-13003) and all subjects gave their written informed consent (ClinicalTrials.gov NCT01777360).

Histopathological studies

Epithelial immuno-localisation of MUC5AC, MUC5B, IL-13Rα2, IL-33, IFN-α/β and the number of mucosal cells positive for major basic protein (eosinophils), IL-13, IL-17A, elastase (neutrophils), tryptase (mast cells), and IL-33 were determined on sections from four paraffin-embedded bronchial biopsies per patient (details in the online repository, Table E2). We also quantified intra-muscular mast cells, ASM area and SBM thickening, as described

145 previously.^{7,8}

146 *Statistical analysis*

147 Qualitative clinical variables are reported as numbers and percentages and compared using t
148 test, χ^2 -test, or Fisher exact test (2-tailed). Non-normal variables are reported as median and
149 25-75 interquartile range (IQR) and compared using Kruskal-Wallis analysis, followed by
150 Mann-Whitney U-test, or by Wilcoxon matched-pairs rank test.

151 Associations between histopathological and clinical variables were assessed using the non-
152 parametric Spearman's rank correlation coefficient (rho) and the Benjamini and Hochberg
153 correction with a family-wise error rate of 0.05 for multiple comparisons. Statistical analyses
154 were performed using Prism (GraphPad Software, San Diego, CA) and R 2.15.2 software (R
155 Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical characteristics of the overall recruited severe asthmatics

The 23 recruited severe asthmatics had uncontrolled asthma at baseline, despite optimal management and maximal medications (Table E1). They also showed airflow obstruction and ≥ 3 annualised exacerbations requiring OCS bursts.^{7,8} Of the 23 asthmatics, 22 were treated regularly with OCS, with a mean dose of oral prednisone of 29.4 mg per day (Table E1).

In agreement with our previous study,⁷ treatment with BT significantly improved scores on ACT and on asthma quality of life questionnaire (AQLQ) and decreased the annualised rate of severe exacerbations and of hospitalisations for asthma at 3 and 12 months (Table E1). In addition, the proportion of severe asthmatics requiring OCS was significantly lower 3 and 12 months after BT, with a reduction in the mean daily dose of oral prednisone at both time points (Table E1).

Clinical characteristics of BT-responders and -partial responders

At baseline, the 15 BT-responders were approximately 8 years younger ($P=0.02$) and had a higher incidence of atopy ($P=0.03$), higher numbers of peripheral blood eosinophils ($P=0.04$), and higher levels of total serum IgE ($P=0.04$) than BT-partial responders (Table 1).

Baseline exacerbation rates, ACT and AQLQ scores were not statistically different between BT-responders and -partial responders (Table 1). The proportion of patients treated with long-lasting β_2 -agonists, leukotriene modifiers, OCS and long acting muscarinic antagonists, and the daily doses of ICS and of oral prednisone were comparable between the two groups (Table 1).

Twelve months after BT treatment, responders had reduced numbers of peripheral blood eosinophils ($P=0.02$) and lower levels of total IgE ($P=0.04$), whereas no changes were observed in partial responders (Table 1). The number of neutrophils was not statistically different across groups ($P=0.65$). As expected, BT also decreased the number of exacerbations by 99% and increased the ACT score by more than 2.5-fold in responders ($P<0.0001$, for both comparisons) (Table 1). The annual exacerbation rate was also reduced

after BT in partial responders (66%, $P=0.02$), but the score on ACT failed to augment significantly (1.4-fold increase, as compared to pre-BT, $P=0.19$). However, the reduction in exacerbations was more marked in BT-responders compared to -partial responders ($P<0.0001$). A similar difference was found for annualised hospitalisations for asthma, for which rates were reduced by BT in both patient groups, although less markedly in partial responders than in responders (72 and 94% respectively, $P=0.02$) (Table 1).

The score on AQLQ was higher 12 months after BT than before BT in responders ($P=0.0002$), but not in partial responders ($P=0.35$). This difference was statistically significant ($P=0.005$) (Table 1).

The proportion of BT-responders necessitating long-term OCS and the daily dose of oral prednisone were reduced at 12 months ($P=0.01$ and $P=0.007$, respectively) (Table 1). In contrast, BT-partial responders required similar doses of OCS to those taken before BT ($P=0.35$) (Table 1). The use of leukotriene modifiers, or of long-acting muscarinic antagonists and the daily doses of ICS were similar in BT-responders and -partial responders (Table 1).

The overall baseline parameters of respiratory function did not differ between BT-responders and -partial responders, although a non-significant trend towards lower values of pre- and post-bronchodilator FEV₁ ($P=0.37$ and $P=0.17$, respectively) and a worse reversibility to β_2 -agonists ($P=0.37$) was observed in BT-partial responders (Table 1).

Histopathology of the overall recruited severe asthmatics

Before BT, the overall group of 23 severe asthmatics showed higher epithelial expression of MUC5AC (but not MUC5B), of IL-13R α 2 and IFN- α/β , and a greater MUC5AC to MUC5B ratio, as compared to controls (Table E3). These patients also had elevated numbers of mucosal eosinophils, IL-13-, IL-17A- and IL-33-positive cells, and of intra-muscular (but not mucosal) mast cells (Table E3). The epithelial expression of IL-33 and the number of mucosal neutrophils were not statistically different across the study groups, although a trend towards an increase in epithelial IL-33 expression was noted in severe asthmatics before and after BT, as compared to controls (Table E3). SBM thickening and ASM area were also higher in severe asthmatics before BT than in controls (Table E3).^{7,23}

Treatment with BT was followed by a significant increase in the epithelial expression of MUC5AC and MUC5B ($P=0.05$ and $P=0.009$, respectively) and of IFN- α ($P=0.04$), as compared to values measured before BT (Table E3). The number of mucosal IL-13-positive cells and of mucosal and intra-muscular mast cells was reduced after BT ($P=0.008$, and $P=0.04$, respectively), whereas mucosal IL-17A+ cells were not significantly modified (Table E3). ASM area and SBM thickening were significantly lower 3 months after BT than before BT ($P<0.0001$, for both comparisons) (Table E3).^{7,8}

Histopathology in BT-responders and -partial responders

At baseline, BT-responders showed higher epithelial expression of IFN- $\alpha 1$ ($P=0.01$, Fig. 1C,D) and elevated numbers of mucosal eosinophils ($P=0.02$, Fig. 2C,D) and IL-33-positive cells ($P=0.04$, Fig. 2A,B) than BT-partial responders (Figs. 1 and 2). No other hallmarks of airway inflammation or remodelling differentiated BT-responders from -partial responders at baseline (Table 2).

BT increased the epithelial expression of MUC5AC, but not of MUC5B, in responders (Fig. 1A,B and Table 2) and reduced MUC5AC to MUC5B ratio by 58% ($P=0.06$, Table 2). In these patients, BT also downregulated the extent of epithelium area positive for IL-13R $\alpha 2$ ($P=0.03$, Table 2), the number of mucosal eosinophils ($P=0.02$, Fig. 2C,D), and of IL-13- ($P=0.04$, Table 2) and IL-33-positive cells ($P=0.004$, Fig. 2A,B), as well as mucosal and intra-muscular mast cells ($P<0.001$ and $P=0.04$, respectively) (Fig. 2E,F and Table 2). This was concomitant with a higher epithelial expression of IFN- α/β after BT than before BT in this patients' group ($P=0.04$ and $P=0.007$, respectively, Fig. 1C-F). In contrast, these histopathological parameters were not modified in partial responders (Table 2 and Figs. 1 and 2).

