Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms

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Background: Asthma is a chronic inflammatory disease involving diverse cells and mediators whose interconnectivity and relationships to asthma severity are unclear. Objective: We performed a comprehensive assessment of T_{H}{}^{17} cells, regulatory T cells, mucosal-associated invariant T (MAIT) cells, other T-cell subsets, and granulocyte mediators in asthmatic patients. Methods: Sixty patients with mild-to-severe asthma and 24 control subjects underwent detailed clinical assessment and provided induced sputum, endobronchial biopsy, bronchoalveolar lavage, and blood samples. Adaptive and invariant T-cell subsets, cytokines, mast cells, and basophil mediators were analyzed. Results: Significant heterogeneity of T-cell phenotypes was observed, with levels of IL-13–secreting T cells and type 2 cytokines increased at some, but not all, asthma severities. T_{H}{}^{17} cells and γδ T cells, proposed drivers of neutrophilic inflammation, were not strongly associated with asthma, even in severe neutrophilic forms. MAIT cell frequencies were strikingly reduced in both blood and lung tissue in relation to corticosteroid therapy and vitamin D levels, especially in patients with severe asthma in whom bronchoalveolar lavage regulatory T-cell numbers were also reduced. Bayesian network analysis identified complex relationships between pathobiologic and clinical parameters. Topological data analysis identified 6 novel clusters that are associated with diverse underlying disease mechanisms, with increased mast cell mediator levels in patients with severe asthma both in its atopic (type 2 cytokine–high) and nonatopic forms. Conclusion: The evidence for a role for T_{H}{}^{17} cells in patients with severe asthma is limited. Severe asthma is associated with a striking deficiency of MAIT cells and high mast cell mediator levels. This study provides proof of concept for disease mechanistic networks in asthmatic patients with clusters that could inform the development of new therapies. (J Allergy Clin Immunol 2015;136:323-33.)

Key words: Asthma, T lymphocytes, cytokines, mast cells, phenotype, endotype, regulatory T, T_{H}{}^{17}, T_{H}{}^{2}, mucosal-associated invariant T-cell

Asthma is characterized by airways inflammation and remodeling. Based on initial studies in animal models1 and human T-cell clones5 and bronchoscopic studies in patients with mild steroid-naïve asthma,3 it has been viewed as a disease driven by activated T_{H}{}^{2} cells producing the type 2 interleukins IL-4, IL-5, and IL-13. These cytokines are believed to orchestrate the functions of mast cells, eosinophils, and IgE-producing B cells/plasma cells. This concept has been challenged with increasing recognition of considerable heterogeneity of asthma mechanisms and definable patient subpopulations associated with immunopathology that cannot be explained by T_{H}{}^{2} inflammation alone.6-11 Discoveries of novel T-cell subsets, notably anti-inflammatory regulatory T (Treg) cells T_{H}{}^{2} and proinflammatory IL-17–secreting (T_{H}{}^{17}) and invariant natural killer T cells, which are implicated in asthma pathogenesis on the basis of studies in animal models and limited evidence in human asthma, have added complexity to our understanding of immunoregulation. Recent studies have revived interest in mast cells, a subject of intense research in the 1980s and early 1990s but not widely seen as candidate targets, possibly because of limited evidence of their role in severe disease.12-18

Most studies of asthma pathobiology to date have focused on individual or limited numbers of inflammatory cell types, making it difficult to appreciate the cell-cell interactions within complex networks that characterize inflammatory diseases, such as asthma. Therefore we have undertaken a comprehensive assessment of bronchial and circulating T cells, including

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Supported by a Wellcome Trust Clinical Research Fellowship (088362/z/09/z; to T.S.C.H.). Infrastructure support was funded by the NIHR Southampton Respiratory Biomedical Research Unit. X.Z. was supported by the Medical Research Council (G0500729), Asthma UK (06/0026), and the Foundation for the Study of Infant Death (254). K.J.S. was supported by a project grant from Asthma UK (08/0026). We acknowledge the support of the National Institute for Health Research through the Primary Care Research Network and through an Academic Clinical Fellowship awarded (to T.S.C.H.). Disclosure of potential conflict of interest: X. Zhou has received funding from Novartis and holds patents EP 1982191 and USPA 12/161409. K. J. Staples has received funding from Asthma UK and GSK Bio, as well as personal fees from Novartis. A. Manta is employed part-time by Roche, which has received funding from MantaMatics UG. T. Petrossian has received stock options from Ayasdi. P. Y. Lum has received stock options from Ayasdi. P. H. Howarth has received funding from the Medical Research Council. A. F. Walls has received funding from Thermo Fisher and Novartis and receives money from patents EP 1982191 and USPA 12/161409. S. Gadola is employed by Novartis. R. Djukanovic holds stock in Synagenix and has received consultancy fees from TEVA Pharmaceuticals, payment for delivering lectures from Novartis, and compensation for travel and other meeting-related expenses from Boehringer Ingelheim. The rest of the authors declare that they have no relevant conflicts of interest. Received for publication June 24, 2014; revised January 12, 2015; accepted for publication January 16, 2015, Available online March 5, 2015.

