Journal of ASTHMA

http://informahealthcare.com/jas
ISSN: 0277-0903 (print), 1532-4303 (electronic)

J Asthma, Early Online: 1–6
© 2014 Informa Healthcare USA, Inc. DOI: 10.3109/02770903.2014.975357

informa healthcare

ORIGINAL ARTICLE

Characteristics of eosinophilic and non-eosinophilic asthma during treatment with inhaled corticosteroids

Toshiki Furukawa, MD[1], Takuro Sakagami, MD, PhD[1], Toshiyuki Koya, MD, PhD[1], Takashi Hasegawa, MD, PhD[2], Hidenori Kawakami, MD, PhD[1], Yosuke Kimura, MD[1], Yoshifumi Hoshino, MD[1], Hirotaka Sakamoto, MD[1], Kenjiro Shima, MD[1], Keisuke Tsukioka, MD[1], Mio Toyama, MD[1], Masachika Hayashi, MD, PhD[1], Hiroshi Kagamu, MD, PhD[1], Ei-ichi Suzuki, MD, PhD[2], and Ichiei Narita, MD, PhD[1]

[1]*Division of Respiratory Medicine, Department of Homeostatic Regulation and Development, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, and* [2]*Department of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan*

J Asthma Downloaded from informahealthcare.com by University of Liverpool on 12/12/14
For personal use only.

Abstract

Objective: Eosinophilic inflammation in the respiratory tract is a hallmark of bronchial asthma. In naïve cases, the inflammatory profile is associated with disease severity and reactivity to inhaled corticosteroids (ICS). Sustained airway eosinophilia has been reported during ICS treatment. However, the immunological characteristics of these cases are not known and it is unclear if this situation contributes to asthma control. This study was performed to determine the answer of these questions. *Methods*: To compare phenotypes of eosinophilic and non-eosinophilic asthma (EA and NEA, respectively) under ICS treatment, clinical data were obtained from asthmatic subjects ($n = 22$) and healthy controls ($n = 10$), and the leukocyte compositions of induced sputum and peripheral blood were determined. T lymphocyte profiles in systemic blood were assessed by flow cytometry. *Results*: A higher frequency of emergency room visits was observed in the NEA group, which had a higher neutrophil count relative to the total inflammatory cell population in induced sputum than the EA group (59.5 versus 36.6%; $p < 0.01$). The fraction of helper T (Th)17 lymphocytes as well as the ratio of Th17 to regulatory T cells (Treg) in the peripheral blood was higher in the NEA than in the EA group (0.24 versus 0.13; $p < 0.05$). *Conclusions*: Th17 were more prevalent than Treg cells in the peripheral blood of NEA patients under ICS treatment, corresponding to neutrophil-dominant airway inflammation and a severe asthmatic phenotype. Thus, an imbalance in Th17/Treg may be associated with the pathogenesis of NEA in patients undergoing ICS treatment.

Keywords

Eosinophilic asthma, inhaled cortico-steroid, non-eosinophilic asthma, regulatory T cell, Th17 cell

History

Received 22 April 2014
Revised 2 October 2014
Accepted 6 October 2014
Published online 11 November 2014

Introduction

Asthma is a complex disease for which precise underlying mechanisms are difficult to identify due to its heterogeneity. Eosinophilic and non-eosinophilic asthma (EA and NEA, respectively) have distinct airway inflammatory profiles characterized by the presence or absence, respectively, of airway eosinophilia. The asthma type is typically diagnosed before an anti-inflammatory, inhaled corticosteroid (ICS) treatment is prescribed. A literature review reveals that fewer than 50% of asthma cases are attributable to eosinophilic airway inflammation [1–3]. Phenotypic differences between the two types of asthma have been well described, and most treatment strategies have focused on allergic/eosinophilic asthma.

Address for correspondence: Takuro Sakagami, Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Niigata, Japan. Tel: +81-25-2272200. Fax: +81-25-2270775. E-mail: stakuro@med.niigata-u.ac.jp

It is well known that NEA is characterized by poor response to ICS, which is associated with impaired asthma control [4]. In addition, in a recent report, a group of patients classified as NEA exhibited an airway reaction to albuterol, indicating that they had airway reversibility [5]. From the point of view of airway inflammation, 28% of these patients exhibited continuous airway eosinophilia during the observation period for reasons that were unclear. The asthma control status in NEA patients upon ICS treatment has never been previously described.