Other histopathological differences between the two patients' groups included a number of mucosal and intra-muscular mast cells ($P=0.05$, Fig. 2E,F and Table 2) and an epithelial expression of IFN- α/β that persisted low ($P=0.04$) after BT in partial responders (Fig. 1C-F). Overall, there were no changes in the epithelial expression of IL-33 across the study groups (Table 2).

Finally, SBM thickening and ASM area were reduced to a similar extent following BT in both responders and partial responders ($P \leq 0.003$ for all comparisons) (Table 2).

Correlation analyses in BT-responders and -partial responders

We next examined potential correlations between clinical parameters at 12 months and hallmarks of airway inflammation and remodelling at 3 months in BT-responders and -partial responders (Tables 3 and E4).

In BT-responders, the number of exacerbations negatively correlated with epithelial MUC5AC and IFN- α , whereas a positive association was found with mucosal eosinophils, IL-13-positive cells, mucosal and intra-muscular mast cells, SBM thickening, and ASM area (Table 3). Scores on ACT and AQLQ positively correlated with epithelial MUC5AC expression and were negatively associated with the number of mucosal mast cells and with parameters of airway remodelling (Table 3).

In contrast, the only correlation found in BT-partial responders was a positive association of the number of exacerbations with SBM thickening and ASM area (Table E4).

Finally, BT-responders showed positive correlations between epithelial IFN- α and MUC5AC and MUC5B expression ($\rho=0.53$, $P=0.006$ and $\rho=0.49$, $P=0.01$, respectively), between MUC5AC and mucosal IL-17A-positive cells ($\rho=0.43$, $P=0.03$) and neutrophils ($\rho=0.50$, $P=0.005$). In addition, IL-13-positive cells were associated with mucosal mast cells ($\rho=0.43$, $P=0.02$), and eosinophils correlated with epithelial IL-33 expression ($\rho=0.45$, $P=0.03$). These correlations were not observed in BT-partial responders (data not shown).

No significant correlations were found between any of the histopathological parameters and the daily doses of OCS (Table 3), respiratory function, or the number of heat activations in these patients overall (Tables 3 and E4 and data not shown).

Discussion

This study aimed at characterising phenotypes and endotypes of patients with severe refractory asthma associated with optimal response to BT. Indeed, severe asthmatics do not respond homogeneously to BT and the major current challenge in the clinical practice is to define eligibility criteria for BT. To this end, we studied hallmarks of epithelial and mucosal inflammation which have never been explored before, including epithelial and mucosal IL-33, epithelial expression of IL-13R α 2 and of type I (α/β) IFN, and the number of mucosal and intra-muscular mast cells. Our study provides new insights on the characteristics of patients who are more likely to benefit from BT and adds novel findings on BT effects on mucosal inflammation and epithelial inflammatory and anti-viral responses in relation with clinical parameters.

We showed that, at baseline, severe asthmatics who had an optimal response to BT (BT-responders) were younger, had higher incidence of atopy, numbers of peripheral blood eosinophils and total serum IgE than BT-partial responders.

Twelve months after BT, blood eosinophils and IgE levels were reduced exclusively in responders, despite a decrease in OCS use, and this was associated with a significant improvement in asthma control and quality of life. These findings support the hypothesis that the clinical benefit of BT in responders involved a downregulation of allergic/T2-type responses. Notably, 14 out of these 15 BT-responders failed to improve with omalizumab, suggesting that BT may provide an additional beneficial effect for atopic severe asthmatics who are refractory to this biologic.

The bronchial epithelium is a major driver of airway inflammation and remodelling in asthma and recent studies have shown that BT causes alterations in its morphology and functions.

^{10,14,29} The degree of patients' response to BT in our study was mirrored by differences in epithelial characteristics at baseline, with BT-responders showing higher numbers of mucosal eosinophils and of IL-33-expressing cells and greater epithelial expression of the anti-viral cytokine IFN- α .

Both BT-responders and -partial responders had elevated numbers of mucosal IL-17A-positive cells before BT, as compared to controls, suggesting that these patients exhibited a dual T2/T17 endotype.

After BT, T2 markers such as eosinophils, IL-13- and IL-33-positive cells, and epithelial IL-13R α 2 expression, as well as the number of mucosal and intra-muscular mast cells, were reduced in responders, but not in partial responders. Although these changes cannot prove cause and effect, they support the hypothesis that an attenuation of T2 responses in the airways and of mast cell accumulation and attraction within the ASM may participate in the clinical benefit of BT in responders. In agreement with this, lower mucosal T2 markers and mast cell numbers correlated with fewer exacerbations in this group and reduced mucosal mast cells were also significantly associated with improved ACT and AQLQ scores. These data suggest that BT may dampen the production of mast cell chemotactic factors from different cell types present in the airways, including the ASM itself, leading to a reduction of the overall inflammatory response.

Conversely, the persistence of intra-muscular mast cells after BT in partial responders could explain the non-significant decrease in pre- and post-bronchodilator FEV₁ values and a worse reversibility to β_2 -agonists observed in these patients. Indeed, mast cell-derived mediators play an important role in ASM contraction,³⁰ which may lead to airway narrowing and lower FEV₁. This hypothesis, however, should be tested in a larger number of patients.

Epithelial MUC5AC expression increased after BT in responders, but not in partial responders, and its levels negatively correlated with exacerbations. Our findings seem to contradict the general notion that MUC5AC overexpression contributes to airway obstruction, but not to poor asthma control,³¹ but agrees with data in mice showing that upregulated *Muc5ac* expression in the lung epithelium is associated to protection against influenza virus-mediated infection.³² Also, of note, MUC5AC is upregulated in response to allergic inflammatory challenge or viral infections.^{33,34}

This increase in MUC5AC after BT contradicts a recent report showing epithelial MUC5AC reduction in severe asthmatics following BT.¹⁰ This discrepancy may originate from differences in the clinical characteristics of the patients included in these two trials, in their

medication, and in the time-point selected after BT to study epithelium alterations. Furthermore, no correlations between decrease in MUC5AC and clinical outcomes were investigated in the latter study. Of interest, however, a recent proteomic analysis showed that treatment of 8 severe asthmatics with BT was followed by an increase in the alveolar levels of MUC5AC.¹⁴ These and our current observations indicate that BT may differentially regulate the signals involved in the expression of this mucin. Although IL-13 is considered a major driver of MUC5AC production,^{10,11} IL-17A may also stimulate MUC5AC production in human airway epithelial cells *via* IL-17RA, IL-17RC and act1/MAPK pathway.³⁵ This is supported by our findings showing that MUC5AC epithelial expression positively correlated with the number of mucosal IL-17A-positive cells in BT-responders, but not in partial responders, despite a lack of modulatory effects of BT on the overall T17-type response.