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mucosal-associated invariant T (MAIT) cells, a novel cell type not yet studied in patients with asthma or other chronic lung diseases, and relevant TH1/TFH/Tfd7 cytokines, as well as mediators released in the lungs by mast cells and basophils. We have combined these pathobiologic findings with standard clinical parameters used to define asthma severity20 and interrogated the rich multidimensional clinicopathobiologic data set using the novel techniques of the machine learning approach (ie, Bayesian network analysis [BNA] and topological data analysis [TDA]).21,22 This enabled an in-depth investigation of the roles of individual cell types in relation to asthma severity, characterization of the complex interconnectivity between the diverse clinical and pathobiologic parameters, and identification of clinicopathobiologic clusters that could point to novel asthma endotypes.

**METHODS**

**Participants**

Eighty-four participants (18-70 years) were enrolled from the Wessex Severe Asthma Cohort, NIHR Southampton Respiratory Biomedical Research Unit, and outpatient clinics at University Hospital Southampton: 24 healthy nonatopic participants, 15 patients with mild asthma receiving β2-agonists alone, 23 patients with moderate asthma receiving inhale corticosteroids (ICs), and 22 patients with severe asthma with persistent symptoms despite high-dose ICs (n = 16) and oral corticosteroids (n = 6, Table I and see Fig E1 in this article’s Online Repository at www.jacionline.org) classified on enrollment according to previously used criteria (see Table E1 in this article’s Online Repository at www.jacionline.org).15 One hundred seven participants were consented to achieve an approximate minimum group size of 15, according to the physician’s assessment of severity at enrollment, which was estimated to provide 80% power at the .05 significance level to detect differences in the primary outcomes of TH17 and MAIT cell frequencies in each tissue compartment after loss of missing data.

**Study procedures**

Participants were assessed based on history, examination results, skin prick test responses to common aeroallergens, spirometric results, exhaled nitric oxide (enO) levels, serum IgE levels, and (except for patients with severe asthma) methacholine responsiveness. Lung samples were obtained by means of spurt induction,23 bronchoalveolar lavage (BAL), and endobronchial biopsies.16,17 By using flow cytometry, the following T-cell subsets were characterized by their surface markers and intracellular cytokines18 in the circulation (blood) and lungs (spurtum and BAL fluid); and biopsy specimens for cell counts, providing airway and tissue composite readouts, respectively, with a matrix of 62 participants and 26 pathobiologic and 26 clinical parameters (see the Methods section and Tables E2 and E3 in this article’s Online Repository at www.jacionline.org for definitions of terms). Interconnectivity between clinical and pathobiologic parameters was first explored with BNA (Genie 2.0; Decision Systems Laboratory, Pittsburgh, Pa). Data were discretized to describe nonlinear correlations into 2 bins for binary variables or 5 to 9 bins for continuous variables.

**TDA**

To use the full range of available clinical and pathobiologic data simultaneously to identify multidimensional features within the data set, which might not be apparent with traditional methods, we used the novel technique of TDA, which is particularly suited to complex biological data sets. This approach represents a high dimensional data set as a structured 3-dimensional network in which each “node” comprises subjects similar to each other in multiple dimensions. Lines or “edges” are drawn between nodes that contain shared data points. Statistical tests can then be performed on groups or features that emerge from the inherent structure of the data set. This method combines features of standard clustering methodologies and

**Abbreviations used**

ACQ: Asthma Control Questionnaire
BAL: Bronchoalveolar lavage
BNA: Bayesian network analysis
eNO: Exhaled nitric oxide
FOXp3: Forkhead box protein 3
GINA: Global Initiative for Asthma
ICs: Inhaled corticosteroid
ICS: Inhaled corticosteroid
IQR: Interquartile range
MAIT: Mucosal-associated invariant T
Tc: Cytotoxic T
TDA: Topological data analysis
Treg: Regulatory T

The study was approved by the Southampton and South West Hampshire Research Ethics Committee B. All participants provided informed consent.

**Statistical analysis**

Data were first analyzed by using standard statistical methods, classifying subjects as healthy or having mild, moderate, or severe asthma as defined above and in the Methods section in this article’s Online Repository.15 BNA was then applied to all the pathobiologic and clinical features to seek association in relation to asthma severity. Finally, TDA was applied to the same data to create a network of distinct clinicopathobiologic clusters.