There is increasing evidence that multiple mechanisms contribute to the enhanced bronchial activity and reversible airway obstruction that characterize asthma, and immunologic profiles may be particularly relevant to asthma phenotypes. Regulatory T (Treg) cells have an anti-inflammatory role and maintain immunologic self-tolerance, while helper T (Th)17 cells play critical roles in the development of autoimmunity and allergic reactions [6–8] as well as NEA [9–13]. The elevated expression of interleukin (IL)-17 in the airway – which is correlated with airway hyper-responsiveness and

RIGHTSLINK

neutrophilia – has been reported in asthma [9,14], and in conjunction with Th17 may contribute to therapeutic response by inhibiting the development of tolerance in an ovalbumin-sensitized mouse model of asthma [15]. A disequilibrium between Th17 and Treg cells may therefore be a factor in inflammatory and allergic diseases [16–19], and, as such, the determination of an immunologic profile is critical not only for elucidating pathophysiological mechanisms but also for devising novel therapeutic strategies to treat bronchial asthma.

The aim of this study was to characterize the features of asthmatic patients undergoing ICS treatment, including the asthma control status and immunologic profiles of the airway and peripheral blood.

Materials and methods

Subjects

The study was performed according to the principles expressed in the Declaration of Helsinki and with the approval of the Ethics Committee at the School of Medicine of Niigata University (#1027). Physician-diagnosed, adult asthmatic outpatients ($n = 22$) treated at Niigata University Hospital were recruited and gave their written consent for participation in the study. All patients were treated with ICS. Age- and gender-matched healthy controls ($n = 10$) were also recruited. All subjects were lifetime non-smokers or ex-smokers with less than 5 pack-years, and their asthma status met the criteria for controlled or partly controlled as defined by the Global Initiative for Asthma (GINA). Subjects with uncontrolled asthma, asthma exacerbation, infectious diseases, other lung diseases (interstitial lung disease, cancer or autoimmune disorders), who were taking systemic corticosteroids 4 weeks prior to the start of the study, receiving treatment with immune-modulating drugs, or pregnant were excluded.

Clinical assessment of asthma

The Asthma Control Test (ACT) [20,21] was performed by interview when patients visited the hospital to be extracted blood for this study. Clinical data from the preceding year, such as history of treatment and asthma exacerbation, were obtained from medical records. The use of any substance for controlling asthma, such as long-acting beta 2-agonist and leukotriene receptor antagonist, was also determined from medical records. The fraction of exhaled nitric oxide (F_ENO) was measured using a nitrogen oxide analyzer (NA-623N; Kimoto Electric Co. Ltd., Osaka, Japan). The pulmonary function test was administered with a spirometer (Chestgraph Jr. HI-101; CHEST Ltd., Tokyo, Japan) following the manufacturer's protocol. Airway reversibility was determined as the forced expiratory volume in 1 s (FEV1) ratio from before to after procaterol inhalation. The fraction of eosinophils and neutrophils in the peripheral blood, and serum concentrations of immunoglobulin E (IgE) and 12 allergen-specific IgEs (multiple fungus antigen, house dust, *Dermatophagoides pteronyssinus*, *D. farina*, orchard grass, ragweed, wormwood, Japanese cedar pollen, cockroach, cat dander, dog dander and moth) were measured. High-sensitivity C-reactive protein (hs-CRP) levels in serum were measured by latex nepherometry (BML Inc., Tokyo, Japan).

Evaluation of airway inflammation

Induced sputum and peripheral blood were collected at the same time to evaluate inflammatory cell content. Sputum induction was performed according to the methods previously validated for consistency and quality [22]. Briefly, after inhalation of 20 μg procaterol, subjects inhaled 5% hypertonic saline solution for up to 15 min. Sputum samples were processed, and the number of various cell types (eosinophils, neutrophils, basophils, macrophages, epithelial cells and lymphocytes) was calculated as a percentage of non-squamous cells. Based on previous reports [4,23–25], subjects with eosinophil counts of $\geq 2\%$ and $<2\%$ were defined as the EA and NEA groups, respectively.