Previous studies have shown that impaired clearance of viruses in patients with uncontrolled asthma was associated with a deficient induction of IFN- α/β , with worsening of airway symptoms, and with higher frequency of exacerbations.^{22,36} Furthermore, a recent study demonstrated elevated expression of IFNs and T2 cytokines analysed from bronchosorption and nasosorption in asthma patients infected with rhinovirus.³⁷ Although we have no direct clinical evidence that respiratory viruses precipitated the exacerbations in our patients, these observations suggest that epithelial IFN- α/β overexpression in BT-responders may act as a protective factor against virus-induced exacerbations. Further studies should be conducted to address this specific point.

Interestingly, treatment with BT led to a rise in the number of mucosal IL-33-positive cells in partial responders. Different cell types may express this cytokine, including mast cells and monocytes/macrophages.²⁰ Given the persistence of mucosal and intra-muscular mast cells observed after BT in this patients' group, it can be hypothesised that these cells sustain airway inflammation and ASM activation through IL-33 expression.³⁸

Previous studies have demonstrated that the release of this alarmin is triggered by mechanisms commonly associated with asthma pathology, particularly viral infections,³⁹ and evidence has supported the ability of IL-33 to dampen IFN-driven anti-viral immunity.^{40,41} Together, these and our current observations suggest that repeated viral infections promote sustained IL-33 overexpression by mucosal cells which, in turn, participates in the persistence of severe exacerbations and symptoms in BT-partial responders.

We also found that in BT-responders, the proportion of patients requiring OCS after BT and the doses of oral prednisone taken were lower than those observed for partial responders. Since corticosteroids have been shown to downregulate MUC5AC in bronchial epithelial cells⁴² as well as IFN- α 1 in monocytes,⁴³ there is the possibility that a reduction in OCS use in BT-responders contributed to the increased expression of these molecules. However, this hypothesis is not supported by our data showing a lack of correlation between daily use of OCS and levels of epithelial MUC5AC, IFN- α , and with inflammatory cells. In contrast, IL-33 is considered as a relatively steroid-insensitive target⁴⁴ and this mechanism involves the IL-33-dependent inhibition of glucocorticosteroid receptor nuclear translocation *via* its phosphorylation.⁴⁵ This and the lack of correlation between mucosal IL-33-positive cells and OCS use reported here suggest that a direct effect of steroid therapy on the elevation in IL-33-positive cells in BT-partial responders is unlikely.

Most of the patients in this study would fall into the classification of steroid-resistant asthma, which is characterised by persistent airway inflammation despite treatment with high doses of corticosteroids. Although steroid-resistant asthmatics showed increased numbers of circulating and mucosal neutrophils,⁴⁶ we found no difference in airway and peripheral neutrophils between BT-responders and -partial responders. These data suggest that mechanisms other than steroid resistance may be involved in the different response to BT in these patients.

We acknowledge that our current study has certain limitations. The relative low number of severe asthmatics included in the study may have reduced the power of some statistical analyses. In addition, immuno-histochemical studies at later time points after BT would allow to determine the persistence of the observed tissue alterations. Also, the detection of MUC5AC was performed only as stainable mucin stored in the goblet cells of the bronchial epithelium and not as a secreted form, since our protocol did not include the collection of sputum or bronchoalveolar lavage samples. A quantification of secreted mucins would strengthen the link between MUC5AC and the formation of airway mucus gels during asthma exacerbations.¹⁷ The association between increased mucins and clinical indicators of sputum and cough could also be investigated by adding a validated cough and sputum questionnaire

(e.g. CASA-Q score).⁴⁷ Finally, the determination of viral load in the airways would be useful to investigate potential correlations between epithelial IFN- α/β expression and exacerbations.³⁴

In conclusion, the current study demonstrated that BT improved clinical outcomes in atopic eosinophilic severe steroid-dependent asthmatics, in association with a reduction in T2-type inflammation. On the other hand, mucosal IL-33 overexpression in BT-partial responders may dampen anti-viral responses and sustain eosinophilic- and mast cell-type inflammation. Thus, the use of anti-IL-33 blocking antibodies may represent a novel therapeutic option for these patients.

Acknowledgements

We thank the patients who participated in the study, Olivier Thibaudeau and Laure Wingertsmann (Morphology platform, Inserm UMR1152) for the preparation of bronchial tissue sections and haematoxylin & eosin staining, and the Investissements d'Avenir programme ANR-11-IDEX-0005-02, Sorbonne Paris Cité, Laboratoire d'Excellence INFLAMEX.

References

1. Mainardi AS, Castro M, Chupp G. Bronchial Thermoplasty. *Clin Chest Med*. 2019;40:193-207.
2. Tan LD, Yoneda KY, Louie S, Hogarth DK, Castro M. Bronchial Thermoplasty: A Decade of Experience: State of the Art. *J Allergy Clin Immunol Pract*. 2019;7:71-80.
3. Pavord ID, Cox G, Thomson NC, Rubin AS, Corris PA, Niven RM, et al. Safety and efficacy of bronchial thermoplasty in symptomatic, severe asthma. *Am J Respir Crit Care Med* 2007;176:1185-91.
4. Cox G, Thomson NC, Rubin, AS, Niven RM, Corris PA, Siersted HC, et al. Asthma control during the year after bronchial thermoplasty. *N Engl J Med* 2007;356:1327-37.
5. Castro M, Rubin AS, Laviolette M, Fiterman J, De Andrade Lima M, Shah PL, et al. AIR2 Trial Study Group. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010;181:116-24.
6. Wechsler ME, Laviolette M, Rubin AS, Fiterman J, Lapa e Silva JR, Shah PL et al. Asthma Intervention Research 2 Trial Study Group. Bronchial thermoplasty: Long-term safety and effectiveness in patients with severe persistent asthma. *J Allergy Clin Immunol* 2013;132:1295-302.
7. Pretolani M, Bergqvist A, Thabut G, Dombret MC, Knapp D, Hamidi F, et al. Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: Clinical and histopathologic correlations. *J Allergy Clin Immunol*. 2017;139:1176-85.
8. Pretolani M, Dombret MC, Thabut G, Knapp D, Hamidi F, Debray MP, et al. Reduction of airway smooth muscle mass by bronchial thermoplasty in patients with severe asthma. *Am J Respir Crit Care Med*. 2014;190:1452-4.
9. Thomson NC. Recent developments in bronchial thermoplasty for severe asthma; *J Asthma Allergy* 2019;12:375-87.
10. Haj Salem I, Gras D, Joubert P, Boulet LP, Lampron N, Martel S, et al. Persistent Reduction of mucin Production following bronchial thermoplasty in severe Asthma. *Am J Respir Crit Care Med*. 2019;199:536-8.
11. Seibold MA. Interleukin-13 stimulation reveals the cellular and functional plasticity of the airway epithelium. *Ann Am Thorac Soc*. 2018;15(Suppl 2):S98-S102.
12. Chakir J, Haj-Salem I, Gras D, Joubert P, Beaudoin ÈL, Biardel S, et al. Effects of Bronchial Thermoplasty on Airway Smooth Muscle and Collagen Deposition in Asthma. *Ann Am Thorac Soc*. 2015;12:1612-8.
13. Denner DR, Doeing DC, Hogarth DK, Dugan K, Naureckas ET, White SR. Airway Inflammation after Bronchial Thermoplasty for Severe Asthma. *Ann Am Thorac Soc*. 2015;12:1302-9.