**Data elaboration and standard statistics**

Data distribution was tested by using the Shapiro-Wilk test, and data were logarithmically transformed if they were not normally distributed. For all analyses, 2-tailed P values of less than .05 were considered significant. Data were compared between the healthy and control groups (Mann-Whitney U or Student t tests) and between each asthma severity group and the control subjects (Kruskal-Wallis test or ANOVA), depending on the distribution of the data. For the latter, an overall 5% significance level was adjusted for multiple comparisons by using the Bonferroni method. Groups ranked according to disease severity were tested for linear trend by using polynomial contrasts (or the Jonckheere-Terpstra test, if not normally distributed). Data are expressed as medians with interquartile ranges (IQRs) unless stated otherwise. Correlations were tested by using the Spearman r coefficient. Kolmogorov-Smirnov tests identified significant differences between distributions within a single cluster. Data were analyzed with Prism 6.0 (GraphPad Software, San Diego, Calif) and SPSS 21.0 (IBM, Armonk, NY) software.
| Parameters                                      | Healthy control subjects | Patients with mild asthma | Patients with moderate asthma | Patients with severe asthma |
|------------------------------------------------|--------------------------|---------------------------|-------------------------------|----------------------------|
| No.                                            | 24                       | 15                        | 23                           | 22                         |
| Demographics                                   |                          |                           |                               |                            |
| Sex (M/F), no. (%)                             | 14 (58)/10 (42)          | 8 (53)/7 (47)             | 10 (43)/13 (57)              | 8 (36)/14 (64)             |
| Age (y), median (range)                        | 28 (20-65)               | 26 (21-64)                | 36 (21-56)                   | 53 (23-67)                 |
| Pulmonary function                             |                          |                           |                               |                            |
| FEV1, (% predicted)                            | 108 (105-113)            | 88 (86-103)               | 99 (86-107)                  | 65 (49-82)                 |
| FEV1 reversibility (%)                         | 3.3 (1.8-7.4)            | 13 (11-19)                | 10 (2.2-17)                  | 13 (2.6-25)                |
| PEFR (%)                                       | 108 (97-116)             | 98 (89-107)               | 95 (85-100)                  | 70 (53-82)                 |
| PEFR variability (%)                           | 0 (0-11)                 | 17 (10-25)                | 22 (17-32)                   | 17 (12-24)                 |
| PD20 (mg methacholine)                        | Negative                 | 0.19 (0.05-0.79)          | 0.25 (0.063-0.73)            | Not done                   |
| eNO (ppb [at 50 L/s])                         | 16 (11-21)               | 53 (27-107)               | 26 (15-51)                   | 20 (13-38)                 |
| Clinical                                       |                          |                           |                               |                            |
| Atopy (positive skin test result, Y/N), no. (%)| 0 (0)/24 (100)           | 15 (100)/0 (0)            | 20 (87)/3 (13)               | 15 (68)/7 (32)             |
| No. of positive skin test results to allergens | 0 (NA)                   | 6 (4-7)                   | 3 (2-5)                      | 3.5 (0-5.3)                |
| Peripheral eosinophil count (10^9/L)           | 0.1 (0.1-0.2)            | 0.2 (0.1-0.6)             | 0.2 (0.15-0.3)               | 0.2 (0.1-0.3)              |
| Total IgE (IU/mL)                              | 26 (10-61)               | 172 (21-451)              | 105 (35-188)                 | 84 (31-669)                |
| Body mass index (kg/m^2)                       | 24.4 (22.5-28.1)         | 23.6 (22.7-26.5)          | 25.3 (23.3-30.9)             | 31.0 (27.1-40.9)           |
| Smoking status                                 |                          |                           |                               |                            |
| Never smoker, no. (%)                          | 21 (88)                  | 14 (93)                   | 19 (83)                      | 17 (77)                    |
| Former smoker, no. (%) (mean pack-years)       | 3 (13 [4.2])             | 1.7 (6 [7.6])             | 4 (17 [5.8])                 | 4 (18 [26])                |
| Current smoker, no. (%) (mean pack-years)      | 0 (0)                    | 0 (0)                     | 0 (0)                        | 1 (5 [49])                 |
| Duration of asthma (y)                         | NA                       | 18 (15-26)                | 22 (9-27)                    | 36 (21-49)                 |
| ACQ score                                      | NA                       | 0.60 (0.43-1.3)           | 1.0 (0.60-1.4)               | 2.8 (2.2-3.5)              |
| GINA level of control, no. (%)                 |                          |                           |                               |                            |
| Controlled                                     | NA                       | 8 (53)                    | 5 (22)                       | 0 (0)                      |
| Partly controlled                              | NA                       | 6 (40)                    | 15 (65)                      | 2 (9.5)                    |
| Uncontrolled                                   | NA                       | 1 (6.7)                   | 3 (13)                       | 19 (90)                    |
| Treatment                                      |                          |                           |                               |                            |
| Inhaled steroids                                | No                       | No                        | Yes                          | Yes                        |
| Dose (equivalent µg BDP)                       | NA                       | NA                        | 400 (200-400)                | 1600 (1280-2000)           |
| Maintenance oral corticosteroids (Y/N), no. (%)| No                       | No                        | No                           | 6 (27)/16 (73)             |
| Mean dose if taken (mg prednisolone/d)         | NA                       | NA                        | 400 (200-400)                | 1600 (1280-2000)           |
| Short-acting β-agonist (Y/N), no. (%)          | No                       | Yes                       | Yes                          | Yes                        |
| Long-acting β-agonist (Y/N), no. (%)           | No                       | No                        | 10 (43)/13 (57)              | 22 (100)/0 (0)             |
| Leukotriene receptor antagonist (Y/N), no. (%) | No                       | No                        | 1 (4)/22 (96)                | 15 (68)/7 (32)             |
| Step on GINA treatment algorithm               | NA                       | 1                         | 2-3                          | 4-5                        |
| Inflammatory subtype, no. (%)                  |                          |                           |                               |                            |
| Total with valid data                          | 16                       | 13                        | 18                           | 21                         |
| Neutrophil                                     | 4 (25)                   | 2 (15)                    | 2 (11)                       | 10 (48)                    |
| Eosinophil                                     | 1 (6.3)                  | 3 (23)                    | 3 (17)                       | 6 (29)                     |
| Mixed granulocytic                             | 0 (0)                    | 0 (0)                     | 0 (0)                        | 1 (4.8)                    |
| Paucigranulocytic                              | 11 (69)                  | 8 (62)                    | 13 (72)                      | 4 (19)                     |
| Sputum cell differential (%)                   |                          |                           |                               |                            |
| Macrophages                                    | 52 (31-66)               | 49 (35-64)                | 47 (30-62)                   | 30 (19-43)                 |
| Neutrophils                                    | 31 (11-65)               | 34 (22-54)                | 33 (16-56)                   | 61 (32-76)                 |
| Epithelial                                    | 3.6 (2.0-24)             | 4.3 (1.7-10)              | 4.1 (1.1-21)                 | 2.9 (0-7.8)                |
| Eosinophils                                    | 0.38 (0-0.94)            | 1.5 (0.75-1.8)            | 0.75 (0.25-1.5)              | 0.69 (0-6.1)               |
| Lymphocytes                                   | 0.1 (0-0.75)             | 0.3 (0.075)               | 0 (0-0.68)                   | 0.0 (0-0.25)               |
| BAL cell differential (%)                      |                          |                           |                               |                            |
| Macrophages                                    | 84 (74-89)               | 70 (60-80)                | 81 (73-89)                   | 72 (46-94)                 |
| Neutrophils                                    | 2.5 (1.0-5.9)            | 2.5 (1.6-4.8)             | 3.5 (1.8-6.4)                | 6.5 (1.4-29)               |
| Epithelial cells                               | 9.9 (3.9-18)             | 21 (13-35)                | 11 (5.6-19)                  | 8.7 (3.3-11)               |
| Eosinophils                                    | 0.25 (0.0-0.56)          | 2.0 (0.75-3.6)            | 1.0 (0-3.0)                  | 0.1 (0-1.6)                |
| Lymphocytes                                   | 1.4 (0.94-2.4)           | 1.5 (0.38-3.0)            | 1.3 (0.5-2.3)                | 1 (0-1.6)                  |
| Relevant comorbidities, no. (%)                |                          |                           |                               |                            |
| Allergic rhinitis                              | 0 (0)                    | 12 (80)                   | 11 (58)                      | 10 (46)                    |
| Nasal polyps                                   | 0 (0)                    | 0 (0)                     | 1 (5.3)                      | 5 (23)                     |
| Eczema                                        | 3 (13)                   | 7 (47)                    | 6 (32)                       | 4 (19)                     |
| Bronchiectasis                                 | 0 (0)                    | 0 (0)                     | 1 (5.3)                      | 1 (4.5)                    |