Analysis of systemic T cell profiles

After whole blood phlebotomy, peripheral blood mononuclear cells (PBMCs) were collected by density gradient centrifugation in a cell preparation tube (Becton Dickinson, Franklin Lakes, NJ). PBMCs were stimulated with 50 ng/ml phorbol myristate acetate, 1 μg/ml ionomycin and 10 μg/ml brefeldin A (all from Sigma, St. Louis, MO) at 37 °C for 4 h. After labeling with a fluorescein isothiocyanate-conjugated anti-human antibody against the T cell marker cluster of differentiation (CD)4 (Beckman Coulter, Brea, CA), cells were fixed and permeabilized, then incubated for 8 h at −4 °C in the dark with R-phycoerythrin-conjugated anti-human antibodies against IL-17 A, interferon (IFN)-γ, IL-4 or Foxp3 (all from eBioscience, San Diego, CA), to label Th17, Th1, Th2 and Treg cells, respectively. Fluorescence intensity was quantified using a FACSCalibur flow cytometer and analyzed with CellQuest software (BD Biosciences, San Joe, CA). Results are expressed as a fraction of the total $CD4^+$ cell population.

Statistical analysis

Statistical analysis was performed with Sigmaplot 12 software (Systat Software Inc., San Jose, CA). The χ^2 and t tests for parametric continuous data and the Kruskal–Wallis test for non-parametric continuous data were used to compare measurements for each phenotype. A p value <0.05 was considered statistically significant.

Results

Clinical manifestations of EA and NEA

The clinical characteristics of the study subjects in the EA and NEA groups are summarized in Table 1. There were no differences in age, sex, body mass index, duration of asthma, positive numbers of 12 allergen specific IgEs, ACT score or asthma control status according to GINA criteria. All subjects were treated with the same dose of ICS in the year preceding the study. However, the NEA group had a higher (albeit non-significant) frequency of systemic steroid burst usage (50 versus 20%) (Figure. 1a) and a higher number of emergency room visits (40 versus 0%; $p = 0.027$)

J Asthma Downloaded from informahealthcare.com by University of Liverpool on 12/12/14
For personal use only.

RIGHTSLINK

J Asthma Downloaded from informahealthcare.com by University of Liverpool on 12/12/14
For personal use only.

(Figure 1b) in the year preceding the study than the EA group, indicating greater disease severity.

Leukocyte profiles and systemic inflammation

In the induced sputum of the NEA group, eosinophil counts were lower (0.5 versus 22.6%; $p<0.001$) and neutrophil counts were higher (59.5 versus 36.5%; $p=0.012$), than in the EA group (Figure 2a and b). Eosinophil counts were also lower in the peripheral blood of NEA patients [2.4% (146 ml^{-1}) versus 9.2% (570 ml^{-1}); $p<0.01$] (Figure 2d and f). The neutrophil compositions and counts in systemic blood were similar in all patients (Figure 2c and e). F_ENO was higher in the EA than in the NEA group (90.4 versus 38.5; $p=0.037$) (Figure 3a), corresponding to the higher representation of eosinophils in the induced sputum of EA subjects. Serum Hs-CRP levels did not differ between the two types of asthma (Figure 3b).

Table 1. Characteristics of study subjects.

	NEA	EA	
Number of subjects	12	10	
Age	40.5 (14.1)	55.8 (18.7)	N.S.
Sex (F/M)	8/4	8/2	N.S.
BMI	25.3 (8.2)	23.2 (2.1)	N.S.
Age of onset	30 (12.7)	45.7 (24.4)	N.S.
Disease duration (years)	10.5 (11.9)	10.3 (11.7)	N.S.
IgE concentration in serum (IU/L)	337 (403)	460 (409)	N.S.
Positive numbers of specific IgE	2.9 (2.2)	2.1 (1.8)	N.S.
Asthma treatment			
ICS (μg fluticasine equivalent/day)	346 (147)	419 (208)	N.S.
LABA induction rate (%)	75	80	N.S.
LTRA induction rate (%)	50	40	N.S.
Asthma control status			
ACT score	23.8±1.6	23.8±1.9	N.S.
Levels of asthma control by GINA			
Uncontrolled	0	0	
Partly controlled	6	3	
Controlled	6	7	N.S.
FVC (L)	3.45 (0.89)	3.15 (0.82)	N.S.
FEV1 (L)	2.89 (0.92)	2.48 (0.71)	N.S.
Predicted FEV1 (%)	96.7 (16.6)	103.5 (13.8)	N.S.
Airway reversibility (%)	3.8 (6.8)	2.7 (2.4)	N.S.