- 431 14. Sun Q, Fang L, Roth M, Tang X, Papakonstantinou E, Zhai W, et al. Bronchial
432 thermoplasty decreases airway remodelling by blocking epithelium-derived heat
433 shock protein 60 (HSP60) secretion and protein arginine methyltransferase 1
434 (PRMT1) in fibroblasts. *Eur Respir J*. 2019;54(6).
- 435 15. d'Hooghe JNS, Ten Hacken NHT, Weersink EJM, Sterk PJ, Annema JT, Bonta PI.
436 Emerging understanding of the mechanism of action of Bronchial Thermoplasty in
437 asthma. *Pharmacol Ther*. 2018;181:101-7.
- 438 16. Evans CM, Kim K, Tuvim MJ, Dickey BF. Mucus hypersecretion in asthma: causes
439 and effects. *Curr Opin Pulm Med*. 2009;15:4-11.
- 440 17. Martínez-Rivera C, Crespo A, Pinedo-Sierra C, García-Rivero JL, Pallarés-Sanmartín
441 A, Marina-Malanda N, et al. Mucus hypersecretion in asthma is associated with
442 rhinosinusitis, polyps and exacerbations. *Respir Med*. 2018;135:22-8.
- 443 18. Lachowicz-Scroggins ME, Yuan S, Kerr SC, Dunican EM, Yu M, Carrington SD, et al.
444 Abnormalities in MUC5AC and MUC5B Protein in Airway Mucus in Asthma. *Am J*
445 *Respir Crit Care Med*. 2016;194:1296-9.
- 446 19. Yang SJ, Allahverdian S, Saunders ADR, Liu E, Dorscheid DR. IL-13 signaling
447 through IL-13 receptor $\alpha 2$ mediates airway epithelial wound repair. *FASEB J*.
448 2019;33:3746-57.
- 449 20. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than
450 just signaling the alarm. *J Clin Invest*. 2019;129:1441-51.
- 451 21. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33
452 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma.
453 *J Allergy Clin Immunol*. 2013;132:676-85.
- 454 22. Denney L, Ho LP. The role of respiratory epithelium in host defence against influenza
455 virus infection. *Biomed J*. 2018;41:218-33.
- 456 23. Aubier M, Thabut G, Hamidi F, Guillou N, Brard J, Dombret MC, et al. Airway smooth
457 muscle enlargement is associated with protease-activated receptor 2/ligand
458 overexpression in patients with difficult-to-control severe asthma. *J Allergy Clin*
459 *Immunol*. 2016;138:729-39.
- 460 24. Andersson CK, Adams A, Nagakumar P, Bossley C, Gupta A, De Vries D, et al.
461 Intraepithelial neutrophils in pediatric severe asthma are associated with better lung
462 function. *J Allergy Clin Immunol*. 2017;139:1819-29.
- 463 25. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell
464 infiltration of airway smooth muscle in asthma. *N Engl J Med*. 2002;346:1699-705.
- 465 26. Fernandes J, Hamidi F, Leborgne R, Beau R, Castier Y, Mordant P, et al. Penetration
466 of the Human Pulmonary Epithelium by *Aspergillus fumigatus* Hyphae. *J Infect Dis*.
467 2018;218:1306-13.

27. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59-65.
28. Schatz M, Sorknesss CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *J Allergy Clin Immunol* 2006;117:549-56.
29. Bonser LR and Erle DJ. The airway epithelium in asthma. *Adv Immunol.* 2019;142:1-34.
30. S  fholm J, Manson ML, Bood J, Al-Ameri M, Orre AC, Raud J, et al. Mannitol triggers mast cell-dependent contractions of human small bronchi and prostacyclin bronchoprotection. *J Allergy Clin Immunol.* 2019; 144:984-992.
31. Dunican EM, Elicker BM, Gierada DS, Nagle SK, Schiebler ML, Newell JD, et al. National Heart Lung and Blood Institute (NHLBI) Severe Asthma Research Program (SARP). Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction. *J Clin Invest.* 2018;128:997-1009.
32. Ehre C, Worthington EN, Liesman RM, Grubb BR, Barbier D, O'Neal WK, et al. Overexpressing mouse model demonstrates the protective role of Muc5ac in the lungs. *Proc Natl Acad Sci USA.* 2012;109:16528-33.
33. Bonser LR, Erle DJ. Airway Mucus and Asthma: The Role of MUC5AC and MUC5B. *J Clin Med.* 2017;6:112.
34. Ridley C, Thornton DJ. Mucins: the frontline defence of the lung. *Biochem Soc Trans.* 2018;46:1099-1106.
35. Xia W, Bai J, Wu X, Wei Y, Feng S, Li L, Zhang J, Xiong G, Fan Y, Shi J, Li H. Interleukin-17A promotes MUC5AC expression and goblet cell hyperplasia in nasal polyps via the Act1-mediated pathway. *PLoS One.* 2014;9:e98915.
36. Zhu J, Message SD, Mallia P, Keadze T, Contoli M, Ward CK, et al. Bronchial mucosal IFN- α/β and pattern recognition receptor expression in patients with experimental rhinovirus-induced asthma exacerbations. *J Allergy Clin Immunol.* 2019;143:114-25.
37. Hansel TT, Tunstall T, Trujillo-Torralbo MB, Shamji B, Del-Rosario A, Dhariwal J, et al. A Comprehensive Evaluation of Nasal and Bronchial Cytokines and Chemokines Following Experimental Rhinovirus Infection in Allergic Asthma: Increased Interferons (IFN- γ and IFN- λ) and Type 2 Inflammation (IL-5 and IL-13). *EBioMedicine.* 2017;19:128-38.
38. Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. *Allergy.* 2015;70:556-67.

39. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med*. 2014;190:1373-82.
40. Werder RB, Zhang V, Lynch JP, Snape N, Upham JW, Spann K et al. Chronic IL-33 expression predisposes to virus-induced asthma exacerbations by increasing type 2 inflammation and dampening antiviral immunity. *J Allergy Clin Immunol*. 2018;141:1607-19.
41. Ravanetti L, Dijkhuis A, Dekker T, Sabolgal Pineros YS, Ravi A. et al. IL-33 drives influenza-induced asthma exacerbations by halting innate and adaptive antiviral immunity. *J Allergy Clin Immunol*. 2019;143:1355-70.
42. Chen Y, Nickola TJ, DiFronzo NL, Colberg-Poley AM, Rose MC. Dexamethasone-mediated repression of MUC5AC gene expression in human lung epithelial cells. *Am J Respir Cell Mol Biol*. 2006;34:338-47.
43. Norbiato G, Bevilacqua M, Vago T. Glucocorticoids and the immune system in AIDS. *Psychoneuroendocrinology*. 1997;22 Suppl 1:S19-S25.
44. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol*. 2013;132:676-85.
45. Hirahara K, Mato N, Hagiwara K, Nakayama T. The pathogenicity of IL-33 on steroid-resistant eosinophilic inflammation via the activation of memory-type ST2⁺ CD4⁺ T cells. *J Leukoc Biol*. 2018;104:895-901.
46. Macdowell AL, Peters SP. Neutrophils in asthma. *Curr Allergy Asthma Rep*. 2007;7:464–8.
47. Crawford B, Monz B, Hohlfeld J, Roche N, Rubin B, Magnussen H, et al. Development and validation of a cough and sputum assessment questionnaire. *Respir Med*. 2008;102:1545-55.