Values are medians with IQRs, unless stated otherwise. The inflammatory subtype is based on sputum differentials using the following cut points: neutrophilic, >61%; eosinophilic, >3%. Percentages given are derived from subjects with valid data.

BDP, Beclomethasone dipropionate; NA, not available; PEFR, peak expiratory flow rate.
also provides a geometric representation of the data. In contrast to most other techniques that depend on prior hypotheses and that focus on pairwise relationships within the data, this geometric visualization allows recognition of multidimensional features (patterns) within the data in a less supervised, data-driven manner to identify meaningful subgroups that become apparent (self-define themselves) on visualization (please see the TDA plots). In addition, TDA does not require an *a priori* definition of the number of clusters anticipated.

TDA was performed, as previously described, with IRIS 2.0 software (Ayasdi, Palo Alto, Calif), constructing networks with parameters from Table E3. Three inputs were used: a distance metric, 1 or more filter functions, and 2 resolution parameters (‘resolution’ and ‘percent overlap’ or ‘gain’). A network of nodes with edges between them was created by using a force-directed algorithm. The nodes represent bins or ‘microclusters’ of data points, and 2 nodes are connected if their corresponding collections of data points have a point in common. Variance-normalized Euclidean distance was used as a distance metric, with 2 filter functions: principal and secondary metric singular value decomposition (for further explanation, see the Methods section in this article’s Online Repository). Resolution and gain settings were selected where the network structure permits identification of subgroups. Kolmogorov-Smirnov tests identified parameters that differentiate each subgroup from the rest of the structure and create clusters. Comparisons between multiple clusters used 1-way ANOVA, with *post hoc* tests with the Bonferroni correction.

For additional methods used, see the Methods section in this article’s Online Repository.