Numbers in parentheses indicate SDs.

ACT, asthma control test; BMI, body mass index; EA, eosinophilic asthma; FVC, forced volume capacity; FEV1, forced expiratory volume in 1 s; F/M, female/male; GINA, global initiative for asthma; ICS, inhaled corticosteroids; IgE, immunoglobulin E; LABA, long-acting beta2-agonists; LTRA, leukotriene receptor antagonist; NEA, non-eosinophilic asthma; N.S., not significant

Th lymphocyte fraction in PBMCs

The fraction of $CD4^+IL17A^+$ lymphocytes, defined as Th17 cells, was higher in the NEA group than in healthy controls, but similar among NEA and EA patients (Figure 4a). No differences were observed in the $CD4^+Foxp3^+$ Treg cell population between the three groups (Figure 4b). The ratio of $CD4^+IL17A^+/CD4^+Foxp3^+$ cells was higher in the NEA group, indicating a predominance of Th17 cells in these patients (Figure 4c). In contrast, the proportion of $CD4^+IFN\text{-}\gamma^+$ Th1 and $CD4^+IL\text{-}4^+$ Th2 cells was similar among all asthmatic patients (data not shown), demonstrating that the prevalence of Th17 lymphocytes in the NEA group was independent of theTh1/Th2 cell balance in peripheral blood.

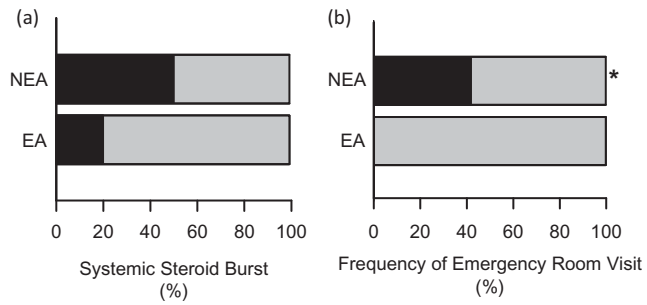

Figure 1. Asthma control in the year preceding the study in non-eosinophilic asthma (NEA) and eosinophilic asthma (EA) cases. (a) Systemic steroid bursts were used more frequently by the NEA than the EA group, but the difference was non-significant. (b) Emergency room visits were more frequent in the NEA than in the EA group. *$p<0.05$. The black bar represents the fraction of subjects with each type of asthma relative to the total patient population (grey bars).

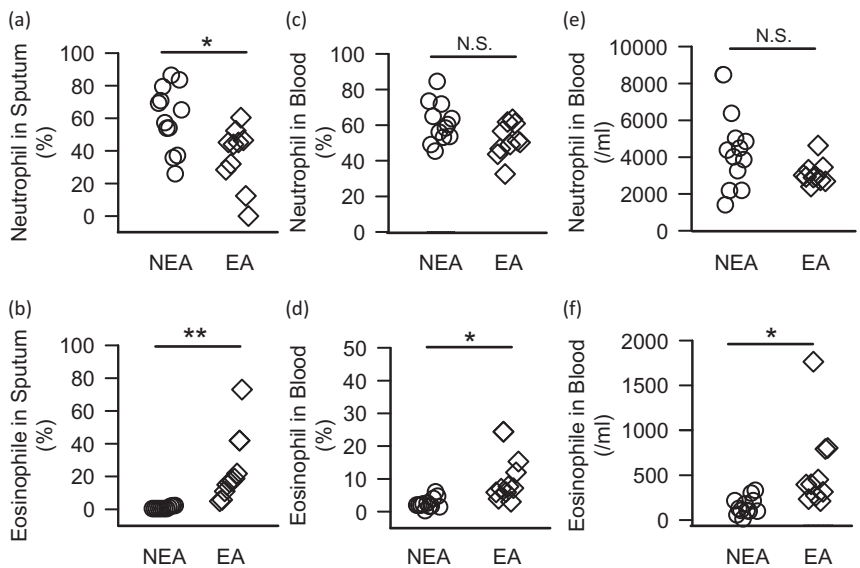

Figure 2. Airway and blood leukocyte profiles in non-eosinophilic asthma (NEA) and eosinophilic asthma (EA) cases.
(a) Neutrophil composition in the sputum were higher in the NEA than in the EA group. (b) Eosinophil composition in the sputum were lower in NEA group than in the EA group, since by definition, NEA and EA subjects were classified as having eosinophil counts of <2% or ≥2% in sputum, respectively. (c–f) Systemic (blood) neutrophil counts were similar between patient groups, but blood eosinophil counts were higher in the EA group. *$p<0.05$; **$p<0.01$.