Figure legends

FIG. 1

Epithelial alterations and mucosal inflammation before and after BT in responders and in partial responders severe asthmatics.

Exemplary photographs showing the epithelial immunolocalisation of (A), MUC5AC, (C) IFN- α and (E) IFN- β before and 3 months after BT in responders and in partial responders severe asthmatics. After BT, the epithelial immunolocalisation of these markers was higher in responders, than in partially-responders severe asthmatics. Original magnifications x 200. (B,D,F) Immuno-staining of each marker was quantified by image analysis and data are expressed as medians (25-75% IQR) of n=15 BT-responders and of n=8 BT-partial responders. $P=0.0003$, for panel B; $P=0.02$, for panel D and $P=0.01$, for panel F (Kruskal-Wallis test). Significances were calculated using Mann-Whitney U-test, between controls and severe asthmatics before BT or after BT, and by Wilcoxon matched-pairs rank test, between severe asthmatics before and after BT.

FIG. 2

Mucosal inflammation before and after BT in responders and in partial responders severe asthmatics.

Exemplary photographs showing the distribution of mucosal cells expressing IL-33, major basic protein (eosinophils) and tryptase (mast cells) (red deposit) before and 3 months after BT in responders and partial responders severe asthmatics. After BT, the density of mucosal of IL-33-positive cells, of eosinophils and of mast cells was lower (arrows) in responders, than in partially-responders severe asthmatics. The frequency of mucosal cells expressing IL-33 was higher after BT in partial responders than in responders. Original magnifications x 200 and x 400, for IL-33 immunostaining. (B,D,F) Immunostaining of each marker was quantified by image analysis and data are expressed as medians (25-75% IQR) of n=15 BT-responders and of n=8 BT-partial responders. $P=0.009$, for panel B; $P=0.02003$, for panel D and $P=0.03$, for panel F (Kruskal-Wallis test). Significances were calculated using Mann-Whitney U-test, between controls and severe asthmatics before BT or after BT, and by Wilcoxon matched-pairs rank test, between severe asthmatics before and after BT.

Table 1: Characteristics of BT-responder and -partial responder severe asthmatics before and 12 months after BT

Parameter	BT-responder severe asthmatics		BT-partial responder severe asthmatics		P value ^a
	Pre-BT	Post-BT	Pre-BT	Post-BT	
Number of subjects	15		8		-
Women – no. (%)	7 (47)	-	6 (75)	-	0.38
Caucasian – no. (%)	13 (87)	-	6 (75)	-	0.59
Age – years	46.7 ± 7.9	-	54.9 ± 6.2	-	0.02
Body mass index - kg per m ²	31.5 ± 9.4	-	26.0 ± 4.1	-	0.13
Smoking history – no. of never smokers/former smokers (%)	9/6 (60/40)	-	5/3 (63/37)	-	1.00
Age of asthma onset – years	20.3 ± 17.7	-	18.8 ± 18.3	-	0.84
Asthma duration – years	26.3 ± 18.2	-	30.4 ± 17.1	-	0.60
With history of atopy – no. (%)	11 (73)	-	2 (25)	-	0.03
Blood eosinophils per mm ³	270 (153-415)	108 (63-243) *	145 (88-192) †	135 (72-315)	0.03
Blood neutrophils per mm ³	10700 (5670-15900)	5940 (4710-8410)	8585 (8108-9062)	8240 (7535-9775)	0.65
Total serum IgE (IU per millilitres)	330 (144-497)	129 (62-168) *	180 (133-317) †	177 (58-203)	0.04
Respiratory function					
Pre-bronchodilator FEV ₁ – Liters	2.11 ± 0.81	2.20 ± 0.74	1.92 ± 0.60	1.56 ± 0.64	0.23
Post-bronchodilator FEV ₁ – Liters	2.23 ± 0.76	2.60 ± 1.14	2.27 ± 0.70	1.76 ± 0.76	0.23
Pre-bronchodilator FEV ₁ – % of predicted	65.1 ± 18.5	68.5 ± 16.1	66.3 ± 20.0	53.9 ± 23.7	0.37
Post-bronchodilator FEV ₁ – % of predicted	73.7 ± 20.3	76.6 ± 16.5	74.1 ± 18.3	57.8 ± 22.0	0.17
Pre-bronchodilator FEV ₁ /FVC – % of predicted	60.9 ± 13.3	64.2 ± 10.4	60.2 ± 10.8	52.3 ± 14.8	0.25
Post-bronchodilator FEV ₁ /FVC – % of predicted	63.0 ± 13.0	63.5 ± 10.1	62.2 ± 11.2	56.6 ± 19.8	0.77
Reversibility to β ₂ -agonists – milliliters	228 ± 226	245 ± 174	373 ± 121	176 ± 108	0.37
Treatments					
On long-acting β ₂ -agonists – number (%)	15 (100)	15 (100)	8 (100)	8 (100)	1.00
Daily dose of ICS – µg of beclomethasone equivalents	2553 ± 256	2000 ± 0	2000 ± 0	2000 ± 0	1.00
On maintenance use of OCS – no. (%)	14 (93)	7 (47) *	8 (100)	8 (100) ‡	0.001
Daily dose of oral prednisone – mg	28.6 ± 13.9	14.7 ± 12.7 *	30.9 ± 23.2	20.3 ± 20.6	0.04
On anti-leukotrienes – no. (%)	6 (43)	7 (47)	3 (38)	3 (38)	0.96
On long-acting muscarinic antagonists – no. (%)	4 (27)	3 (20)	2 (25)	5 (63)	0.18

Table 1 (continued)

Asthma control					
With uncontrolled asthma – no. (%)	15 (100)	4 (27) *	8 (100)	7 (88) ‡	< 0.0001
Score on ACT	6.4 ± 2.0	16.6 ± 5.0 *	6.1 ± 1.6	8.6 ± 3.2 ‡	< 0.0001
Score on AQLQ	1.9 ± 9.7	4.4 ± 1.6 *	1.7 ± 0.8	2.3 ± 1.4 ‡	< 0.0001
Annual number of severe exacerbations	10.7 ± 1.5	0.9 ± 0.2 *	10.0 ± 2.3	3.4 ± 0.3 * ‡	< 0.0001
Annual rate of hospitalisations for asthma	1.8 ± 0.7	0.1 ± 0.1 *	2.9 ± 1.4	0.8 ± 0.4 * ‡	0.003

Abbreviations: BT, bronchial thermoplasty; FEV₁, Forced Expiratory Volume in One second; ICS, inhaled corticosteroids; OCS, oral corticosteroids; ACT, asthma control test; AQLQ, asthma quality of life questionnaire.

Data are n (%), or means ± SD, or median (25-75 IQR). Results on annual rates of severe exacerbations and hospitalisations for asthma are expressed as means ± SE

^a One-way ANOVA, or χ^2 -squared test, or Kruskal-Wallis test

* $P < 0.05$, as compared to values obtained before BT in each group; † $P < 0.05$, between BT-responder and -partial responder severe asthmatics before BT; ‡ $P < 0.05$, between BT-responder and -partial responder severe asthmatics after BT (Wilcoxon matched-pairs test, Student's t test, Fisher exact test, two-tailed, or Poisson test).