RESULTS

Data were first analyzed by using standard statistical methods without imputation or composite averages, classifying subjects as healthy or as having mild, moderate, or severe asthma (see Table E1 and the Results section in this article’s Online Repository at www.jacionline.org). Previous observations that mild steroid-naive asthma is characterized by a bias toward type 2 inflammation were confirmed, with increased numbers of IL-13–secreting CD4^+ (TH2) cells in sputum, BAL fluid, and endobronchial biopsy specimens from patients with mild asthma (Fig 1, A) and ratios of IL-13– to IFN-γ–secreting CD4^+ (TH2) cells (see Fig E3 in this article’s Online Repository at www.jacionline.org). However, this bias was not seen in patients with severe asthma, in whom frequencies of IL-13–secreting TH2 cells were not significantly different from those in healthy subjects, although we did not measure frequencies of IL-4– or IL-5–secreting T cells. Similarly, in patients with mild asthma, there were significant increases in median concentrations of the type 2 cytokines IL-5 (median,
0.13 pg/mL; IQR, 0.05-0.19 pg/mL) compared with those in healthy subjects (0.003 pg/mL; IQR, 0.001-0.006 pg/mL; P < .001) and IL-13 (0.009 pg/mL; IQR, 0.0009-0.026 pg/mL) compared with those in healthy subjects (0 pg/mL [IQR, 0-0.0008 pg/mL]; P < .05) in BAL fluid (see Fig E4 in this article’s Online Repository at www.jacionline.org). In sputum neither IL-5 nor IL-13 levels were increased in patients with mild or moderate asthma. In patients with severe asthma, IL-5 levels were also significantly increased in both BAL fluid (0.015 pg/mL; IQR, 0.007-0.19 pg/mL; P < .05) and sputum (6.18 pg/mL; IQR, 3.13-14.8 pg/mL) compared with levels seen in healthy subjects (1.19 pg/mL; IQR, 1.0-2.2 pg/mL; P = .005; see Fig E4, B), suggesting that its secretion might be relatively steroid insensitive by a physician on enrollment or based on Global Initiative for Asthma (GINA) criteria, and patients were mostly not receiving ICSs. They had increased IL-13–secreting TH2 cell numbers and lower IL-13 and tryptase levels and were predominantly paucigranulocytic (sputum neutrophils, ≤61%; eosinophils, ≤3%; Fig 4, A, and see Table E4).

To look further for novel associations between clinical and pathobiologic features, we applied TDA to all acquired clinical and pathobiologic data (Figs 4 and 5 and see Table E4 in this article’s Online Repository at www.jacionline.org). Data were treated as composite averages for T-cell subsets (across blood, sputum, BAL fluid, and biopsy specimens), cytokines and eosinophils (across blood, sputum, and BAL fluid), neutrophils, macrophages, lymphocytes, mast cell mediators, and basogranulin (across sputum and BAL fluid). One healthy and 6 asthma clusters were identified.

The TDA-derived cluster 1, comprising predominantly patients with mild atopic asthma, had (compared with other asthmatic patients) better lung function, lower Asthma Control Questionnaire (ACQ) scores (mean, 0.88), and lower severity, as assessed by a physician on enrollment or based on Global Initiative for Asthma (GINA) criteria, and patients were mostly not receiving ICSs. They had increased IL-13–secreting TH2 cell numbers and lower IL-13 and tryptase levels and were predominantly paucigranulocytic (sputum neutrophils, ≤61%; eosinophils, ≤3%; Fig 4, B, and see Table E4).

Cluster 2 consisted of patients with well-controlled asthma (mean ACQ score, 0.5), with little evidence of inflammation (the only abnormality being eosinophilia) and lower frequencies of Treg cells and IFN-γ-secreting CD8+ T cells (Fig 4).

Cluster 3 consisted of patients with moderately severe (defined by enrollment criteria) and partially controlled asthma (based on GINA criteria) despite ICSs; they had the highest bronchodilator reversibility and eNO levels. Their pathobiologic profile consisted of type 2 inflammation, with the highest levels of IL-5 and IL-13 and high frequencies of IL-13–secreting Treg cells in bronchial biopsy specimens (Fig 5, A, and see Figs E10 to E12 in this article’s Online Repository at www.jacionline.org) but also other T-cell subsets, Tfh1 cells, Tfh17 cells, and Treg cells. Cluster 4 was a small group with later-onset (mean, 28 years) moderately severe disease based on a physician’s assessment, nasal polyposis, salicylate sensitivity, and low IL-17 levels.

Cluster 5 asthmatic patients were older (mean age, 50 years), with high BMI (mean, 32.6 kg/m²), poor lung function, high symptom scores (mean ACQ score, 2.1), and high treatment requirements (predominantly GINA step 4-5 and a mean of 1500 μg/d beclomethasone dipropionate equivalent). Their pathobiologic profile was high type 2 cytokine levels (IL-5 and IL-13), IL-13–secreting CD8+ T (Tfh2) cells (see Fig E13, D, in this article’s Online Repository at www.jacionline.org), and high tryptase, chymase, and carboxypeptidase A3 levels (Figs 4 and 5, C, and see Figs E10 to E12 in this article’s Online Repository). However, when compared with cluster 3 (also type 2 cytokine high), cluster 5 had fewer Treg cells and higher ICS use (mean difference, 1250 μg/d).