RIGHTSLINK

J Asthma Downloaded from informahealthcare.com by University of Liverpool on 12/12/14
For personal use only.

Discussion

This study examined airway inflammatory profiles in ICS-treated asthmatic patients to determine whether immune status was associated with asthma control. Previous studies have found that patients who respond well to ICSs show improvement in eosinophilic inflammation in the airway tract upon treatment, but in resistant cases, airway eosinophilia persists even after receiving ICS [3,26–31]. In addition, the non-eosinophilic type of asthma is usually more severe and refractory to ICS treatment [5,25]. Here, we reported that an exacerbation of asthma was observed in the NEA group during ICS treatment (Figure 1), indicating that the asthma was poorly controlled. Therefore, the NEA group presents a clinically important phenotype because a conventional treatment approach failed to improve their condition.

There are several possible reasons for the elevated sputum neutrophil level in the NEA group that was observed upon ICS treatment. First, an eosinophilic airway inflammation may have been absent, whereas neutrophilic inflammation may have existed initially. Although the presence of chronic obstructive pulmonary disease – characterized by neutrophilic airway inflammation – should be considered, it can be excluded in our patients because only non-smokers or ex-smokers with less than 5 pack-years were selected for the study. Second, eosinophilic inflammation may have been improved by ICSs, with a corresponding increase in neutrophil-related respiratory tract inflammation. These two factors could lead to a diagnosis of NEA, although there could be other contributing factors that would vary for each individual. In addition, an infectious disease harbored by the patient could be a cause of inflammation; however, this was a remote possibility, since EA and NEA patients showed no differences in blood neutrophil composition and systemic hs-CRP levels (Figures 2 and 3), both of which would be elevated in the case of an infection. Taken together, these results indicate that asthma is less controlled in cases of NEA that present a neutrophilic airway inflammation profile during ICS treatment. These subjects may be easily determined by measuring FeNO [32–34].

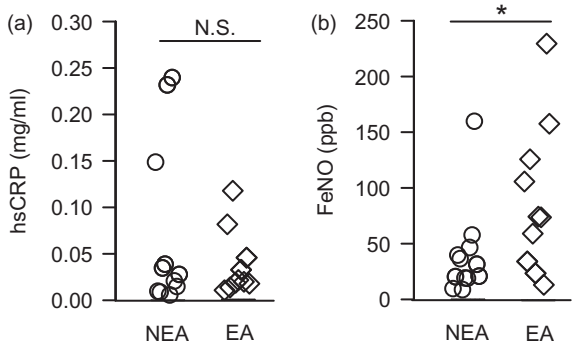

Figure 3. Airway and systemic inflammation markers in non-eosinophilic asthma (NEA) and eosinophilic asthma (EA) cases. (a) The fraction of exhaled nitric oxide (FeNO) was lower in the NEA than in the EA group, reflecting the airway eosinophilia present in the latter. (b) Expression levels of high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, were similar between the two types of asthma. $*p < 0.05$.

TThe Th17 population in systemic blood was significantly increased in the NEA group relative to healthy subjects in the present study (Figure 4), resulting in a higher Th17/Treg ratio. The observation that neutrophil was recruited to the airway in significant numbers and was also predominant in the blood suggests a reason for which the Th17 population was increased in NEA. Airway neutrophilia has been well characterized in the mouse Th17 cell adoptive transfer model [35]; human Th17 lymphocytes directly recruit neutrophils via granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-α and IFN-γ release. Moreover, activated neutrophils enhance chemotaxis in Th17 cells [36]. These findings suggest a possible association between circulating Th17 lymphocytes and NEA with systemic inflammation.