Bold denotes statistical significance

Table 2: Histopathological parameters in controls and in BT-responder and BT-partial responder severe asthmatics

Parameter	Controls	BT-responder severe asthmatics		BT-partial responder severe asthmatics		P value ^a
		Pre-BT	Post-BT	Pre-BT	Post-BT	
Number of patients	10	15		8		
Epithelium activation and remodelling						
MUC5B-positive area - %	3.0 (1.6-5.4)	4.1 (2.6-5.4)	5.8 (4.6-12.2)	2.2 (1.0-6.0)	3.2 (2.3-8.7)	0.10
MUC5AC to MUC5B ratio	1.5 (0.7-1.7)	5.5 (2.8-7.1) *	2.3 (1.3-7.1)	6.1 (4.3-17.2) *	4.7 (2.0-8.4) *	0.003
IL-13R α 2-positive area - %	1.5 (0.5-1.9)	19.6 (16.4-29.1) *	9.5 (8.1-13.7) *†	17.8 (5.6-23.3) *	17.6 (5.0-28.9) *	0.008
IL-33-positive cells per mm ²	225.4 (0.0-410.8)	716.0 (266.7-964.8)	759.0 (475.3-1206.3)	658.1 (423.9-1302.6)	679.3 (176.6-986.6)	0.18
Mucosal inflammation						
Neutrophils per mm ²	26.6 (18.9-40.1)	30.6 (22.7-38.6)	30.8 (18.5-51.5)	40.0 (16.4-109)	23.3 (15.9-37.1)	0.44
IL-13-positive cells per mm ²	2.8 (1.0-4.4)	8.9 (5.4-11.0) *	3.9 (2.9-6.5) †	9.1 (3.7-14.5) *	8.8 (3.6-12.7)	0.01
IL-17A-positive cells per mm ²	20.5 (13.5-37.6)	66.3 (59.8-115.4) *	63.4 (44.5-124.0) *	87.4 (74.2-111.1) *	77.7 (58.2-107.4) *	0.03
Intra-muscular mast cells per mm ²	0.0 (0.0-0.0)	47.1 (25.8-74.8) *	25.2 (6.3-50.2) *†	51.3 (32.2-88.4) *	48.4 (30.4-52.2) *§	0.0002
Airway remodelling						
SBM thickening - μ m	2.9 (2.4-3.0)	5.4 (3.7-6.8) *	3.1 (2.4-4.0) †	5.6 (5.2-6.5) *	3.6 (2.5-4.4) †	< 0.0001
ASM area - % of total biopsy area	6.6 (4.6-7.0)	18.0 (16.0-19.0) *	6.1 (4.2-8.0) †	20.3 (17.9-22.8) *	6.6 (5.2-9.5) †	0.0002

Abbreviations: BT, bronchial thermoplasty; MUC, mucin; IL, interleukin; IL-13R α 2, IL-13 receptor α 2; IFN, interferon; SBM, sub-epithelial basement membrane; ASM, airway smooth muscle.

Data are medians (25-75 IQR)

^a Kruskal-Wallis test

* $P < 0.05$, as compared to controls; † $P < 0.05$, between pre- and post-BT in each group; ‡ $P < 0.05$, between BT-responsive and -partial responsive severe asthmatics pre-BT; § $P < 0.05$, between BT-responsive and -partial responsive severe asthmatics post BT (Mann-Whitney U-test, or Wilcoxon matched-pairs test).

Bold denotes statistical significance

Table 3: Correlation analyses between clinical and histopathological parameters at 12 months in BT-responder severe asthmatics^a

Parameter	Exacerbations		Score on ACT		Score on AQLQ		Daily dose of OCS	
	rho	P value	rho	P value	rho	P value	rho	P value
Epithelium MUC5AC-positive area	- 0.47	0.009	0.54	0.002	0.43	0.02	- 0.17	0.45
Epithelium MUC5B-positive area	- 0.27	0.15	0.28	0.13	0.21	0.27	- 0.22	0.34
Epithelium IL-13R α 2-positive area - %	0.21	0.27	- 0.22	0.25	- 0.29	0.12	0.24	0.29
Epithelium IL-33-positive area - %	0.00	0.99	0.12	0.56	0.02	0.93	0.08	0.75
Epithelium IFN- α -positive area - %	- 0.39	0.03	0.13	0.53	0.13	0.52	- 0.27	0.28
Epithelium IFN- β -positive area - %	- 0.31	0.07	0.27	0.21	0.15	0.48	- 0.31	0.24
Mucosal eosinophils per mm ²	0.36	0.03	- 0.16	0.44	- 0.29	0.15	0.09	0.73
Mucosal neutrophils per mm ²	- 0.14	0.48	0.14	0.45	- 0.03	0.88	0.06	0.80
Mucosal IL-13-positive cells per mm ²	0.35	0.04	- 0.14	0.45	- 0.09	0.65	0.09	0.70
Mucosal IL-17A-positive cells per mm ²	- 0.03	0.86	0.09	0.62	- 0.20	0.28	0.16	0.56
Mucosal mast cells per mm ²	0.47	0.008	- 0.38	0.02	- 0.39	0.04	0.07	0.77
Intra-muscular mast cells per mm ² ASM	0.37	0.03	- 0.12	0.52	- 0.16	0.41	- 0.15	0.52
Mucosal IL-33-positive cells per mm ²	0.16	0.39	- 0.19	0.32	- 0.21	0.27	- 0.18	0.44
SBM thickening - μ m	0.43	0.02	- 0.57	0.001	- 0.56	0.001	0.32	0.12
ASM area - % of total biopsy area	0.82	< 0.0001	- 0.76	< 0.0001	- 0.70	< 0.0001	0.15	0.53

Abbreviations: BT, bronchial thermoplasty; ACT, asthma control test; AQLQ, asthma quality of life questionnaire; OCS, oral corticosteroids; MUC, mucin; IL, interleukin; IL-13R α 2, IL-13 receptor α 2; IFN, interferon; SBM, sub-epithelial basement membrane; ASM, airway smooth muscle.

^a Spearman's rank-order method and the Benjamini and Hochberg correction (n = 15 BT-responder severe asthmatics, before and 12 months after BT)

Bold denotes statistical significance

Table E1: Characteristics of the severe asthmatics before, 3 and 12 months after BT