Cluster 6 was predominantly female, obese (mean BMI, 35 kg/m²), and nonatopic, with salicylate sensitivity and later onset (mean age, 25 years). They were the most severe cluster based on GINA classification, physician’s assessment, symptom scores (mean ACQ score, 3.2), and lung function (mean prebronchodilator FEV1, 62%), despite high-dose ICSs (see Fig E12, B, in this article’s Online Repository at www.jacionline.org) and, frequently (50% of the group), maintenance oral corticosteroids (mean, 14 mg/d prednisolone). Their key pathobiologic features were high carboxypeptidase A3 levels and profound MAIT cell deficiency (Figs 4, E, and 5, D, and see Fig E9, D). They also had low Tc1, Th17, and IL-13–secreting Tfh2 cell numbers.
A MAIT cells

B PBMC

C Sputum

D Bronchoalveolar lavage

E Bronchial biopsies

FIG 2. MAIT cells (Vα7.2+CD161+) as proportions of CD3+ T cells in blood, sputum, BAL fluid, and endobronchial biopsy specimens in healthy subjects and asthmatic patients (A) and stratified by disease severity (B). Horizontal lines show medians. Unpaired t tests were used for log-transformed data. MAIT cell deficiency correlates with severity by linear trends across groups using residuals on log-transformed data (where P < .05). *P < .05, **P < .01, and ***P < .001, post hoc Dunnett test compared with healthy subjects.

DISCUSSION

Asthma is a common disease with a clinical severity that ranges from mild forms controlled with β2-agonists alone or low doses of ICSs to very severe forms requiring high doses of ICSs and oral corticosteroids and, increasingly, biologics, such as the anti-IgE mAb omalizumab. In this study comprehensive analysis of T cells, granulocytes, cytokines, and mast cell mediators across the airway lumen, mucosa, and blood compartments pointed to their relative roles within the asthma syndrome that have not been recognized before: reduced MAIT cell frequencies as a striking feature that is related to asthma severity, reduced Treg cell frequencies in severe disease, and increased mast cell mediator levels in patients with severe disease, which is consistent with corticosteroid-insensitive mast cell activation. This study shows that the asthma spectrum can be broken down into several multidimensional clusters defined by combined clinical parameters and underlying mechanisms (pathobiology), which provides proof of concept for endotyping asthma for better understanding of its mechanisms and more focused drug development.

Original descriptions of asthma pathobiology suggested a key role for T(H)2 mechanisms. Consistent with this concept,
when all the asthmatic patients in this study were compared as a group with healthy participants, the most significant asthma discriminators were airway eosinophilia and higher levels of mast cell mediators (carboxypeptidase A3, chymase, and tryptase), IL-5, IL-13, eNO, and serum IgE but lower IFN-γ levels, a pathobiologic profile classically associated with TH2 inflammation (Fig 1, A, and see Fig E11). The application of BNA showed high connectivity between the asthma severity node and nodes for mast cell mediators and IL-13– and IFN-γ–secreting CD8+ cytotoxic T cells (Tc2 and Tc1 cells, respectively) and a strong negative association between MAIT cell frequencies and asthma severity and ICS consumption. Applying the recently developed TDA method to the same data set showed complex multidimensional clusters (ie, possible endotypes defined by a combination of clinicopathobiologic features). The advantage of TDA over standard clustering methodologies is that it provides geometric representations of complex and multidimensional data sets that reveal and stratify distinct subgroups. TDA can deal with both linear and nonlinear associations and identifies significant subgroups in a data-driven manner, allowing for finer stratification. Furthermore, TDA is sensitive to both large- and small-scale patterns that other techniques, such as clustering and multidimensional scaling, often do not detect because they sometimes obscure geometric features captured by using topological methods. Hierarchical clustering cannot easily identify these subgroups because it tends to separate points that might in fact be close in the data.

The finding of clinicopathobiologic clusters in the data set in this study should improve our understanding of asthma and inform drug development. Overexpression of the T_{H2} cytokine network in cluster 1 is similar to the original reports in corticosteroid-naive asthmatic patients, highlighting the role of type 2 mechanisms in asthmatic patients. Clusters 3 and 5 share many clinical features, including atopy, allergic rhinitis, and emotion-related symptoms (see this article’s Online Repository). Both are characterized by type 2 inflammation, with the highest levels of IL-5, IL-13, and IL-10, suggesting that these clusters reflect asthma endotypes that might be particularly suitable for biologics, such as mepolizumab and lebrikizumab, that currently use indirect biomarkers (ie, eosinophil counts and serum periostin levels) to select patients to maximize clinical efficacy. However, important differences between these 2 clusters were identified: higher Treg cell frequencies in cluster 3 might explain their lower corticosteroid requirements, whereas lower IL-13–secreting T_{H2} cell frequencies and higher tryptase levels in cluster 5 suggest distinct, steroid-insensitive mechanisms. It should be noted that we stained only for IL-13, and therefore we cannot