Th17 lymphocytes and activated neutrophils interact in autoimmunity and allergic reactions, and several studies indicate that they may be important in the attenuation of allergic airway inflammation in asthma [11,37–39]. The expression of FOXp3 – a transcription factor required for Treg cell differentiation – in PBMCs was lower in asthmatic patients not receiving ICS than those treated with ICS [40,41]. According to these reports, ICSs induce FOXp3 expression and thereby improve the immunologic imbalance in asthma. As such, NEA and EA groups may respond differently to ICSs, with ICS-induced upregulation of FoxP3 levels potentially attenuated in NEA subjects through an unknown mechanism. Indeed, although the difference was not statistically significant, FoxP3 expression in $CD4^+$ lymphocytes was lower in NEA patients relative to other groups (Figure 4), corresponding to the increased Th17/Treg ratio. Immune responses in asthma, particularly in allergic phenotypes, are mediated by a balance between Th17 and Treg cell populations [42], and a lower ratio provides beneficial effects for airway inflammation in house dust mite-sensitized mice [43]. Thus, a systemic imbalance of Th17/Treg could be a characteristic feature of NEA during ICS treatment.

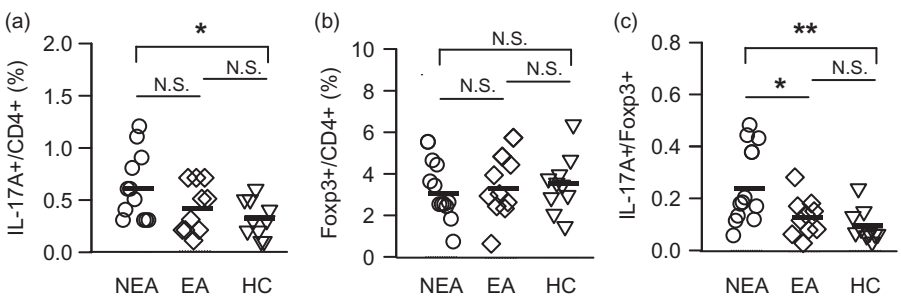

Figure 4. Immunologic profiles in non-eosinophilic asthma (NEA), eosinophilic asthma (EA) and healthy controls (HC). (a) $IL17A^+$ Th17 represented a higher fraction of the total $CD4^+$ peripheral blood mononuclear cell population in the NEA group than in the EA or control groups. (b) The fraction of $Foxp3^+$ Treg cells was similar between groups. (c) An imbalance in the ratio of Th17 to Treg cells was observed in the NEA relative to the other groups. $*p < 0.05$; $**p < 0.01$; *N.S.* non-significant.

RIGHTSLINK

One limitation of this study is that because it concerned subjects who received ICS treatment, inflammatory profiles of asthmatic subjects before ICS induction were not analyzed. These two situations – before or during ICS treatment – must be discussed separately. Additional studies are needed to determine whether the inflammatory profiles observed in this study reflect events that occur initially; the results can potentially lead to an improvement in asthma treatment approaches.

Conclusion

There are no reports to date of an association between the control of asthma and immune balance during ICS treatment. The results of this study suggest that a Th17/Treg imbalance in the peripheral blood may contribute to asthma status, which was poorly controlled in NEA relative to EA cases. Given that the present investigation was limited by a relatively small sample size, future studies will include a larger study population and a closer examination of the mechanism of immunologic induction by ICS, with the objective of developing more effective asthma management and treatment strategies.

Acknowledgements

The authors thank Asako Takiguchi for expert help in the physiological examinations.

Declaration of interest

The authors declare that they have no competing interests.

J Asthma Downloaded from informahealthcare.com by University of Liverpool on 12/12/14
For personal use only.