Parameter	Pre-BT	3 months post-BT	12 months post-BT	P value ^a
Number of subjects	23	23	23	
Women – no. (%)	12 (52)	—	—	—
Age – years	47.8 ± 8.9	—	—	—
Caucasian origin	18 (78)	—	—	—
Body mass index - kg per m ²	30.0 ± 8.3	—	—	—
No. of never smokers/former smokers (%)	14/9 (61/39)	—	—	—
With history of atopy – no. (%)	13 (56)	—	—	—
Age of asthma onset – years	19.8 ± 17.5	—	—	—
Asthma duration – years	27.7 ± 17.5	—	—	—
Blood eosinophils per mm ³	240 (125-440)	160 (68-320)	125 (63-248)	0.20
Blood neutrophils per mm ³	8205 (5298-13320)	7830 (6850-10310)	7630 (5325-8975)	0.79
Total serum IgE (IU/milliliters)	244 (143-497)	192 (98-345)	134 (56-320)	0.40
Respiratory function				
Pre-bronchodilator FEV ₁ – Liters	2.04 ± 0.74	2.01 ± 0.71	1.98 ± 0.76	0.96
Post-bronchodilator FEV ₁ – Liters	2.24 ± 0.72	2.18 ± 0.68	2.28 ± 1.08	0.93
Pre-bronchodilator FEV ₁ – % of predicted	65.5 ± 18.6	64.5 ± 18.0	63.4 ± 19.9	0.93
Post-bronchodilator FEV ₁ – % of predicted	71.6 ± 19.5	70.4 ± 18.5	69.9 ± 20.5	0.96
Pre-bronchodilator FEV ₁ /FVC – % of predicted	61.3 ± 11.1	62.3 ± 14.0	60.0 ± 13.1	0.82
Post-bronchodilator FEV ₁ /FVC – % of predicted	61.3 ± 11.9	63.8 ± 13.5	60.5 ± 13.9	0.71
Reversibility to β2-agonists – milliliters	265 ± 254	191 ± 164	258 ± 273	0.67
Treatments				
On long-acting β2-agonists – number (%)	23 (100)	21 (91)	23 (100)	0.13
Daily dose of ICS – μg of beclomethasone equivalents	2261 ± 915	2043 ± 878	1774 ± 338	0.11
On maintenance use of OCS – no. (%)	22 (96)	14 (61)	15 (64)	0.02
Daily dose of oral prednisone – mg	29.4 ± 17.3	18.6 ± 12.8 *	18.1 ± 19.7 *	0.03
On anti-leukotrienes – no. (%)	9 (39)	11 (48)	10 (43)	0.35
On long-acting muscarinic antagonists – no. (%)	7 (30)	6 (26)	8 (35)	0.14
Asthma control				
With uncontrolled asthma – no. (%)	23 (100)	10 (43) *	11 (48) *	< 0.0001
Score on ACT	6.3 ± 1.8	14.1 ± 5.3 *	13.8 ± 5.8 *	< 0.0001
Score on AQLQ	1.8 ± 0.7	3.3 ± 1.4 *	3.7 ± 1.8 *	< 0.0001
Annual rate of severe exacerbations requiring OCS bursts	10.5 ± 1.2	0.9 ± 0.3 *	1.7 ± 0.3 *†	< 0.0001
Annual rate of hospitalisations for asthma	2.5 ± 0.7	0.1 ± 0.1 *	0.3 ± 0.1*	< 0.0001

Abbreviations: BT, bronchial thermoplasty; BMI, body mass index; IU, international units; FEV₁, Forced Expiratory Volume in One second; FVC, Forced Vital Capacity; ICS, inhaled corticosteroids; OCS, oral corticosteroids; ACT, asthma control test; AQLQ, asthma quality of life questionnaire.

Data are n (%), or means ± SD, or medians (interquartile range). Results on annual rates of severe exacerbations and hospitalisations for asthma are expressed as means ± SE

^a ANOVA or Kruskal-Wallis test of χ^2 test

* $P < 0.05$, as compared to pre-BT, † $P < 0.05$ as compared to 3 months post-BT (Student's t test for paired value, or Wilcoxon matched-pairs rank test, or Fisher test- 2-tailed, or Poisson test)

Bold denotes statistical significance

Table E2. Primary antibodies used for immunohistochemistry

Primary antibody	Isotype	Clone	Dilution	Manufacturer
Anti-MUC5AC	Mouse IgG1 κ	45M1	1:500	Invitrogen
Anti-MUC5B	Rabbit IgG	H-300	1:200	Santa Cruz Biotechnology
Anti-eosinophil major basic protein	Rabbit IgG	Polyclonal	1:50	Abcam
Anti-neutrophil elastase	Mouse IgG1 κ	NP57	1:100	Dako
Anti-mast cell tryptase	Mouse IgG1 κ	AA1	1:50	Dako
Anti-IL-17A	Goat IgG	Polyclonal	1:100	R&D Systems
Anti-IL-13	Rabbit IgG	Polyclonal	1:200	Abcam
Anti-IL-13R α 2	Goat IgG	Polyclonal	1:20	R&D Systems
Anti-IL-33	Goat IgG	Polyclonal	1:100	R&D Systems
Anti-IFN- α	Mouse IgG2b κ	F-7	1:10	Santa Cruz Biotechnology
Anti-IFN- β	Rabbit IgG	Polyclonal	1:2000	Life Technologies
Anti-smooth muscle α -actin	Mouse IgG2a	1A4	1:200	Sigma Aldrich

Abbreviations: MUC, mucin; IL, interleukin; IL-13R α 2, IL-13 receptor α 2; IFN, interferon.

Table E3: Airway inflammation and remodelling in controls and severe asthmatics before and 3 months after BT

Parameter	Controls	Severe asthmatics		<i>P</i> value ^a
		Pre-BT	3 months post-BT	
Number of subjects	10	23	23	-
Epithelium alterations				
MUC5AC-positive area - %	7.2 (2.3-12.2)	18.6 (13.0-27.5) *	24.4 (18.2-33.2) *†	0.001
MUC5B-positive area - %	3.0 (1.6-5.4)	3.4 (2.2-5.5)	7.6 (3.5-12.0) †	0.02
MUC5AC to MUC5B ratio	1.5 (0.7-1.7)	5.0 (1.9-12.4) *	3.0 (1.3-11.2)	0.01
IL-13Rα2-positive area - %	1.5 (0.5-1.9)	19.3 (5.4-33.6) *	10.5 (6.5-25.0) *	0.001
IL-33-positive cells per mm ²	225.4 (0.0-810.8)	682.7 (273.7-1103.7)	743.5 (300.2-1227.3)	0.07
IFN-α-positive area - %	0.2 (0.2-1.6)	6.4 (0.5-13.8) *	10.2 (2.7-25.1) *†	0.01
IFN-β-positive area - %	1.6 (0.9-2.5)	9.3 (5.7-24.9) *	20.2 (8.5-35.7) *	0.004
Airway inflammation				
Eosinophils per mm ²	0.5 (0.0-2.7)	11.6 (4.1-30.2) *	7.9 (0.0-16.6) *	0.01
Neutrophils per mm ²	26.6 (18.9-40.1)	30.6 (20.4-83.2)	27.9 (18.5-57.1)	0.47
IL-13-positive cells per mm ²	2.8 (1.0-4.4)	7.1 (5.3-10.4) *	4.9 (2.9-5.8) †	0.002
IL-17A-positive cells per mm ²	20.5 (13.5-37.6)	82.1 (64.1-115.4) *	74.9 (44.5-111.0) *	0.006
Mast cells per mm ²	47.7 (37.7-84.6)	43.8 (27.9-58.7)	21.0 (11.5-45.3) †	0.004
Intra-muscular mast cells per mm ²	0.0 (0.0-0.0)	51.3 (25.9-77.1) *	33.2 (12.6-55.8) †	0.004
IL-33-positive cells per mm ²	0.0 (0.0-0.3)	21.0 (7.5-51.5) *	14.0 (1.9-44.4) *	0.01
Airway remodelling				
SBM thickening - μm	2.9 (2.4-3.0)	5.5 (4.4-6.8) *	3.3 (2.4-4.1) †	< 0.0001
ASM area - % of total biopsy area	6.6 (4.6-7.0)	18.3 (16.0-23.0) *	5.6 (4.4-8.0) †	< 0.0001

Abbreviations: BT, bronchial thermoplasty; IL, interleukin; IL-13Rα2, IL-13 receptor α2; IFN, interferon; SBM, sub-epithelial basement membrane; ASM, airway smooth muscle.