FIG 3. Bayesian belief network showing the strongest interactions between pathobiologic parameters across a range of clinical severities of asthma or health. Nodes without strong interactions are excluded. Line thickness represents strength of interaction (Euclidean distance). Line colors: green, positive associations; red, negative associations; black, nonlinear associations. Asthma severity is based on overall physician’s assessment at enrollment (see Table E1). BMI, Body mass index; Tc1, CD8+ IFN-γ T cells; Tc2, CD8+IL-13+ T cells.
exclude an increase in T_{H}2 cells secreting IL-4 or IL-5 in the more severe asthma clusters. We observed some differences between patterns of cytokine secretion in sputum and BAL fluid, which might arise because BAL samples the distal airways and alveoli, whereas sputum measurement reflects changes in more proximal airways.

The strong association between asthma severity and mast cell mediator levels in clusters 5 and 6 suggests that severe asthma is a disease in which mast cell activation plays an important role. Our data add to evidence implicating mast cells in patients with severe asthma, providing additional confirmation that should stimulate the development of drugs that target mast cells. Brightling et al.6

![FIG 4](image)
have described increased numbers of tryptase-positive mast cells infiltrating the airway smooth muscle in patients with mild and severe asthma in numbers that correlate with airway hyperresponsiveness. In the Severe Asthma Research Program Balzar et al reported that severe asthma was associated with an increase in numbers of bronchial mast cells staining positive for both tryp- tase and chymase and with BAL concentrations of PGD2, a lipid mediator associated with mast cells and shown to increase after allergen challenge.

The current study adds to the evidence that mast cell activation is insensitive to corticosteroids and suggests that patients with severe asthma, in whom mast cell mediator levels are increased, can be stratified further by clinical features, such as atopic status and also by evidence of type 2 cytokine–mediated mechanisms in cluster 5 but not cluster 6. We speculate that the anti-IgE antibody omalizumab might exhibit some of its benefi- cial effects in patients with severe atopic asthma through inhibition of IgE-mediated mast cell activation. This finding might yield a prognostic biomarker for this biologic, which is currently missing, and could extend the indication for omalizumab to non-atopic asthma, in which a preliminary trial has suggested clinical efficacy.

Several asthma studies have reported increased IL-17 levels, but this study found only limited evidence for T\textsubscript{H}17 cells and none for γδ\textsuperscript{-}17 cells during either a period of clinical stability or an exacerbation. This is consistent with the findings of a recent trial in which the anti–IL-17 receptor A mAb brodalumab had no effect on symptoms or lung function in patients with moderate-to-severe asthma. However, we observed associations between IL-17 concentrations and levels of traditional type 2 biomarkers (airways eosinophils and serum IgE) that have not been reported before because IL-17 has mainly been implicated in neutrophilic inflammation in asthmatic patients.

Our study also identified, for the first time, reduced numbers of CD3\textsuperscript{+}CD4\textsuperscript{+}FOXP3\textsuperscript{-} Treg cells in patients with severe asthma. In human subjects some upregulation of the nuclear transcription factor FOXP3 has been observed in nonsuppressive T cells on T-cell receptor stimulation. Although we were not able to further validate the identity of these FOXP3\textsuperscript{-} T cells as Treg cells with additional surface markers, we observed low rates of spontaneous T-cell activation, suggesting that activated T cells will comprise only a small proportion of the CD3\textsuperscript{+}CD4\textsuperscript{+}FOXP\textsuperscript{+} T cells enumerated.

An important finding in this study is the striking deficiency of MAIT cells in both the circulation and lungs, which correlated strongly with clinical severity. To our knowledge, MAIT cells have not yet been studied in any airways disease. This study suggests that they are more abundant than invariant natural killer T cells, comprising up to 10% of blood and airway T cells. Their marked evolutionary conservation implies an important role in immunity. MAIT cells are the most abundant T-cell subset able to detect and kill bacteria-infected cells. Recent animal models of bacterial airways infection indicate their critical role in lung host defense. We found MAIT cell frequencies to be associated with serum vitamin D3 concentrations and in pilot data could be suppressed by 1 week of treatment with prednisolone (see Fig E14 in this article’s Online Repository at www.jacionline.org). The lack of a significant deficiency of MAIT cells in BAL fluid might result from low peripheral deposition of ICSs in the more distal airways and alveolar compartments sampled by...
means of lavage. Their deficiency in patients with severe asthma, whether primary or resulting from chronic corticosteroid use, can contribute to increased susceptibility to bacterial infection recognized in patients with severe asthma and to changes in the airway microbiome and might thus effect asthma pathology.

In summary, this study sheds light on previously unreported observations in asthma in relation to disease severity. The observation of clusters composed of clinical and pathobiologic parameters will need to be reproduced before these clusters can be accepted as novel endotypes of asthma. However, this paves the way for future asthma studies in large patient cohorts, such as the Severe Asthma Research Program and Unbiased BIOmarkers in PREDiction of respiratory disease outcomes (U-BIOPRED), in which distinct asthma endotypes could be identified and subsequently validated, allowing translation to clinical trials and routine clinical practice.