References

1. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001; 119:1329–1336.
2. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet 1999;353: 2213–2214.
3. Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. Am J Respir Crit Care Med 1999;160):1532–1539.
4. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 2002;57:875–879.
5. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, Fahy JV. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. Am J Respir Crit Care Med 2012;185:612–619.
6. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol 2007;8:345–350.
7. Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. Allergol Int 2008;57:121–134.
8. Fouser LA, Wright JF, Dunussi-Joannopoulos K, Collins M. Th17 cytokines and their emerging roles in inflammation and auto-immunity. Immunol Rev 2008;226:87–102.
9. Bullens DM, Truyen E, Coteur L, Dilissen E, Hellings PW, Dupont LJ, et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? Respir Res 2006;7:135.
10. Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, Lemiere C, Hamid Q. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. J Allergy Clin Immunol 2009;123: 1185–1187.
11. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. Am J Respir Crit Care Med 2009;180:720–730.
12. Alcorn JF, Crowe CR, Kolls JK. TH17 cells in asthma and COPD. Annu Rev Physiol 2010;72:495–516.
13. Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 cells: new players in asthma pathogenesis. Allergy 2011;66: 989–998.
14. Barczyk A, Pierzchala W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. Respir Med 2003;97:726–733.
15. Kawakami H, Koya T, Kagamu H, Kimura Y, Sakamoto H, Yamabayashi C, Furukawa T, et al. IL-17 eliminates therapeutic effects of oral tolerance in murine airway allergic inflammation. Clin Exp Allergy 2012;42:946–957.
16. Wang W, Shao S, Jiao Z, Guo M, Xu H, Wang S. The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. Rheumatol Int 2012;32:887–893.
17. Eastaff-Leung N, Mabarrack N, Barbour A, Cummins A, Barry S. Foxp3+ regulatory T cells, Th17 effector cells, and cytokine environment in inflammatory bowel disease. J Clin Immunol 2010; 30:80–89.
18. Yang J, Chu Y, Yang X, Gao D, Zhu L, Wan L, Li M, et al. Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. Arthritis Rheum 2009;60:1472–1483.
19. Cheng X, Yu X, Ding YJ, Fu QQ, Xie JJ, Tang TT, Yao R, et al. The Th17/Treg imbalance in patients with acute coronary syndrome. Clin Immunol 2008;127:89–97.
20. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, Murray JJ, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol. 2004;113: 59–65.
21. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, Kosinski M, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol 2006;117:549–556.
22. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, Dolovich J, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;47: 25–29.
23. Spanevello A, Confalonieri M, Sulotto F, Romano F, Balzano G, Migliori GB, Bianchi A, et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. Am J Respir Crit Care Med 2000;162:1172–1174.
24. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med 2000;161:475–478.
25. Haldar P, Pavord ID. Noneosinophilic asthma: a distinct clinical and pathologic phenotype. J Allergy Clin Immunol 2007;119: 1043–14052; quiz 53–54.
26. Synek M, Beasley R, Frew AJ, Goulding D, Holloway L, Lampe FC, Roche WR, et al. Cellular infiltration of the airways in asthma of varying severity. Am J Respir Crit Care Med 1996; 154:224–230.
27. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax 1996;51: 496–502.
28. Lim S, Jatakanon A, John M, Gilbey T, O'Connor BJ, Chung KF, Barnes PJ. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999;159:22–30.
29. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000;161:9–16.
30. Tattersfield AE, Knox AJ, Britton JR, Hall IP. Asthma. Lancet 2002;360:1313–1322.
31. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J Allergy Clin Immunol 2004;113: 101–108.
32. Silkoff PE, Lent AM, Busacker AA, Katial RK, Balzar S, Strand M, Wenzel SE. Exhaled nitric oxide identifies the persistent

RIGHTSLINK

eosinophilic phenotype in severe refractory asthma. J Allergy Clin Immunol 2005;116:1249–1255.
33. Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. Thorax 2010;65:384–390.
34. Matsunaga K, Yanagisawa S, Hirano T, Ichikawa T, Koarai A, Akamatsu K, Sugiura H, et al. Associated demographics of persistent exhaled nitric oxide elevation in treated asthmatics. Clin Exp Allergy 2012;42:775–781.
35. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, Goldman SJ, et al. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. J Immunol 2007;179:7791–7799.
36. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, Cosmi L, et al. Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 2010;115:335–343.
37. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8–27.
38. Laan M, Cui ZH, Hoshino H, Lotvall J, Sjostrand M, Gruenert DC, Skoogh BE, et al. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. J Immunol 1999;162: 2347–2352.
39. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, McCormick M, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. Chest 2010; 138:1140–1147.
40. Provoost S, Maes T, van Durme YM, Gevaert P, Bachert C, Schmidt-Weber CB, Brusselle GG, et al. Decreased FOXP3 protein expression in patients with asthma. Allergy 2009;64:1539–1546.
41. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, Mantel PY, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol 2004;114:1425–1433.
42. Martin H, Taube C. Regulatory T cells and regulation of allergic airway disease. Am J Clin Exp Immunol 2012;1:166–178.
43. McGee HS, Edwan JH, Agrawal DK. Flt3-L increases CD4+CD25+ Foxp3+ICOS+ cells in the lungs of cockroach-sensitized and -challenged mice. Am J Respir Cell Mol Biol 2010;42:331–340.

J Asthma Downloaded from informahealthcare.com by University of Liverpool on 12/12/14
For personal use only.

RIGHTSLINK