Data are medians (25-75 % interquartile range).

^a Kruskal-Wallis test

* *P* < 0.05, as compared to controls (Mann-Whitney U-test); † *P* < 0.05, as compared to severe asthmatics pre-BT (Wilcoxon matched-pairs rank test)

Bold denotes statistical significance

Table E4: Correlation analyses between clinical and histopathological parameters before at 12 months in BT-partial responder severe asthmatics ^a

Parameter	Exacerbations		Score on ACT		Score on AQLQ		Daily dose of OCS	
	rho	P value	rho	P value	rho	P value	rho	P value
Epithelium MUC5AC-positive area	- 0.36	0.17	0.34	0.19	0.24	0.29	- 0.31	0.24
Epithelium MUC5B-positive area	- 0.06	0.83	0.11	0.67	- 0.01	0.98	0.07	0.80
Epithelium IL-13R α 2-positive area - %	- 0.18	0.50	0.23	0.39	0.20	0.47	0.29	0.28
Epithelium IL-33-positive area - %	- 0.06	0.83	0.20	0.50	0.41	0.15	- 0.05	0.88
Epithelium IFN- α -positive area - %	- 0.12	0.68	- 0.19	0.51	- 0.23	0.43	0.34	0.24
Epithelium IFN- β -positive area - %	0.15	0.65	0.00	0.99	- 0.08	0.80	0.26	0.41
Mucosal eosinophils per mm ²	0.34	0.23	- 0.19	0.51	- 0.26	0.37	0.17	0.55
Mucosal neutrophils per mm ²	0.22	0.42	- 0.17	0.53	- 0.11	0.69	0.18	0.51
Mucosal IL-13-positive cells per mm ²	0.14	0.60	0.16	0.53	0.25	0.29	- 0.20	0.45
Mucosal IL-17A-positive cells per mm ²	0.13	0.63	- 0.05	0.85	- 0.14	0.60	0.10	0.72
Mucosal mast cells per mm ²	0.19	0.51	0.03	0.93	- 0.17	0.56	0.16	0.58
Intra-muscular mast cells per mm ² ASM	0.20	0.54	- 0.28	0.38	- 0.39	0.21	0.07	0.83
Mucosal IL-33-positive cells per mm ²	- 0.24	0.38	0.12	0.65	0.09	0.74	- 0.04	0.89
SBM thickening - μ m	0.78	0.0003	- 0.40	0.12	- 0.31	0.24	0.16	0.58
ASM area - % of total biopsy area	0.75	0.0009	- 0.30	0.26	- 0.15	0.59	0.22	0.41

Abbreviations: BT, bronchial thermoplasty; ACT, asthma control test; AQLQ, asthma quality of life questionnaire; OCS, oral corticosteroids; MUC, mucin; IL, interleukin; IL-13R α 2, IL-13 receptor α 2; IFN, interferon, SBM, sub-epithelial basement membrane; ASM, airway smooth muscle.

^a Spearman's rank-order method and the Benjamini and Hochberg correction (n = 8 BT-partially responder severe asthmatics, before and 12 months after BT)

Bold denotes statistical significance

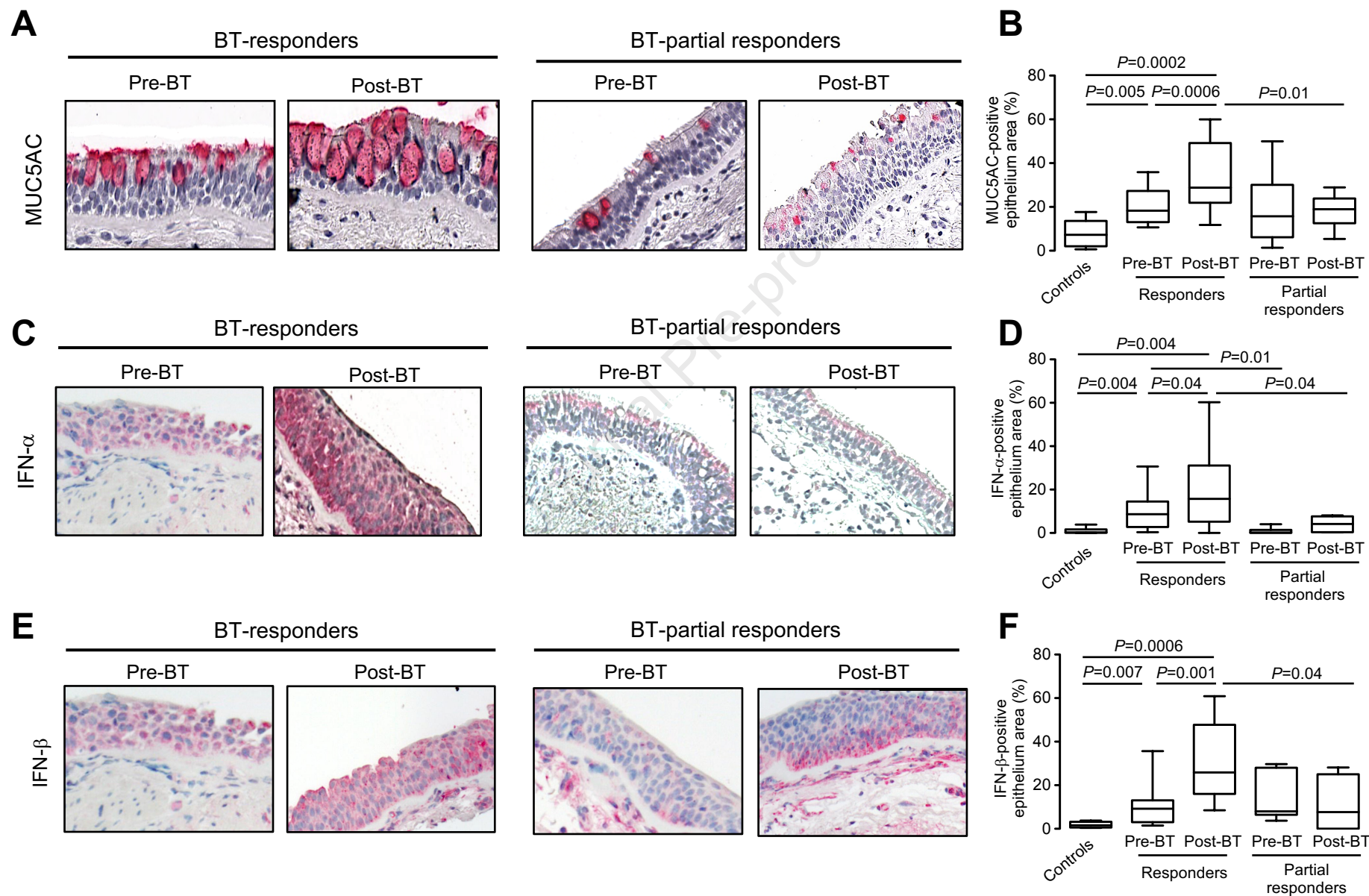
Fig. 1

Fig. 2