We thank the staff of the NIH Wellcome Trust Southampton Clinical Research Facility. We thank Professor Alan Jackson and Dr Stephen Wootton for their advice on measurements of vitamin D3 and the laboratories of University Hospital Southampton for conducting the assays. We also thank all the volunteers who gave of their time and enthusiasm to make this research possible.

Key messages

- We provide proof of concept for a powerful new analytic approach to defining multidimensional clinical and pathobiologic clusters: TDA. This underlines the role of mast cells in 2 distinct subgroups of patients with severe asthma characterized by the presence or absence of type 2 responses.
- Evidence supporting a role for T_{H}17 cells in patients with severe asthma is limited.
- We describe a striking deficiency of mucosal-associated T cells, as well as a mild reduction in Treg cell numbers, in patients with severe asthma.

REFERENCES

1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348-57.
2. Parronchi P, Macchiar D, Piccinni MP, Biwas P, Simonelli C, Maggi E, et al. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci U S A 1991;88:4538-42.
3. Robinson DS, Hamid Q, Ying S, Tiscopulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchial vault T-lymphocyte population in atopic asthma. N Engl J Med 1992;326:298-304.
4. Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am Rev Respir Dis 1990;142:863-71.
5. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Measurement of severe allergic asthma. J Allergy Clin Immunol 2001;108:184-90.
6. Corren J, Laner BQ, McClary M, Fowler-Taylor A, Cioppa GD, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol 2012;130:1404-12.e7.
7. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respir Res 2006;11:54-61.
8. Brussell GG, Maes T, Bracke KR. Eosinophils in the spotlight: eosinophilic airway inflammation in nonallergic asthma. Nat Med 2015;19:977-9.
9. Staples KJ, Hinks TS, Ward JS, Gunn V, Smith C, Djukanovic R. Phenotypic characteristics of lung macrophages in asthmatic patients: overexpression of CCL17. J Allergy Clin Immunol 2012;130:1404-12.e7.
10. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parise MV, Arron JR, et al. Leukotriene inhibition in adults with asthma. N Engl J Med 2011;365:1088-98.
11. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 2008;372:1107-19.
12. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2011;183:355-63.
13. Wenzel SE, Schwartz LB, Langmack LB, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001-8.
14. Brightling CE, Bradling P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med 2002;346:1699-705.
15. Boussiotis V, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. N Engl J Med 1990;323:1033-9.
35. Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. Am J Respir Crit Care Med 2005;171:431-9.
36. Garcia G, Magnan A, Chiron R, Contin-Bordes C, Berger P, Taille C, et al. A proof-of-concept, randomized, controlled trial of omalizumab in patients with severe, difficult-to-control, nonatopic asthma. Chest 2013;144:411-9.
37. Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J Allergy Clin Immunol 2001;108:430-8.
38. Bullens DM, Truyen E, Couteur L, Couteur L, Dilissen E, Hellings PW, et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? Respir Res 2006;7:135.
39. Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med 2013;188:1294-302.
40. McKinley L, Alcorn JP, Peterson A, Dupont RB, Kapadia S, Logar A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. J Immunol 2008;181:4089-97.
41. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003;4:330-6.
42. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Lui L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 2012;49:717-23.
43. Brossay L, Chioda M, Burdin N, Koezuka Y, Casorati G, DellaPena P, et al. CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. J Exp Med 1998;188:1521-8.
44. Meierovics A, Yankelevich WJ, Cowley SC. MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. Proc Natl Acad Sci U S A 2013;110:E3119-28.
45. Talbot TR, Hartert TV, Mitchel E, Halasa NB, Arbogast PG, Poehling KA, et al. Asthma as a risk factor for invasive pneumococcal disease. N Engl J Med 2005;352:2082-90.
46. Klemets P, Lyytikainen O, Ruutu P, Ollgren J, Kaajalainen T, Leinonen M, et al. Risk of invasive pneumococcal infections among working age adults with asthma. Thorax 2010;65:698-702.
47. Green BJ, Wiriyachaiporn S, Grainge C, Rogers GB, Kehagia V, Lau L, et al. Potentially pathogenic airway bacteria and neutrophilic inflammation in treatment resistant severe asthma. PLoS One 2014;9:e100645.
48. Auffray C, Adcock IM, Chung KF, Djukanovic R, Pison C, Sterk PJ. An integrative systems biology approach to understanding pulmonary diseases. Chest 2010;137:1410-6.
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Title: 
Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms.

Date: 
2015-08

Citation: 
Hinks, T. S. C., Zhou, X., Staples, K. J., Dimitrov, B. D., Manta, A., Petrossian, T., Lum, P. Y., Smith, C. G., Ward, J. A., Howarth, P. H., Walls, A. F., Gadola, S. D.  &  Djukanovi, R. (2015). Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms.. J Allergy Clin Immunol, 136 (2), pp.323-333. https://doi.org/10.1016/j.jaci.2015.01.014.

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